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Using Historical Biogeography Models to Study Color Pattern Evolution

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Abstract.—Color is among the most striking features of organisms, varying not only in spectral properties like hue and brightness, but also in where and how it is produced on the body. Different combinations of colors on a bird's body are important in both environmental and social contexts. Previous comparative studies have treated plumage patches individually or derived plumage complexity scores from color measurements across a bird's body. However, these approaches do not consider the multivariate nature of plumages (allowing for plumage to evolve as a whole) or account for interpatch distances. Here, we leverage a rich toolkit used in historical biogeography to assess color pattern evolution in a cosmopolitan radiation of birds, kingfishers (Aves: Alcedinidae). We demonstrate the utility of this approach and test hypotheses about the tempo and mode of color evolution in kingfishers. Our results highlight the importance of considering interpatch distances in understanding macroevolutionary trends in color diversity and demonstrate how historical biogeography models are a useful way to model plumage color pattern evolution. Furthermore, they show that distinct color mechanisms (pigments or structural colors) spread across the body in different ways and at different rates. Specifically, net rates are higher for structural colors than pigment-based colors. Together, our study suggests a role for both development and selection in driving extraordinary color pattern diversity in kingfishers. We anticipate this approach will be useful for modeling other complex phenotypes besides color, such as parasite evolution across the body. [Evolutionary rates, feathers, plumage, RevBayes, spectrophotometry.]

Ornaments used in display or social competition are among the most diverse traits in nature (West-Eberhard 1983). Colorful feathers in birds can evolve rapidly through sexual selection (Eliason et al. 2015) and can play a role in the speciation process by causing reproductive isolation between populations (West-Eberhard 1983; Ritchie 2007; Seddon et al. 2013). Feather colors in birds are caused by diverse mechanisms, including light absorption by pigments, coherent scattering of light by organized feather tissue, or both (Shawkey and D'Alba 2017). In many cases, birds are not uniformly colored but show variable colors across the body organized into distinct feather patches. These patches may follow known feather tract boundaries (Lucas and Stettenheim 1972) or spread across whole body regions (Prum and Williamson 2002). Ornithologists have long realized the biological importance of plumage patterns and their complex, yet modular nature (Prum and Dyck 2003). There are two schools of thought for how plumage patterns might evolve: through divergent selection in different social or environmental contexts (Marchetti 1993) or through developmental constraints limiting the range of possible plumage patterns (Price and Pavelka 1996). It is likely that both of these mechanisms operate together. To understand the biological role of color patterns, we need to know how color patches change through time and space (across a bird's plumage) in a comparative framework.

Studying plumage pattern evolution presents several analytical challenges, including the multivariate nature of plumages, variability and difficulty in scoring color mechanisms, and definition of patch boundaries. There are generally two classes of approaches scientists have used in modeling the evolution of plumage color patterns: i) distance-based methods using continuous color data (e.g., reflectance spectra) and ii) continuous time Markov chain (CTMC) methods (O'Meara 2012) using discrete color data. Distance-based methods consider the average color distance among plumage patches for an individual bird, with lower values indicating more uniform plumages (Maia et al. 2016). This approach effectively treats plumage pattern complexity as a single trait. Several recent studies have used this approach to look at diversity of color mechanisms, both within and among species (Stoddard and Prum 2008; Shultz and Burns 2013; Maia et al. 2016). In contrast to distance-based methods, CTMC methods consider the presence or absence of a color pattern or mechanism in a body region as an independent trait and models their evolution using Markov models (Price and Pavelka 1996; Omland and Lanyon 2000). These analyses can show whether patterning mechanisms are ancestral (Price and Pavelka 1996), and how often they turn on or off during the course of evolution in different lineages. CTMC approaches have also be applied at a finer scale to look at pattern transitions within feathers (Gluckman and Mundy 2016). Importantly, both classes of methods used in modeling plumage pattern evolution are either univariate or do not consider possible nonindependence of color among plumage patches (e.g., caused by shared developmental pathways or correlated selection for concerted changes in nearby body regions). Thus, our understanding of how plumages evolve might be hindered by our limited ability to draw inferences from methods that do not take into account the multivariate nature of plumage patterns.

TABLE 1. Analogy between historical biogeography models and plumage pattern evolution

Concept	Biogeography	Plumage pattern evolution
Location	Geographic location	Location on a bird's body (i.e., a plumage patch)
Presence/absence	Presence/absence of individuals in a geographic area	Presence or absence of a given color mechanism in a body region
Dispersal	Movement of birds from one geographic region to another	Spreading of color from one plumage patch to another
Extirpation	Local extinction of birds in a geographic region	Loss of a color mechanism in a plumage patch
Allopatric range change	Splitting of geographic range during speciation	Evolutionary change from color presence in one to several patches at speciation
Widespread sympatry	Daughter species retain the same range as the parent during speciation	Daughter species maintain the same color mechanism in a patch as the ancestor
Jump dispersal	Long-distance dispersal over inhospitable areas	Color gain in a distant, non-contiguous plumage patch
Macroevolutionary source	Region that does not receive taxa through dispersal (Goldberg et al. 2005)	Plumage patch that does gain color from surrounding regions
Macroevolutionary sink	Region that obtains taxa through dispersal and without a local origination (Goldberg et al. 2005)	Plumage patch that gains color from surrounding region(s) but does not act as an origin for color in other patches

Recent comparative approaches for inferring historical biogeographic patterns (Ree and Smith 2008; Landis et al. 2013) might be usefully applied to modeling color movement across the body for three important reasons. First, we argue that color spreading across a bird's plumage is analogous to the movement of individuals/species' ranges across a globe (Table 1). Second, a recent Bayesian implementation of historical biogeography models enables analysis of a large number of areas using a data-augmentation approach (Landis et al. 2013), relaxing an analytical constraint in studying plumage evolution. Third, this Bayesian approach can model the effects of distance along with rates of color "dispersal" and loss in a patch. Expansion of pigment patterns at the developmental level can occur by a diffusion mechanism in which pigment concentration, time, and distance play distinct roles (Prud'homme et al. 2007). Distance might also be critical for understanding changes in color patterns at the macroevolutionary scale.

Here, we test the role of distance across the body in understanding color pattern evolution across a color-diverse, cosmopolitan radiation of birds, kingfishers (Aves: Alcedinidae). Kingfishers produce diverse colors in distinct ways, including both carotenoids and melanin pigments as well as spongy nanostructures of air and keratin within feathers (Stavenga et al. 2011; Li et al. 2012; Thomas et al. 2014). Inspired by this diversity, we tested whether a model that predicts expansion of color across patches as a function of distance between patches will be a better statistical fit for modeling how color pattern complexity evolves than a simpler, independent model. We then used this model to test whether evolutionary rates are higher for structural colors compared to pigment-based colors, testing a conclusion of Eliason et al. (2015). Finally, we calculated a color complexity metric taking into account diversity both in *where* and *how* colors are produced on the body and assessed whether this metric covaries with distinct aspects of the signaling environment. Our treatment of plumage patterns as a single evolving phenotype allows us to ask novel questions about color movement among body regions and plumage modularity and defines a framework for testing novel hypotheses about the selective agents driving and maintaining color patterns in birds.

METHODS

Scoring Plumage Color Mechanisms

We defined 22 distinct plumage patches based on observed patch diversity across kingfishers and characterized these patches for 113 species, comprising nearly all of the family's diversity (Andersen et al. 2018). In nearly all cases, these plumage patches followed known feather tract boundaries in birds (see Supplementary Table S1 available on Dryad at http://dx.doi.org/10.5061/dryad.3680n0c). In some cases, we ignored feather tracts either due to observed homogeneity in the clade (e.g., dorsal and humeral feather tracts) or lack of feathers (e.g., metatarsal tract). Following Stoddard and Prum (2011), we scored the presence or absence of six color mechanisms in each patch: noniridescent structural color in feather barb rami, iridescent color in barbules inferred from gloss and angle-dependent coloration, phaeomelanin-based coloration, eumelanin-based coloration, carotenoid coloration, and structural white coloration (see Table 2, Supplementary Figs. S1–S6 available on Dryad for data). We assessed evidence for color mechanisms in both males and females using a combination of museum specimens (see Supplementary Material available on Dryad for specimen list), field guide illustrations, and photos (Fry 1992; Woodall 2016). In most kingfisher species, males are either more colorful or as colorful as females (i.e., monochromatic); therefore, we used

TABLE 2. Plumage color mechanisms in birds

Mechanism	Color(s) produced	Diagnostic features	Evidence in kingfishers
Structural color in barb rami (sr)	Blue, purple, green, and turquoise	Color changes with viewing angle, distinct reflectance peaks	Stavenga et al. (2011)
Structural color in barbules (sb)	Green and blue	Color change with angle, distinct reflectance peaks	Durrer (1977)
Structural white (w)	White	No distinct peaks in reflectance curve	
Phaeomelanin (p)	Light brown, rufous, and yellow	Proportionally more reflectance at long wavelengths in reflectance curves	Stavenga et al. (2011)
Eumelanin (e)	Dark brown, black, and gray	Flat reflectance curves	Li et al. (2012)
Carotenoids (co)	Yellow, orange, red, and purple	Increasing reflectance at long (>600 nm) wavelengths, reflectance dip (from absorption by pigments) from ~400 to 500 nm	Thomas et al. (2014)
Psittacofulvins	Red and yellow		Not observed
Porphyrins (turacin, turacoverdin)	Green and red		Not observed

Notes: Evidence for different color mechanisms in kingfishers in the literature and diagnostic features used to assess color in our data set.

the more diverse male color patterns for this study, in order to maximize our opportunity to find signal in our data. In cases of species with multiple subspecies, we used data for the nominal subspecies (e.g., *Todiramphus chloris chloris*). For purple and blue colors, assessment was straightforward given there are no known pigments in birds that can produce blue colors, therefore we only had to assess whether color emanated from the barbules or barb ramus using a light microscope. For colors more difficult to assess (e.g., yellow or brown colors that can be produced by several mechanisms), we used microscopy (light, scanning electron) and UV–Vis reflectance spectrophotometry (Table 2). Under our scheme, a single patch can contain one or several color mechanisms (e.g., pink coloration produced by phaeomelanin pigments and structural blue coloration; Supplementary Table S2 available on Dryad). This approach avoids problems with polymorphisms (e.g., multiple color mechanisms within a single feather or patch) and is most compatible with our interest in understanding the evolution of color mechanisms rather than spectral properties of individual color patches (e.g., hue, brightness). One potential issue with this approach is that eumelanin can mask the effects of carotenoid or phaeomelanin pigments (Shawkey and D'Alba 2017); this would cause us to underestimate the number of color mechanisms in a patch. Confirming presence of these pigments would require detailed chemical analyses for several species. Expression of brown coloration indicative of phaeomelanin pigments indicates relatively more phaeomelanin than darker eumelanin compared to patches without brown coloration. Given that brown color would be the actual target of selection, we feel our approach of inferring color mechanisms is justified.

Modeling Color Pattern Evolution

Character scoring.—We scored color as a binary character (e.g., presence or absence of a given color mechanism in a body region). We did not consider multistate characters for three reasons: i) color mechanisms are not mutually exclusive (e.g., pink; see Supplementary Table S2), suggesting that color mechanisms can spread across the body independent of other colors; ii) it is computationally infeasible to model 6 color states and 22 patches ($>10^{17}$ possible plumage patterns compared to $\sim10^{6}$ for binary characters); and iii) we were interested in evolutionary origins of color mechanisms and tying color evolution to development of color patterns rather than the spectral qualities of plumage patches (e.g., yellow or red hues). One potential caveat with our binary scoring approach compared to multistate characters is that we may be overestimating rates of evolution (Brazeau 2011).

Accounting for distance.—To account for among-patch distances, we defined an adjacency matrix for all pairs of plumage patches (e.g., crown feathers are adjacent to loral feathers, but not wing feathers). We then used this matrix to perform a principal coordinates analysis (Gower 1966) using the pcoa function in ape (Paradis et al. 2004) to get x and y coordinates (required by the BayArea model implemented in RevBayes) for each patch. These values were then input into RevBayes (Höhna et al. 2016) to account for distance in the analysis. In some bird species, plumage patterns are very different with spread wings compared to folded wings. For example, wing feathers in golden-winged manakins (*Masius chrysopterus*) are black with bright yellow on the inner portion of the feather vane; with wings folded the color patch appears black, but spread out the color is brilliant yellow (Prum and Johnson 1987). Similarly, several duck species have a bright iridescent color patch on the secondary feathers (Eliason and Shawkey 2012) that is only visible with spread wings and *Halcyon* kingfishers incorporate flashes of color in their spread wing during courtship display (Fry 1992). Therefore, we calculated distances for two different

anatomical configurations: a spread-wing posture and a folded-wing posture (see Supplementary Figs. S7 and S8 available on Dryad). A model that fit the folded-wing configuration better would suggest that plumage patch coordinates derived from this wing position better explain evolutionary transitions in color across the body than coordinates derived from a spread-wing configuration.

Model fitting.—We fit two different historical biogeography models for each presumed color mechanism using a time-calibrated phylogeny for kingfishers (Andersen et al. 2018). First, we fit a distance-dependent model in which rates of color dispersal are related to the anatomical distance between patches in a closed-wing configuration. Second, we fit a distance-dependent model for distances derived from a spread-wing configuration. We did not account for cladogenetic change in these models because this option is not currently implemented in the BayArea-type model within RevBayes. For each model, we used a Dirichlet prior for rates of color "dispersal" and "extirpation" and an exponential prior (mean = 0.1) for the parameter describing distance-dependence (β). In traditional historical biogeography models, null ranges (i.e., a species living in no areas) are not allowed. However, with color pattern evolution, it is entirely plausible that a given color mechanism would be absent in a species but genetic machinery would be allow color to appear at a later point in evolution, thus we allowed null ranges. We ran two chains with 10^6 generations each, sampling nodes and branch character histories every 10^3 generations. We assessed chain convergence using diagnostic plots in Tracer v. 1.6 (Rambaut et al. 2014) and the Gelman diagnostic (Gelman and Rubin 1992) calculated in the R package coda (Plummer et al. 2006). Since a distance-independent model is equivalent to the distance-dependent model at $\beta = 0$ (Landis et al. 2013), we were able to compare Bayes factors for each model using the Savage–Dickey ratio—the ratio of the posterior probability to the prior probability at $\beta = 0$ (code available at https://rdrr.io/github/LudvigOlsen/LRO.utilities/man/savage_dickey.html).

Macroevolution of Color Patterns

Comparing tempo and mode.—To determine significance of the difference in inferred parameters (rate of color dispersal, rate of color extirpation, and distance-dependence parameter) among color classes, we uniformly sampled from the posterior distributions and computed 95% credible intervals for the difference between pairs of parameters. We classified parameters as significantly different if their 95% credible intervals failed to overlap zero.

Comparing ancestral state reconstruction methods.—To compare our Bayesian approach to simpler approaches, we used parsimony to reconstruct ancestral states for each plumage patch independently using the ancestral.pars function in phangorn (Schliep 2011). To compare the results, we rounded ancestral states from RevBayes (to 0/1) and summed the differences per node between RevBayes and parsimony reconstructions.

Network analysis of color transitions.—We visualized transitions among different plumage patches in a network framework. A benefit of the data augmentation approach in the BayArea model is that saved sampled histories can be used to tabulate evolutionary changes in traits along branches, not just at nodes in the tree. We wrote custom R code to add up changes along branches. To compare evolutionary transition matrices for different color mechanisms, we used Mantel tests. To further understand the temporal patterns of color acquisition among patches, we calculated the proportion of evolutionary transitions to and from different plumage patches using functions in the igraph package (Csardi and Nepusz 2006). In the context of plumage pattern evolution, a macroevolutionary source patch would indicate an early origin of color in that region followed by spread of color to other parts of the body, while a sink patch would indicate color evolved by dispersal from other parts of the body (see Table 2 for definitions).

Evolution of Plumage Color Complexity

Calculating complexity.—We defined plumage color complexity as the number of unique contiguous plumage regions summed across all color mechanisms (e.g., see Endler 2012). We calculated this metric by multiplying the anatomical adjacency matrix by the color distance matrix for each tip and node in the tree based on reconstructed ancestral states. As an example, a species with a black head would be given a complexity score of 1, while a species with a black head and a blue tail would be given a score of 2 (see Supplementary Fig. S9 available on Dryad). To compare these ancestral estimates of complexity to another approach involving complexity values at the tips (Shultz and Burns 2013; Maia et al. 2016), we estimated complexity values at nodes using ancestral state reconstruction based on tip values using the ace function in ape (Paradis et al. 2004).

Comparing complexity metrics.—Our estimates of color complexity capture variability in the numerous ways of producing color in kingfishers (see Supplementary Table S2) but might not capture more subtle variation in hue. For example, two species with turquoise and violet feathers would both be given a score of 1 (for having a structural color mechanism in feather barbs). To compare our metric with other ways of estimating plumage complexity involving fine-scale spectral data (Stoddard and Prum 2008; Shultz and Burns 2013; Maia et al. 2016), we gathered reflectance data from 22 patches in males of 61 species (see Supplementary Material available on Dryad for specimen list). We then

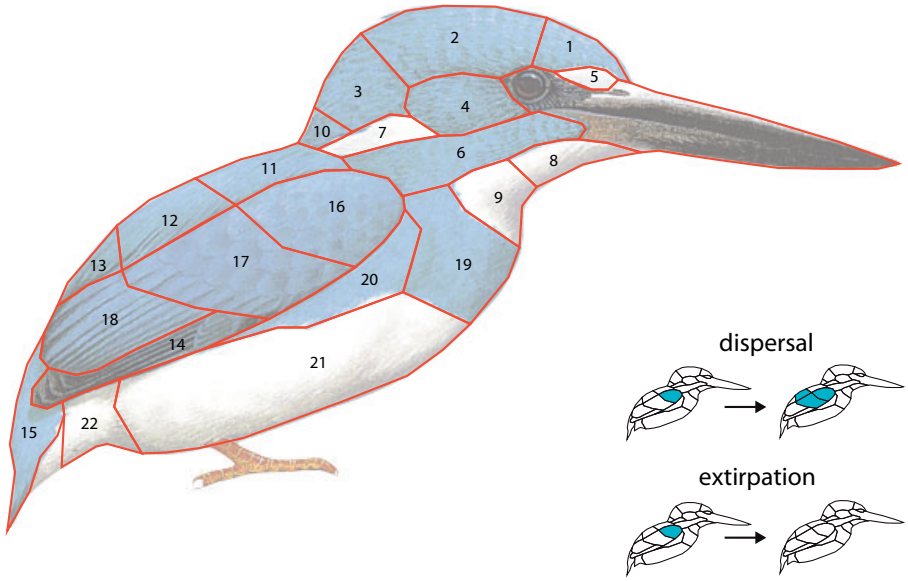

FIGURE 1. Analogy between historical biogeography analyses and plumage pattern evolution. Drawing of a cerulean kingfisher (*Alcedo coerulescens*) with outlines for 22 plumage patches we scored for each species in the kingfisher clade. Insets depict two scenarios: color "dispersal" from the shoulder to the back and wing (upper) and color "extirpation" in the shoulder (lower). Illustration reproduced by permission of Lynx Edicions.

averaged spectra by species and used visual models implemented in the pavo R package (Maia et al. 2013) to estimate the proportion of light stimulating each of the four cones in birds and converted these 4D data into a 3D representation of color diversity (i.e., a tetrahedral colorspace; Supplementary Fig. S10 available on Dryad). We then calculated plumage complexity as the mean Euclidean distance among all plumage regions (i.e., "interpatch chromatic contrast" sensu Maia et al. 2016). To link spectral complexity to our metric of mechanistic complexity, we used Pearson correlation tests and phylogenetic generalized least squares (PGLS) regressions implemented in the phylolm R package (Ho and Ané 2014).

Testing environmental drivers of plumage complexity.— Kingfishers are globally distributed and live in a broad spectrum of habitats and light environments (Fry 1992). The signaling environment might drive the evolution of plumage patterns by acting as a selective agent on where bright colors are produced on the body (Marchetti 1993; Gomez and Théry 2007) or the spectral properties of individual color patches (Gomez and Théry 2004). The light environment hypothesis predicts that species will use different colors in different environments. To test this hypothesis, we obtained data on habitat openness and diet from Woodall (1991) and *Handbook of the Birds of the World* (Woodall 2016). Several kingfisher species forage in open environments by plunge-diving for fish. Relationships between plumage patterns and fish-eating behavior have been previously demonstrated in birds, both experimentally (Göttmark 1987) and statistically (Bretagnolle 1993). We therefore included foraging mode (plunge-diving or not) as an additional factor in our model, based on available data (Woodall 1991, 2016). Predation may also influence plumage pattern evolution, thus we included insularity as an additional covariate, as species living on islands generally experience lower predation pressures (Steadman 2006). Insular species were those whose ranges primarily occur in Oceanic islands, Philippines, and/or Wallacea (Andersen et al. 2018). To test whether these ecological factors explain a significant amount of variation in plumage complexity across kingfishers, we used PGLS multiple regression in the phylolm R package (Ho and Ané 2014).

RESULTS

Modeling Color Pattern Evolution

For all color mechanisms, the distance-dependent model was strongly preferred over a simpler model in which patches evolve independently (Table 3), suggesting that accounting for nonindependence of plumage patches is critical for understanding tempo and mode of color pattern evolution. Distance was generally more important for spread-wing than folded-wing configurations (Table 3). For structural ramus, eumelanin, and phaeomelanin coloration, the spread-wing configuration was strongly preferred (Table 3). In contrast, for structural white colors the spread-wing configuration was preferred (Table 3). For folded-wing configurations, the distance-dependence parameter did not differ significantly among color mechanisms (Fig. 2d). For spread-wing configurations, distance was significantly more important for phaeomelanin and structural white colors compared to eumelanin colors (Supplementary Fig. S11 available on Dryad).

TABLE 3. Model comparison and parameter estimates

Color mechanism	Wing configuration	β	Dispersal rate	Extirpation rate	AICM	ΔAICM	Savage–Dickey ratio
Eumelanin	Folded	0.557 [0.001, 1.142]	0.017 [0.013, 0.02]	0.031 [0.027, 0.035]	22281	856	23*
	Spread	0.553 [0.387, 0.701]	0.016 [0.013, 0.02]	0.032 [0.027, 0.036]	21425	0	3.7E4**
Phaeomelanin	Folded	1.249 [0.902, 1.621]	0.013 [0.01, 0.016]	0.031 [0.026, 0.037]	21275	141	4.8E6**
	Spread	0.911 [0.731, 1.094]	0.013 [0.009, 0.016]	0.032 [0.025, 0.038]	21133	0	4.9E6**
Structural ramus	Folded	1.122 [0.11, 1.752]	0.009 [0.006, 0.011]	0.019 [0.016, 0.022]	13947	126	101**
	Spread	0.736 [0.532, 0.928]	0.009 [0.007, 0.012]	0.019 [0.016, 0.022]	13821	0	2.5E5**
Structural white	Folded	0.846 [0.319, 1.356]	0.009 [0.007, 0.012]	0.023 [0.019, 0.026]	16819	0	251**
	Spread	0.958 [0.741, 1.164]	0.01 [0.007, 0.012]	0.023 [0.02, 0.027]	17020	201	2.7E4**

Notes: Results shown for different color mechanisms (sr = structural ramus, p = phaeomelanin, e = eumelanin, w = structural white) and evolutionary models (folded: folded-wing patch configuration, spread: spread-wing patch configuration). Parameter values are means and 95% credible intervals in square brackets. We compared models using AICM scores (Baele et al. 2012) in Tracer (100 simulations, 25% burn-in). To assess the importance of distance, we used Savage–Dickey ratios, or the ratio of the posterior probability of the distance-dependence parameter (β) being zero compared to the prior (exponential distribution, mean = 0.1) density at zero (Landis et al. 2013). Support for distance-dependence indicated by asterisks, with categories from Jeffreys (1961): strong (10–30, *) and decisive support (>100, **). Limited variation in structural barbule and carotenoid coloration (see Supplementary Figs. S5 and S6 available on Dryad) made it difficult to fit these models, thus results are not shown.

Rates of Evolution

Rates of color loss were significantly higher for eumelanin and phaeomelanin-consistent colors compared to structural ramus colors (Fig. 2b). For spread-wing configurations eumelanin colors were lost at a significantly faster rate than structural ramus or white colors, but not phaeomelanin colors (Supplementary Fig. S11 available on Dryad). Rates of color dispersal to different parts of the body were significantly higher for eumelanin compared to structural ramus or structural white colors, both for folded (Fig. 2a) and spread wing configurations (Supplementary Fig. S11 available on Dryad). Net rates of color dispersal (dispersal minus color loss) did not differ significantly among color mechanisms for either wing configuration (Fig. 2c). The highest number of plumage changes per time occurred in the "*Todiramphus*" clade (including both *Todiramphus* and *Syma* kingfisher species), while the lowest number occurred in the older Cerylininae clade (Fig. 4).

Ancestral States of Color Mechanisms

The common ancestor of kingfishers is estimated as having structural ramus coloration on the tail, back, wings, and head; phaeomelanin coloration on the chest, lores, back of head and flanks; eumelanin coloration on the flight feathers and head; and structural white coloration on the throat, belly, and rump (Fig. 4). Structural barbule coloration and carotenoid orange coloration are inferred to be absent in the common ancestor of kingfishers.

Ancestral states reconstructed with RevBayes were generally comparable to parsimony ancestral states with some notable differences (Supplementary Fig. S12 available on Dryad). For eumelanin coloration, RevBayes reconstructs more color in old lineages, while for phaeomelanin coloration color is reconstructed more in young lineages compared to parsimony. Results for structural barb color are generally similar (Supplementary Fig. S12e,k available on Dryad). White color shows the biggest difference between anatomical configurations and the young "*Todiramphus*" radiation (lower part of tree in Supplementary Fig. S12f,l available on Dryad) suggests parsimony is reconstructing gains in ancestor then subsequent losses, while RevBayes reconstructs absence in ancestor than several gains. This likely results from RevBayes upweighting color spread to a new area depending on how many current plumage regions are colorful.

Spread of Color across the Body

Phaeomelanin-based color occurs primarily on the head compared to the back and wings for structural and eumelanin coloration (Fig. 3, Supplementary Fig. S13 available on Dryad). For structural barb coloration, the tail and wing covert feathers act as "sources" while primary wing feathers act as a "sink" (Fig. 3a). For phaeomelanin-based coloration, breast, flank, and lore feathers act as sources while belly, forehead, crown, and nape feathers act as sinks (Fig. 3b). There were some cases of long-distance color "dispersal" for phaeomelanin-based coloration (e.g., from the back of the head to the rump), but most changes were to nearby patches (e.g., from the crown to the back of the neck; Fig. 3b). For eumelanin-consistent coloration, the primaries act as sources and the "moustachial stripe" acts as a sink

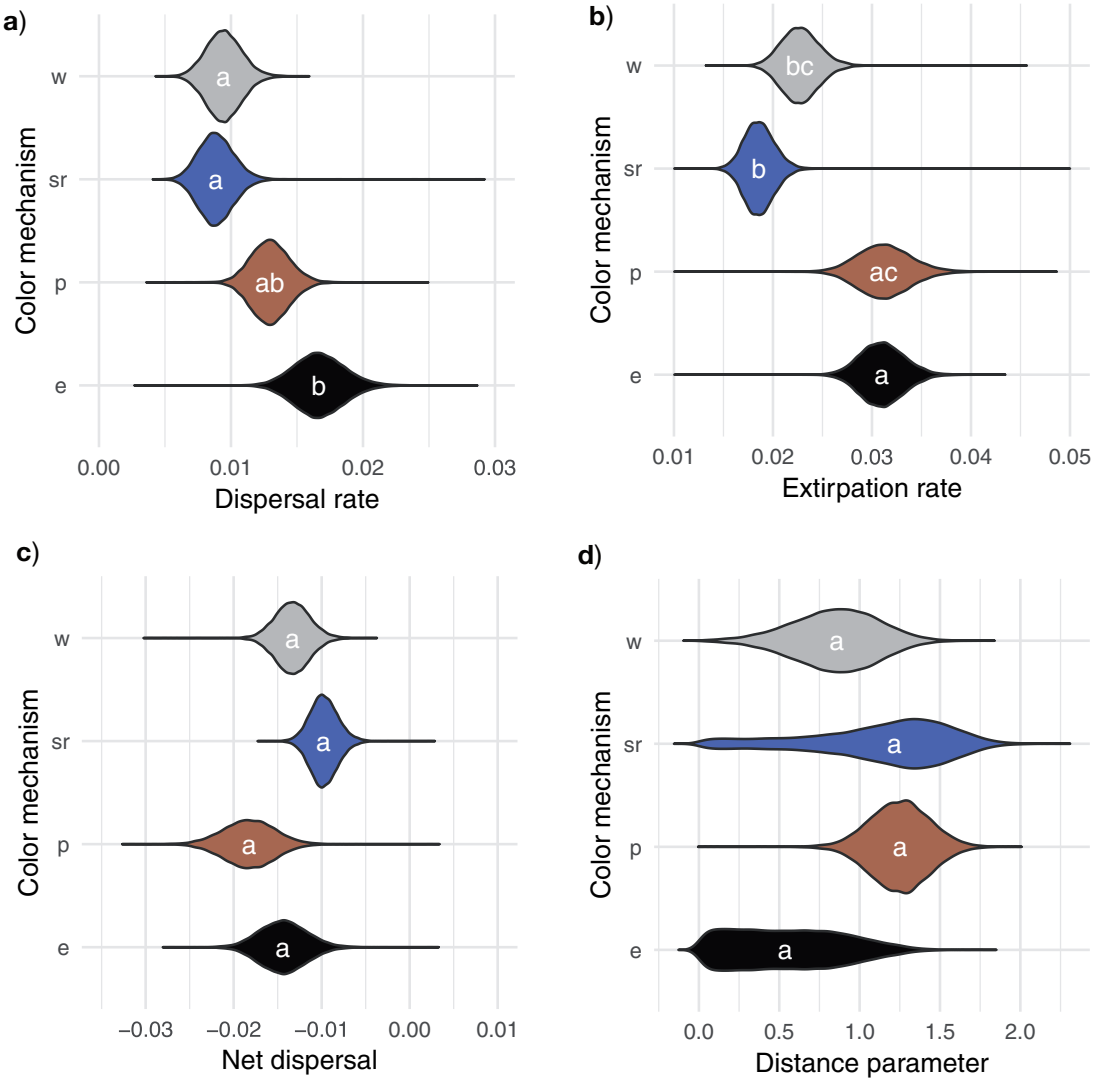

FIGURE 2. Tempo and mode of plumage pattern evolution in birds. Violin plots show distribution of parameters color "dispersal" rate (a), color "extirpation" rate (b), net rate of gain (color dispersal rate minus color extirpation rate (c), and the distance parameter (d). Colors correspond to color-producing mechanisms (beige: structural white coloration, blue: structural barb ramus coloration, brown: phaeomelanin coloration, black: eumelanin coloration). Violin plots sharing similar letters are not significantly different ($P > 0.05$).

(Fig. 3c). For structural white coloration, the chin and throat act as sources and the flank feathers act as a sink (Fig. 3d). Transition matrices were significantly correlated for structural ramus and eumelanin coloration (Mantel test, $P = 0.006$) and phaeomelanin and structural white coloration ($P = 0.006$; see Fig. 3). Other pairs of color mechanisms were not correlated ($P > 0.05$).

Evolution of Plumage Complexity

Interpatch chromatic contrast calculated from reflectance spectra increased significantly with plumage color complexity when considering a folded-wing configuration (Pearson's $r = 0.26, P = 0.049, N = 58$; see Supplementary Fig. S14 available on Dryad). This relationship was not significant for a spread-wing configuration. Neither relationship was significant when we accounted for phylogenetic signal using PGLS regression ($P > 0.05$). We recovered several increases in plumage complexity (e.g., in the species-rich Alcedininae clade and *Halcyon* kingfishers), as well as decreases in the Cerylininae clade, kookaburras, and paradise kingfishers (Fig. 4, Supplementary Fig. S15 available on Dryad). Ancestral state reconstructions based on tip values only overestimated plumage complexity in old nodes and underestimated values in young nodes (Supplementary Fig. S16 available on Dryad). Plumage complexity was significantly higher in fish-eating species and those living in closed habitats for both wing configurations considered (Supplementary Table S3 available on Dryad). Insular species further had significantly more complex plumages based on complexity scores calculated from a spread-wing

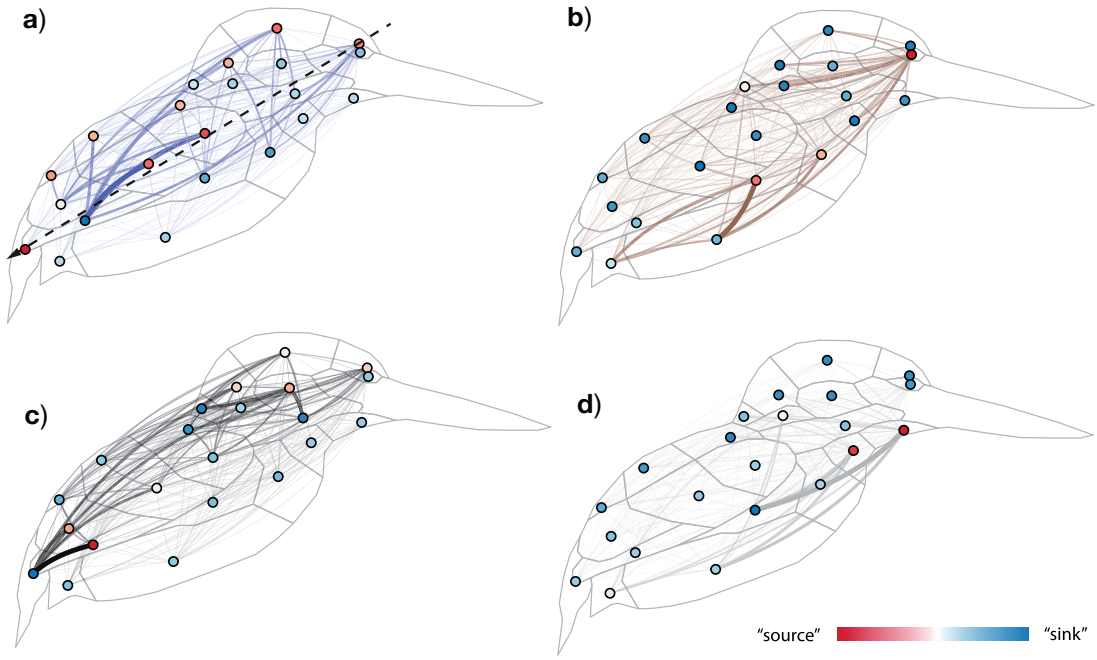

FIGURE 3. Evolutionary transitions of color-producing mechanisms across the body. Results show total number of evolutionary transitions among plumage patches for structural barb ramus coloration (a), phaeomelanin-based coloration (b), eumelanin-based coloration (c), and structural white coloration (d). Results are for color "dispersal" toward the posterior part of the body, defined by axis from the front of the head to the tail (dashed black arrow in A; see Supplementary Fig. S13 available on Dryad for color anterior-ward color dispersal results). Thickness of curved lines indicates number of character changes calculated from stochastic character maps. Filled circles indicate whether a patch acts as a macroevolutionary source (red) or sink (blue; see Table 1 for definitions).

configuration (Supplementary Table S3 available on Dryad).

DISCUSSION

Complex problems demand novel approaches. Here, we use historical biogeography models to shed light on a longstanding question in animal communication—how complex color patterns evolve. Treating color patches as analogous to the geographic range of a lineage allows us to ask new questions about color patterns (e.g., *What is the role of distance across the body in color pattern evolution?*) and visualize complex traits in new ways (e.g., as networks; Fig. 3). Our results demonstrate the importance of accounting for distance in modeling the evolution of color across animals' bodies (Fig. 2, Table 3) and reveal differences in the tempo and mode of color evolution for different color-producing mechanisms (Fig. 2). These findings highlight the importance of considering body orientation in studies of color evolution (Table 3) that might be important in other systems, such as distinct dorsal and ventral wing coloration in butterflies or concealed colors in the bellies of agamid lizards (Stuart-Fox and Ord 2004).

Mechanistically, the spread of color across the body could occur either through broad changes in transcription factors controlling where pigments are expressed or narrow changes in the expression of individual pigment genes (Richardson et al. 1991; Prud'homme et al. 2007). The demonstrated role for diffusion in pigment-based colors of birds (Price and Pavelka 1996; Prum and Williamson 2002) might explain why melanic color spreads more frequently to adjacent plumage regions (Fig. 3b,c). Developmental mechanisms could also influence within-feather patterning of pigments, which we do not treat here owing to analytical constraints. Compared to pigment-based colors, the development of color-producing nanostructures in feathers uniquely depends on concentrations and interactions between keratin molecules and temperature (Dufresne et al. 2009). Structural colors should therefore be more malleable over developmental and evolutionary timescales (Eliason et al. 2015). Interestingly, we find that melanic colors are gained at the same rate (Fig. 2a) but are lost more easily than structural colors (Fig. 2b). This suggests that, while the hue of individual patches displaying structural coloration evolves rapidly (Eliason et al. 2015), at the overall plumage level structural colors in a patch may be constrained or under stabilizing selection. Structural color dispersal to nonadjacent body regions (Fig. 3a) might be further explained by modular expression of keratin genes (Wu et al. 2015) potentially involved in nanostructure development. Macroevolutionary source-sink dynamics reveal the temporal patterns of color acquisition in different body regions. For example, wing feathers act as a sink for structural barb coloration (Fig. 3b) but a source for eumelanin-consistent coloration (Fig. 3c). This suggests that structural color in primary feathers has

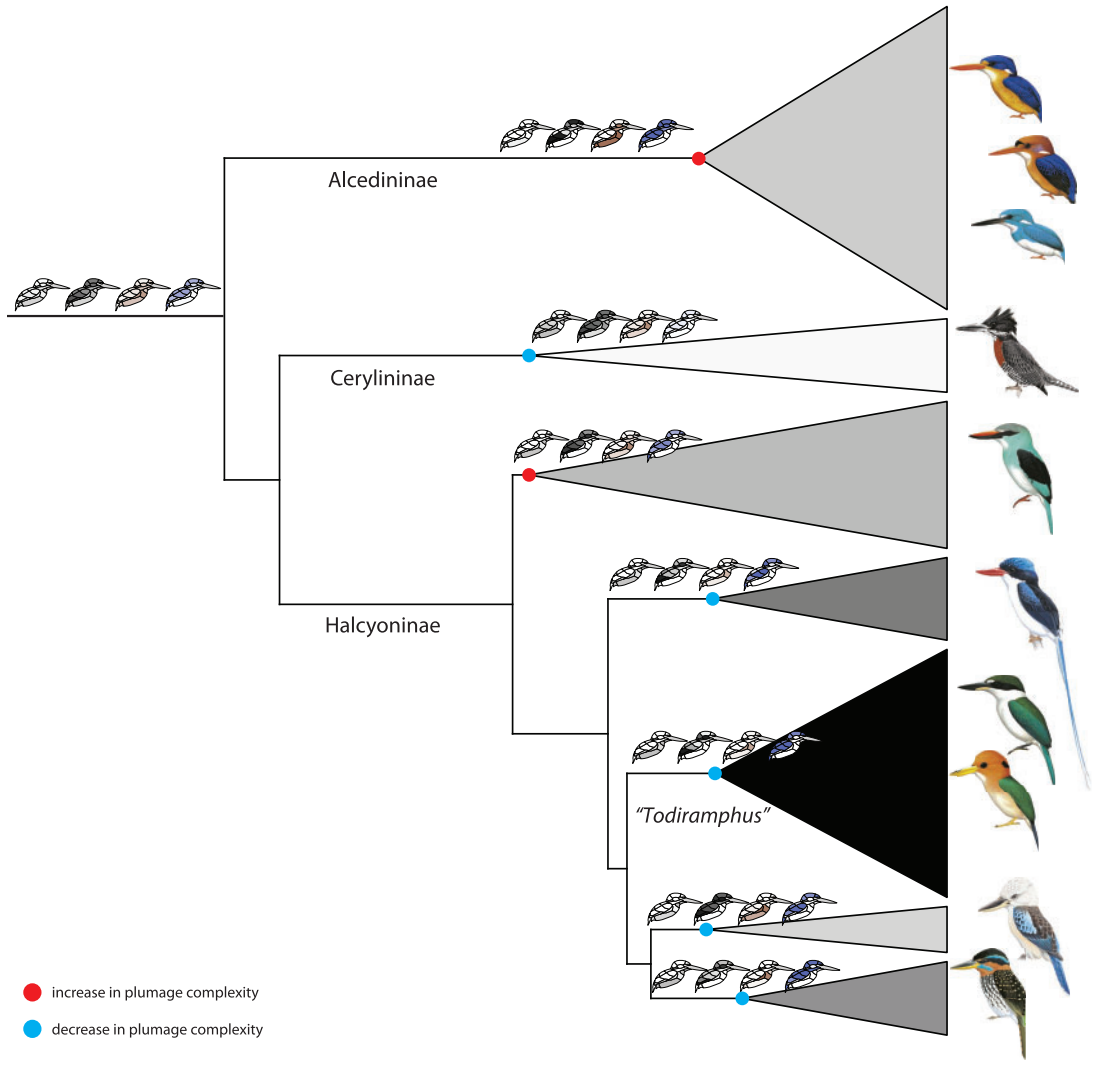

FIGURE 4. Evolution of plumage patterns in kingfishers. Phylogeny of kingfishers (Andersen et al. 2018) with estimated ancestral states of structural white, eumelanin, phaeomelanin, and structural ramus coloration for 22 distinct plumage patches. Other coloration mechanisms are not shown, as they are inferred to be absent in most (structural barbule coloration only occurs in the Cerylininae clade) or all nodes indicated (carotenoid coloration). Triangles represent number of species in each clade (size of triangle) and number of plumage changes per myr (shade of triangle). Cartoons show absence (white) or presence of a given color mechanism in a patch, with shade corresponding to uncertainty integrated over all stochastic character maps (lighter colors indicating higher uncertainty). Circles show changes in plumage complexity along the branches leading to each subclade (see Supplementary Material available on Dryad for details and full ancestral state reconstructions). Illustrations reproduced by permission of Lynx Edicions.

evolved more frequently in young lineages compared to eumelanin-consistent coloration that is inferred to have been present in the ancestral kingfisher (Fig. 4).

The role of distance might explain why some color patterns are more likely to evolve, but social and environmental factors can also contribute to the origin and maintenance of color patterns through time. The light environment hypothesis has received considerable support in birds, but the effects have often been in opposite directions depending on the clade. For example, Marchetti (1993) found that warbler species living in closed dark habitats had brighter plumage patches than species living in more open habitats, whereas McNaught and Owens (2002) found that Australian bird species living in closed habitats were duller than species living in open habitats. In addition to brightness, researchers have also compared plumage color complexity in different light environments. Gomez and Théry (2007) found that color complexity was higher for canopy birds compared to understory species (Gomez and Théry 2007). Shultz and Burns (2013) showed that color diversity is highest in tanagers living in closed environments. Hernández-Palma (2016) found that antbird communities had more diverse plumages in open/high-light environments, whereas Maia et al. (2016) found that plumage complexity was not significantly associated with habitat. Our result showing increased plumage complexity in closed environments

(Supplementary Table S3 and Fig. S17 available on Dryad) is thus most comparable to the evolutionary dynamics of tanagers and warblers. In addition to variation in light environment, kingfishers also have variable mating systems (Fry 1992; Woodall 2016), with ~10% of species showing cooperative breeding strategies (Cockburn 2006). Increased female–female competition might drive female ornamentation in these social species (Rubenstein and Lovette 2009). Recent work shows elevated speciation rates in kingfishers linked to island dwelling (Andersen et al. 2018). The same processes driving species diversification (e.g., limited gene flow among islands) might also be driving the explosive diversification of plumage color patterns seen in the island-dwelling "*Todiramphus*" clade (Fig. 4). It is likely that social system, light environment, and geography all play roles in driving diversity in color patterns in kingfishers.

Kingfishers produce diverse colors in several ways. Bright purple, blue, and turquoise colors are produced by coherent scattering of light by spongy nanostructures within feather barbs (Stavenga et al. 2011). Compared to pigment-based colors, structural color evolution proceeds in a more modular fashion, evolving in disjunct plumage regions (e.g., head and back; Fig. 4). Our analysis reveals several independent gains of structural coloration, for example in the moustachial stripe of the Alcedininae and *Actenoides* clades, and in other cases, structural color elements are shared across species (e.g., flight feathers; Fig. 4). Green colors in some Neotropical kingfishers are produced by coherent light scattering by iridescent nanostructures in feather barbules (Durrer 1977). Brown and black colors in kingfishers are produced by light absorption by melanin pigments (Stavenga et al. 2011; Li et al. 2012), whereas yellow and red colors in paradise kingfishers are produced by carotenoid pigments (Thomas et al. 2014). Phaeomelanin expression is more expansive in Alcedininae compared to other clades showing more limited color expression (Fig. 4). In addition to common blue, orange, and white colors (Supplementary Fig. S10 available on Dryad), kingfishers also have rare colors that can elude traditional (i.e., human observer-based) methods of color assessment. For example, bright white colors in the back feathers of the silvery kingfisher (*Ceyx argentatus*) differ from other white colors in the clade, including white breast feathers of the same species. Distinct reflectance peaks suggest these colors may be produced by organized keratin nanostructures (Supplementary Fig. S18a available on Dryad), perhaps similar to that described in manakins (Igic et al. 2016). Bronze colors in the hook-billed kingfisher (*Melidora macrorrhina*) appear to be phaeomelanin-based in fields guides and photos (Woodall 2016), but distinct reflectance peaks (Supplementary Fig. S18b available on Dryad) and color changes with angle (Supplementary Video S1 available on Dryad) indicate these colors are instead structural in origin. Pink colors in the Alcedininae clade appear to result from a combination of coherent scattering by feather nanostructures and selective absorption of short wavelengths by phaeomelanin pigments (Supplementary Fig. S18c available on Dryad). Further study of these colors will require detailed colorimetric (e.g., UV imaging/reflectance spectrophotometry) and morphological analyses (e.g., scanning electron microscopy). These new observations of color mechanism diversity highlight the importance of museum collections, in addition to field guides and photos, for understanding color diversity in nature.

Understanding how complexity evolves remains a challenge in biology. Our approach of estimating plumage complexity in ancestral lineages reveals two increases and five decreases in color complexity across the tree (Fig. 4). Treating complexity as a single trait can be problematic when reconstructing evolutionary changes. For example, consider a case of two sister species, one with a red head and blue throat and the other with a blue head and red throat. Each species would have a similar interpatch color contrast, so the ancestor would be reconstructed as having the same value. However, there must have been at least two changes to get from one species to another (e.g., from a blue to red head and red to blue throat), but these changes would not be captured by an approach reconstructing the evolution of complexity based on tip values alone (Supplementary Fig. S16 available on Dryad). Reconstructing plumage complexity at nodes, as we do here, would capture these changes. Interestingly, our metric for plumage complexity explains only ~11% of the variation in interpatch chromatic contrasts calculated from reflectance spectra (Supplementary Fig. S14 available on Dryad). This discrepancy can be understood by considering a species with several patches of drab black, eumelanin-based coloration (giving a high complexity score but very low color diversity) or a species with a similar way of producing color all across its body, but with variability in hue (giving a low complexity score but high interpatch contrast; Supplementary Fig. S10 available on Dryad). Future work will be needed to compare the performance of discrete (Price and Pavelka 1996; Omland and Lanyon 2000) versus continuous approaches (Stoddard and Prum 2008; Shultz and Burns 2013; Maia et al. 2016) in elucidating evolutionary trends in color pattern evolution.

Although we treat the body as a static entity across the clade, there is considerable variation in body shape that can be addressed in future work. For example, integrating our historical biogeography approach with geometric morphometrics to model the shape or boundaries of plumage patches would enable new questions about the evolution of plumage patterns. Additionally, the ability to work with within-feather patterning and continuous data (e.g., reflectance spectra) rather than just binary characters would allow us to ask questions about the evolution and biological role of color patterns in the context of developmental constraints and avian visual systems. We also envision including cladogenetic modes of color change in addition to anagenetic changes, as well as allowing for rate variation

across the tree and a bird's plumage. In the context of plumage pattern evolution, allopatric change would indicate a lineage giving rise to daughter lineages with different color pattern configurations. We anticipate the use of historical biogeography models in animal communication will help answer fundamental questions about color patterns—*Does sexual selection drive plumage pattern complexity? Do more complex plumages offer greater opportunity for color diversity?* We also envision other novel applications of historical biogeography models, including modeling the evolution and distribution of parasites across the body. We hope that this framework will inspire further discussion and evolutionary analyses of complex traits in biology.

Supplementary Material

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.3680n0c.

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