TITLE: Macroevolutionary bursts and constraints generate a rainbow in a clade of tropical birds

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RUNNING HEADER: Mosaic Evolution of color in Lorikeets

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Abstract

Bird plumage exhibits a diversity of colors that serve functional roles ranging from signaling to camouflage and thermoregulation. The color of plumage is known to evolve in response to natural selection, but drift and sexual selection play important roles in generating ornate phenotypes. The benefit of colorful sexual signals and the inherent selective cost of conspicuous phenotypes likely interact, but the macroevolutionary patterns which result from this relationship remain unclear. To quantify plumage color and test macroevolutionary models, we imaged museum specimens of an ornate tropical clade, the lorikeets, using visible-light and UV-light photography. We found that lorikeets repeatedly evolved similar plumage colors during their evolutionary history and remarkably occupy a qualitatively similar color space to all birds. Next, we tested both the relative and absolute fit of alternative macroevolutionary models and found that independent processes or rates acting on different plumage regions best explained this exceptional color variation. At a macroevolutionary scale, plumage regions likely under natural selection were constrained while regions known to be involved in sexual signaling underwent late-burst evolution. Overall, patch-specific modeling showed that the extraordinary color diversity in the lorikeets was generated by a mosaic of evolutionary processes acting on distinct plumage regions.

Introduction

Animals and plants express a dazzling range of colors. color has a direct impact on fitness through signaling (Bennett et al. 1997; Hill and McGraw 2006; Edler and Friedl 2010; Gluckman, and Cardoso, 2010; Stevens et al. 2012), camouflage (Hill and McGraw 2006; Gluckman and Cardoso 2010; Stevens et al. 2012), and thermoregulation (Hill, Beaver, and Veghte 1980; Beasley and Davison Ankney 1988; Medina et al. 2018), and is therefore a key component of biological diversification. For birds in particular, plumage color is integral to life history and evolution. Many birds have four color cones and a compound double cone which plays a role in motion and pattern perception. These cone types, alongside wavelength-tuning oil droplets, allow birds to perceive a wide range of colors exhibited by myriad pigments and keratin structures (Stoddard and Prum 2011). While climatic adaptations and crypsis are prominent explanations for the evolution of avian plumage color (Nordén and Price 2018), sexual selection is clearly important in the evolution of elaborate plumage color palettes (Hill and McGraw 2006). Sexual selection is often invoked to explain the evolution of extreme ornamentation and colorfulness seen in various groups of birds (Hill, and McGraw 2006; Saranathan et al. 2007; Irestedt et al. 2009). Signaling and mate choice can drive rapid trait divergence among and within species (Uy and Borgia 2000; Seddon et al. 2013), but the evolutionary patterns of sexually selected traits over deep phylogenetic scales are unclear for most elaborately colored groups. Examining the macroevolution trends of traits within brightly colored clades provides a framework for understanding how the interplay between natural and sexual selection shape the diversification of color (Dunn, Armenta, and Whittingham 2015; Nordén and Price 2018).

Parrots (Order: Psittaciformes) are among the gaudiest of birds. The evolution of avian coloration can be viewed as the outcome of an interplay between natural selection, sexual selection, and stochasticity (Dunn, Armenta, and Whittingham 2015; Nordén and Price 2018). Typical avian clades that have ornamental traits often show extreme sexual dimorphism in which males exhibit exaggerated feathers often with fabulous colors compared to the more unornamented and modestly colored female (Irestedt et al. 2009). In contrast, the brightly colored parrots are predominately monomorphic (Forshaw 2010), indicating that sexual selection alone may not adequately explain their evolution. Parrots harbor one of the largest color palettes in birds (Delhey 2015) and a unique pigment class called psittacofulvins (McGraw and Nogare 2004; McGraw and Nogare 2005). These pigments, along with melanins and UV-reflective physical feather nano-structures, produce a range of colors that rival flowering plants (Stoddard and Prum 2011; Delhey 2015). Psittacofulvin concentration in feathers is linked to antibacterial resistance, and parrot color can provide anti-predator defense (Burtt et al. 2011; Heinsohn, Legge, and Endler 2005). Phylogenetic relationships among all parrots are reasonably well known (Provost, Joseph, and Smith 2018), and some subclades have the dense taxon-sampling (Smith et al. 2018, unpublished manuscript) necessary for detailed comparative analysis, such as the brush-tongued parrots or lories and lorikeets (Tribe: Loriini; hereafter lorikeets). In comparison to other parrots, lorikeets are species-rich given their age (Schweizer et al. 2015), which may be linked to the evolution of their specialized nectarivorous diet and was likely driven by allopatric speciation as they dispersed across Australasia (Schweizer et al. 2014). Lorikeets have radiated into over 100 taxa across the Australasian region (Forshaw 2010) since their origin in the mid-Miocene (Schweizer et al. 2015). An outcome of this radiation is that lorikeets exhibit extraordinary colors which range from vibrant ultraviolet blue to deep crimson

and black, and these colors are organized in discrete “color patches” which in turn vary in size, color, and placement among taxa. The macroevolutionary patterns that underlie the radiation of these color patches in lorikeets can provide context into how diverse and brightly colored animals came to be.

The evolutionary processes of drift, natural selection, or sexual selection may have acted together or independently to produce the brilliant plumages in lorikeets. Separate forces may act upon different color metrics (e.g., hue vs. brightness) to balance a tradeoff between eye-catching ornamentation and cryptic background matching (Heinsohn, Legge, and Endler 2005; Dunn, Armenta, and Whittingham 2015). The overall color variance of lorikeets is large, however because this variance is partitioned in myriad ways both among patches and between taxa with different potential functional roles, we predict that different color patches would be supported by different evolutionary models. Support for a particular model may capture a signature of selection or stochastic processes and indicate whether color evolution was early or late, unconstrained or constrained by phylogeny or constrained by environmental variables.

In this study we quantified and modeled color evolution in the lorikeets. To produce color data, we imaged museum specimens, extracted color data from plumage regions, and summarized color hue, disparity, and volume. We first assessed whether each color patch was correlated with environmental variables to test for climatic adaptation in plumage color. We then tested the fit of alternative evolutionary models that best explain how color has evolved across their radiation using comparative phylogenetic methods. Characterizing the veritable rainbow of colors in the lorikeets and testing processes that could give rise to this variation will help clarify how macroevolutionary patterns underlie the evolution of elaborate colors in an ornate group.

Materials and Methods

Specimen imaging and color extraction

To quantify color, we photographed the lateral, ventral, and dorsal sides of one male museum skin for 98 taxa deposited at the American Museum of Natural History (table S3, supplemental material). This sampling represents 92% of the described diversity in Loriini, all described genera, and all taxa for which phylogenomic data exists. Specimens were photographed using a Nikon D70s with the UV filter removed and a Novoflex 35mm lens. Using baader spectrum filters affixed to a metal slider, specimens were photographed in both "normal" Red/Green/Blue (RGB) color as well as in the UV spectrum (McKay 2013; Schweizer et al. 2015).

We demarcated 35 plumage patches on the images produced for each specimen to fully capture all clade-wise color variance (fig. 1A; fig. S1, supplemental material). Using the multispectral imaging package (MSPEC) in ImageJ (Troscianko and Stevens 2015) we linearized and normalized our images to five gray standards placed alongside each bird and extracted RGB and UV reflectance for each patch (Troscianko and Stevens 2015). color measurements were collected using a visual model which transformed the data from the D70s color space into an objective color space and then into a tetrachromatic avian visual model (Troscianko and Stevens 2015). Data were normalized to sum to one and plotted in tetrahedral color space using the R v. 3.4.3 (R Core Team 2017) package Pavo v. 1.3.1 (Maia et al. 2013). Using Pavo, we extracted statistics (volume, relative volume, hue angle, and hue angle variance) of color spaces at varying

phylogenetic scales within the Loriini. For each specimen, we also measured proxies for body size, wing length and tarsus length.

Plotting color on patch maps and traits on trees

To visualize color data and model output, we created a 2-D schematic of an outline of a generic lory, hereafter referred to as a "patchmap." (fig. 1A) We inputted the red, blue, and green values of the tetrahedral color space (fig. 1B) into the RGB method in the R package grDevices version 3.4.3 to generate hex colors for each of the 35 patches (R Core Team 2017; Dale et al. 2015). Images were plotted as tip labels on a published phylogeny representing 92% of described taxa in Loriini (Smith et al. 2018, unpublished manuscript; fig. 1C) using ggtree v. 1.10.4 in R (Maia et al. 2013; Yu et al. 2017). To convert the branch lengths of the tree to absolute time, we used a secondary calibration from (Schweizer et al. 2015) and specified the age for the node separating the Loriini from their sister taxon, *Melopsittacus undulatus,* to 11-17 million years ago (Mya) using the program reePL (Smith and O'Meara 2012). Patchmap image sizes were scaled to represent relative taxa sizes measured from museum skin wing lengths. To visualize how color and body size evolved across the phylogeny, we used the contMap method in the phytools package (version 0.6.44, Revell, 2011) in R.

Testing for climatic correlates with color

To test for adaptive plumage variation, we examined the relationship between temperature and precipitation variables and color space. Using shapefiles of taxa ranges (Birdlife International 2011) we extracted mean bioclim variables within each taxa range. To get a single

value to approximate "color" we performed a PCA using the prcomp method in R using the four reflectance variables (UV, short-, medium-, and long-wave) as factors across all patch measurements (R Core Team, 2017). A phylogenetic PCA for patch color was not performed because the relationships between the four reflectance variables were phylogenetically independent. We then used the PGLS method in the R package Caper (Orme 2018) to test the relationship between color and climate while accounting for phylogeny.

Macroevolutionary model selection and adequacy test

We used a comparative phylogenetic method to select the relative best-fit evolutionary model for each color patch and checked the absolute fit of the model using a model adequacy approach. To avoid assumptions about whether color patches evolve synchronously, we initially modeled each patch independently. First, we condensed color into one continuous measurement with a centered and scaled principal component analysis using the prcomp function in the base stats package in R (R Core Team 2017) (Version 3.4.3). We used the four relative cone stimulations as factors and included all patch measurements for each bird as individual measurements. For each patch, we calculated interspecific variance, and modeled Brownian Motion rate, delta rate-change (δ), phylogenetic signal (λ), and OU bounding effect (α). To identify the relative best-fit model for each patch, we compared Akaike information criterion (AICc) scores among Brownian Motion, OU, white noise, and delta models fit with the fitContinuous method in the Geiger package in R (Harmon et al. 2008) (version 2.0.6). We considered models with a ΔAICc score greater than 2 to be significantly different. These models were used to test the following expectations: colors evolved randomly with respect to phylogeny (Brownian Motion), color evolved within selective constraints (OU), color evolved in a random

pattern, irrespective of phylogeny (white noise), or color evolved in late or early burst fashion (delta).

Though model selection based on AICc identifies the best relative model, that model may be overfit and empirically unlikely (Pennell et al. 2015). To test absolute model fit, we compared our empirical trait values to simulated trait values using the arbutus package (Pennell et al. 2015) (version 0.1). Based on a fitted evolutionary model, Arbutus creates a unit tree of uniform branch length one, simulates posterior distributions, and compares those simulated distributions of six statistics (table S2, supplemental material) to the empirical trait distribution. When empirical values significantly differ from simulated values, the model has poor absolute fit. We then filtered out the models which failed two or more tests (Pennell et al. 2015; Seeholzer, Claramunt, and Brumfield 2017). The best-fit model for each patch was plotted on the patchmap. For patches with ΔAICc scores of < 2 among top models, the model with fewer parameters was selected. For models with identical complexity, Mahalanobis distance to simulated trait means was used as a post-hoc test in order to pick best-fit models (Revell 2011; Pennell et al. 2015). Because modeling of individual patches may mis-specify models due to separate analysis of correlated traits (Adams and Collyer 2017), we tested for non-independence of patches using the R package Phylocurve (Goolsby 2016). We generated a phylogenetic covariance matrix for all patches in Phylocurve, which fits a single trait evolution model to many high-dimensional phenotypic traits simultaneously, and plotted these covariance matrices using the Corrplot package (Wei 2013). We found best-fit models for PC1, PC2, and full 4-dimensional color data for each patch. Finally, we performed *post-hoc* tests on model fits of patch subsets to check for analysis biases towards more heavily sampled regions with uniform color (e.g., just wing patches).

Results

Color and its correlation with climate

Lorikeets occupied 33.5% of the colors predicted to be perceived by tetrachromatic birds. The average color volume per taxon was 0.00513, which represents a relative volume of around 2.37% (median 2.07%) of the total avian visual space. Individual taxon color volumes ranged between 0.04% to 11.7% of avian visual space. The average largest pairwise distance between two patches for one bird, or average hue disparity, was 0.912 (median: 0.962). The most variant patches were on the crown, forehead, face, and breast, while the least variant were on the wings (fig. 3A). The first and second principal components represented 50-60% and 30-35% of the total-dataset variance, and primarily described long-wave and medium-wave variation, respectively. For PC1, variance was highest within the crown, breast, and rump. PC2 varied most within back, crissum, and lower abdomen. Wing patches had strong covariance, as did breast and face patches (fig. S3). Climatic variables poorly predicted color variation. R^2 values explained between 4% and 0.3%, and none of these relationships were significant.

Patterns of plumage color evolution

Continuous character mapping of both color and wing length showed heterogeneity in the distributions of states within and among clades (fig. 2). Patch color showed a dynamic pattern where colors changed frequently and independently towards similar states (fig. 1B, fig. 2). We found repeated evolution of patch colors across distantly related genera and high color divergence between closely related genera. For comparison, wing length and tarsus length

exhibited less heterogeneous evolutionary rates (fig. 2), largely reflecting that the taxa within genera have similar body sizes.

Fitted λ values equaled one for for most patches, indicating that phylogenetic signal was greater than or equal to the expected signal under a Brownian Motion model of trait evolution (fig. 3B). Back, wrist, and crissal feathers exhibited the lowest phylogenetic signal. All patches fit a delta model with $\delta > 1$, indicating that every patch followed a late-burst pattern of evolution (fig. 3D). For PC1, many fitted δ values were at the default maximum, three. For PC2, δ was three for the wings, body, crown and crissum, but lower on the tail, back and side-throat. OU □ value fits showed a similar pattern. High alpha (□) values, which represent stronger bounding effects, were fit to lower abdomen patches, wings, and wrists. The weakest bounds were fit to the breast, face, and head patches.

Relative-model fitting revealed variability in the evolutionary dynamics between color patches. PC1 for the forehead, crown, and occiput was best fit by Brownian Motion with λ values of one indicating a rate of evolution greater than or equal to the expected signal under a random walk. Face, breast, and tail evolution was best supported by a δ model with δ values > 1 indicating a late-burst pattern of evolution. All other patches were best supported by an OU model. The best-fit model for most patches was selected with high relative support (Δ AICc >4) except for crown, forehead and occiput (Δ AICc < 2) (fig. S1, supplemental material). For PC2, wing, wrist, rump, and breast were best fit by an OU model. The best-fit model of PC2 of lower abdominal patches, lateral neck, and tail was Brownian Motion while an OU model explained half of the wing, wrist, eyeline, and lower breast color. All other patches, which were clustered around the abdomen, head, and face, were best modeled by a late-burst delta model. Most best-fit models passed a four-statistic absolute adequacy filter (table S2). Simulated C*var*, the coefficient

of variation of the paired differences between the estimated node and tip values, was the statistic which most frequently deviated from empirical values (table S2). Wing patches failed one empirical model test for PC1 because the favored OU model did not properly account for rate heterogeneity. Undescribed rate heterogeneity in the PC2 of back and crissum caused model-adequacy to fail for these patches. Multi-trait, non-independent model fitting showed that the highest-likelihood multi-trait model was an OU model, and that the regions fit to alternative models during our individual patch analysis covaried when fit to a single model.

Discussion

In this study we modeled the evolution of color variance in the lorikeets. Despite representing less than 1% of avian diversity we found that that lorikeets manifest a third of the colors predicted to be perceptible by tetrachromatic birds. In turn, the entire Australian avifauna and all birds together represent a fifth (Delhey 2015) and a third (Stoddard and Prum 2011), respectively, of the predicted color space tetrachromatic birds can perceive. This suggests that lorikeets likely contain most of the color diversity found in birds.

The evolutionary origins of this exceptional color variation were best explained by independent processes or rates acting on different plumage regions. Patch-specific analyses showed that some plumage regions evolved randomly with respect to the phylogeny, while in other regions color evolution was constrained through time or rapidly diversified at the tips of the tree. When the non-independence of patches was accounted for and all patches were modeled together, model selection favored an OU model. OU models are sometimes cited as evidence of natural selection because they describe a statistical pattern wherein trait values remain close to local optima through time, but may sometimes best fit evolution of traits under physiological constraint (Cooper et al. 2016, Felice, Randau, and Goswami 2018). As is the case with many

ornamental traits (Hill and McGraw 2006), the plumage of lorikeets was not correlated with climatic variation despite the clade occurring across a large geographic area. Instead, the brilliant colors that evolved during the radiation of the lorikeets appear to have been generated by a mosaic of patterns or rates. If separate models represent patterns produced by discrete processes, then non-climatic selective pressures such as sexual selection, predator avoidance, and drift are leading explanations for macroevolution of plumage colors in the lorikeets.

Colorful groups have recurring colors

When individual clades radiate across a high percentage of the available color space, then the repeated evolution of similar colors may be a common feature (Nordén and Price 2018; Hofmann, Cronin, and Omland 2006). For example, the robust-bodied and short-tailed lorikeets in *Lorius* and the distantly related, slender and small, long-tailed lorikeets in *Charmosyna* both have red bodies and green wings. Ancestral states inferred from the continuous character mapping, while subject to a high degree of uncertainty (fig. 2), suggest that similar colors have repeatedly and independently evolved across this radiation. So, despite being exceptionally colorful, the lorikeet radiation was not characterized by constant gain of new colors. Novel color evolution in birds is modulated by interactions between genes, gene expression patterns, structures, and existing metabolic pathways (Morrison and Badyaev 2016; Cooke et al. 2017, Endler, Cole, and Kranz 2018). Biochemical constraints likely played a role in this plumage convergence because parrot feather color is controlled via regulatory pathways as opposed to dietary pigmentation (Berg and Bennett 2010). Certain trait shifts, such as loss of ancestral yellow/green pigments and gains of red, are common in lorikeets and all parrots (Nemeśio 2001). In carotenoid-based color systems such as in the songbird genus *Icterus*, a relatively small

number of color states rapidly oscillate, leading to convergence in carotenoid and melanosome-based colors (Omland and Lanyon 2000; Morrison and Badyaev 2018). A similar process may be occurring in lorikeets despite the unique pigmentation found in Psittaciformes. Regardless of mechanism, architectural constraints on plumage color or morphological traits, necessarily produce similar looking but distantly-related taxa.

Independent or correlated patches

The developmental architecture that underlies potential concerted evolution among feather regions remains unknown for most birds (Morrison and Badyaev 2016; Cooke et al. 2017). We found that there were three clusters of correlated patches that correspond to adjacent sections on the wing, breast, and face (fig. S3, supplemental material). These regions may be developmentally linked or under similar selective regimes. In the sister taxon to lorikeets, *Melopsittacus undulatus*, a single base-pair change expresses tryptophan, blocking expression of yellow pigment, changing the mostly-green wild-type to a pale-blue across all patches (Cooke et al. 2017). This simple molecular change may explain the evolution of the two brilliant blue taxa in the Loriini; *Vini ultramarina* and *V. peruviana* (Nemeśio 2001). However, the evolution of complex feather color may be due to differential regulation of separate genes across patches (Abolins-Abols et al. 2018, unpublished manuscript). Regulatory controls on feather color may work at patch-level, feather tract-level, or whole bird-level scales (Morrison and Badyaev 2016; Cooke et al. 2017; Schwochow-Thalmann 2018), and understanding how these pathways are connected will elucidate how complex plumage colors and patterns evolve. For example, most lorikeets have all-green or all-red wings with black-tipped primaries, but some *Eos* taxa have

evolved barring and UV coloration on some wing patches, demonstrating a clear interplay between region- and patch-level pigment regulation.

A mosaic of evolutionary processes

Under the assumption that lorikeet plumages patches were independent, our model fitting for each patch demonstrated that three alternative evolutionary models tested were supported depending on the plumage patch examined. This suggests that multiple evolutionary processes are involved in lorikeet plumage evolution. In contrast, our single global multi-trait model fit, which accounted for the covariance among color patches, identified OU as the best fit model, suggesting overarching constraints on plumage color evolution. While seemingly contrary, these results are reconcilable. Plumage color evolution may be constrained by natural selection, sexual selection, phylogeny, and the physiological bounds of color production and perception. In turn, each of these evolutionary processes are predicted to be important for different functional regions of lorikeet plumage based on natural history, social behavior, and physiology. Avian plumage is expected to be subject to multiple evolutionary processes along different color axes and among color patches which will necessarily form distinct macroevolutionary patterns (Dunn, Armenta, and Whittingham 2015; Nordén and Price 2018).

The high likelihood of a global multi-trait OU model also indicates that the model may be biased towards describing the evolutionary patterns of the more prevalent less-variable patches, (fig. 3A), which tended to be green, and were potentially overly subdivided relative to regions of higher variance. To test this prediction, we compared the fit of alternative multi-trait models for regions identified as covariant in the global multi-trait, multi-dimensional model (fig. S3, supplementary material). For instance, the five face patches covaried, and the best-fit multi-trait

model for these five patches (late burst delta model of trait evolution) was similar to when these regions were modeled independently, and different from the global best-fit multi-trait OU model which was identified for all plumage patches combined. This instability of the best-fit multi-trait model when subsampling covarying plumage regions and modeling them together suggests that simultaneously fitting a single evolutionary model to all plumage patches, while robust to transformations, sample size concerns, and trait covariance, may not approximate the biological reality of plumage evolution closely enough in lorikeets. While the multi-trait model for all patches has greater statistical rigor than independent model fitting, it also has acute interpretive limitations. Regardless, the results of both approaches are most consistent with a complex pattern of mosaic evolution.

Functional underpinnings of mosaic evolution

We suggest mosaic evolution as the most biologically realistic explanation for the evolution of traits whose functional roles change depending on their location on an organism. Our model fitting analyses supported this prediction. The selected best-fit models are not only grouped among covariant traits but are readily interpretable based on our functional knowledge of plumage color biology. For instance, the crown, forehead, and lower abdomen, which are areas with high UV variation, were best supported by a model of Brownian Motion which may be because these regions are under a non-deterministic process such as sexual selection. Several *Trichoglossus haematodus* subspecies flare and preen crown and forehead feathers during courtship, indicating that these regions may be important social signals, perhaps for recognition of conspecifics or mate quality assessment (Serpell 1989).

An OU model best fit most wing and body patches, which suggests either a constraint on evolution to new states, or selection not captured by our PGLS analysis with color and climate. In the forest canopy, green body and wing color may serve the purpose of camouflage against predation (Medina et al. 2018; Soma and Garamszegi 2018), while brighter plumage colors may serve as signals. The tradeoff between psittacofulvin-based signaling and crypsis has been observed in the reversed sexually dichromatic parrot *Eclectus roratus*, where bright-red female plumage advertises nesting sites, and green plumage helps foraging males avoid predation through camouflage (Heinsohn, Legge, and Endler 2005). Other highly variable and colorful regions like the face, breast, and tail regions were best explained by a delta model. Our inferred delta parameters were greater than one, which indicates color variance within any patch evolved relatively recently. Although this pattern can be interpreted as evidence for character displacement (Schluter and Schluter 2000), the majority of taxa within clades are currently allopatric (Forshaw 2010) so color evolution was presumably unaffected by interactions with congeneric or contribal taxa. Instead, the recent evolution of many color patches likely reflects the commonly observed pattern of rapid color evolution at the tips of phylogenies (Stoddard and Prum 2008). These regions have likely been changing at a similar rate across the evolutionary history of the lorikeets, but since these patches evolve so fast, the signal that can be recovered is recent.

For traits that are both adaptive and ornamental, a mosaic of evolutionary patterns is a likely outcome. For example, both within the Loriini and across the Order Psittaciformes, green wings are a common phenotype (Stoddard and Prum 2008; Forshaw 2010), as 90% of parrots have green patches and 85% are primarily green (Nemeśio 2001). We found that the wing patches were best explained by an OU model, which may indicate there is a selective cost to

evolving away from green. Species with green wings and backs are predicted to have increased camouflage in trees against aerial and terrestrial predators (Heinsohn, Legge, and Endler 2005). The impact of climatic adaptation appears to be limited because we found no evidence that color was adaptive to climatic conditions. In contrast to monochromatic birds, which may be under strong selection for uniform plumage color (such as the snow-colored winter plumage of Rock Ptarmigans (Montgomerie 2001), lorikeets may be colorful, in part, because much of the color variation is not constrained by natural selection. Highly variable regions, like the breast and face, were not well-fit by an OU model. These regions are often involved in signaling and under sexual selection. Lorikeets, such as *T. haematodus*, engage in plumage-centric courtship displays, preening their brilliant breast and crown feathers and flaring their collars (Serpell 1989). Although only *Trichoglossus* courtship has been quantified, the small, variable facial patches and bright breast patterns present across the Loriini may be important signals to conspecifics while conserved, monochrome green dorsal feathers potentially provide cover from predators.

Model adequacy

Overall, we found that most best-fit models were also a good absolute fit to the patch color data with the exception of a few patches (fig. 3F). We performed model adequacy by the comparison of statistics estimated from empirical and simulated trait values. We used a four-statistic threshold for determining absolute fit, but many patches would have passed a five- or six-statistic threshold. Prior work, based on non-color traits, found that relative best-fit models are frequently a poor absolute fit to empirical trait data (Pennell et al. 2015; Seeholzer, Claramunt, and Brumfield 2017). In our dataset, simulated values of one statistic (C*var*)

frequently deviated from empirical values because of unaccounted-for rate variation in our best-fit, constant rate model. Even at relatively shallow phylogenetic scales, body size and plumage color exhibit rate heterogeneity (Chira and Thomas 2016; Seeholzer, Claramunt, and Brumfield 2017; Felice and Goswami 2018). Accounting for shifts to faster rates was critical for accurately characterizing the evolution of highly variable regions, which may be rapidly shifting between several discrete states or diversifying due to sexual selection.

Challenges in studying plumage color

Quantifying color from museum specimens presented numerous challenges. Using museum specimens instead of hand-painted plates from field guides was preferable to us because skins exhibit UV reflectance, and the three-dimensional variation of the specimen can be captured. However, the variable preparation of museum specimens may expand or obscure certain feather patches. Therefore, we had no objective way to decide whether a certain color belonged to a specific patch and relied on subjective judgement and consultation of multiple skins, plates, and photographs, when outlining patches. Patch outlines had to be drawn by hand to account for preparation style. One possible solution for patch delineation could be through random sampling of patch location (Miller et al. 2018, unpublished manuscript). The potential error in our approach pertains mostly to patch delineation, not the overall color volume of the entire bird. Despite our concerns about the subjectivity in identifying the location of patches on specimens, much of the potential error was likely minimized because of the overall morphological similarity across our focal clade. Additionally, we found that patchmaps and field guide plates were qualitatively similar. In studies that sample across much deeper phylogenetic scales, identifying and sampling homologous patches will be a much more complicated task.

Machine learning approaches, possibly guided by evo-devo data on feather color and pattern regulation (Schwochow-Thalmann 2018), may lead to more objective patch-specific analyses. Delineating high-contrast boundaries would enable patch geometry and boundaries to be objectively quantified (Endler, Cole, and Kranz 2018, Schwochow-Thalmann 2018) and provide a clearer means of interpreting patch colors in the context of sexual or social signaling.

Conclusion

We found that alternative macroevolutionary models best explained the exceptional color variance in the lorikeets. A finding of mosaic evolution is consistent with the view that separate selective and stochastic processes help shape different plumage regions and have enabled lorikeets to evolve extreme colors despite the selective costs of conspicuous coloration. Demonstrating that mosaic evolution operates in birds and other animals will clarify how extreme phenotypic diversification occurred under variable evolutionary pressures.

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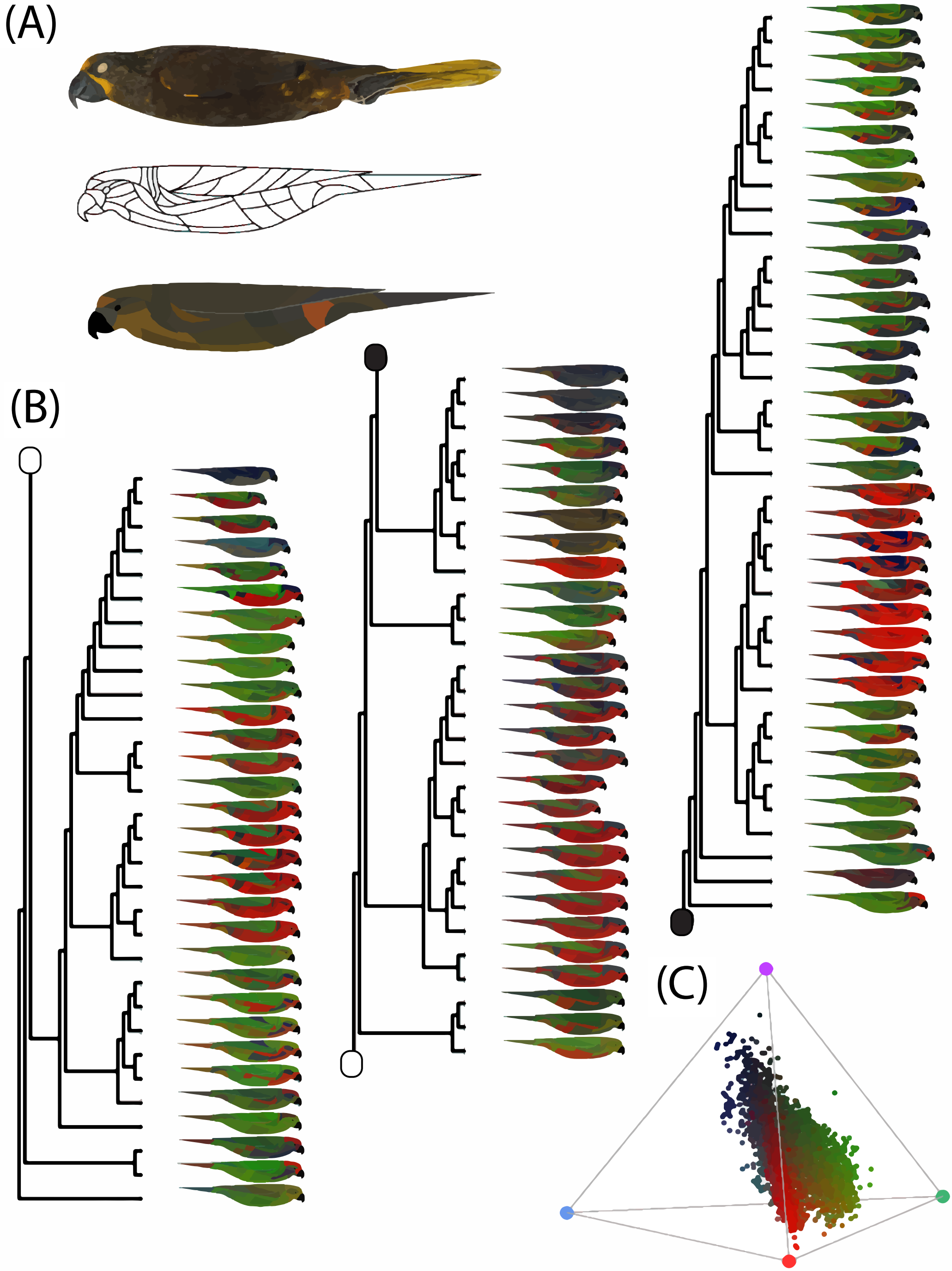
(A)
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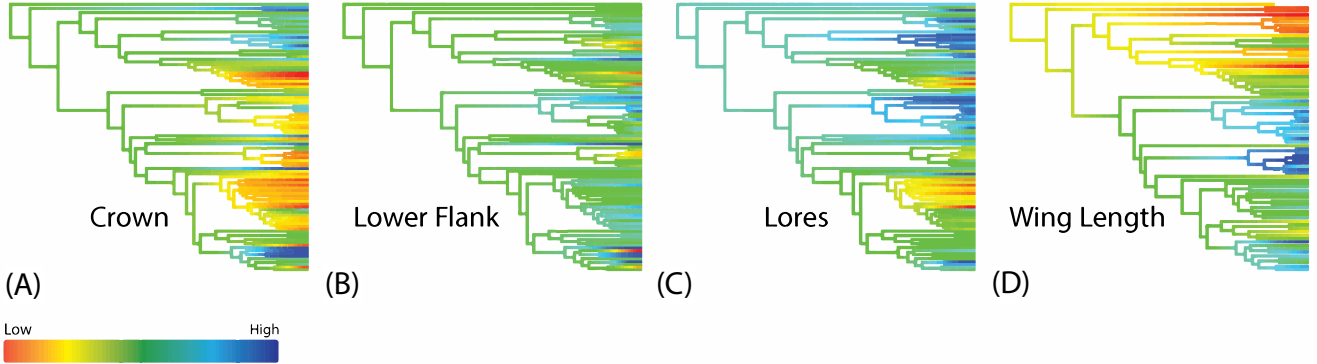
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(A)
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(B)
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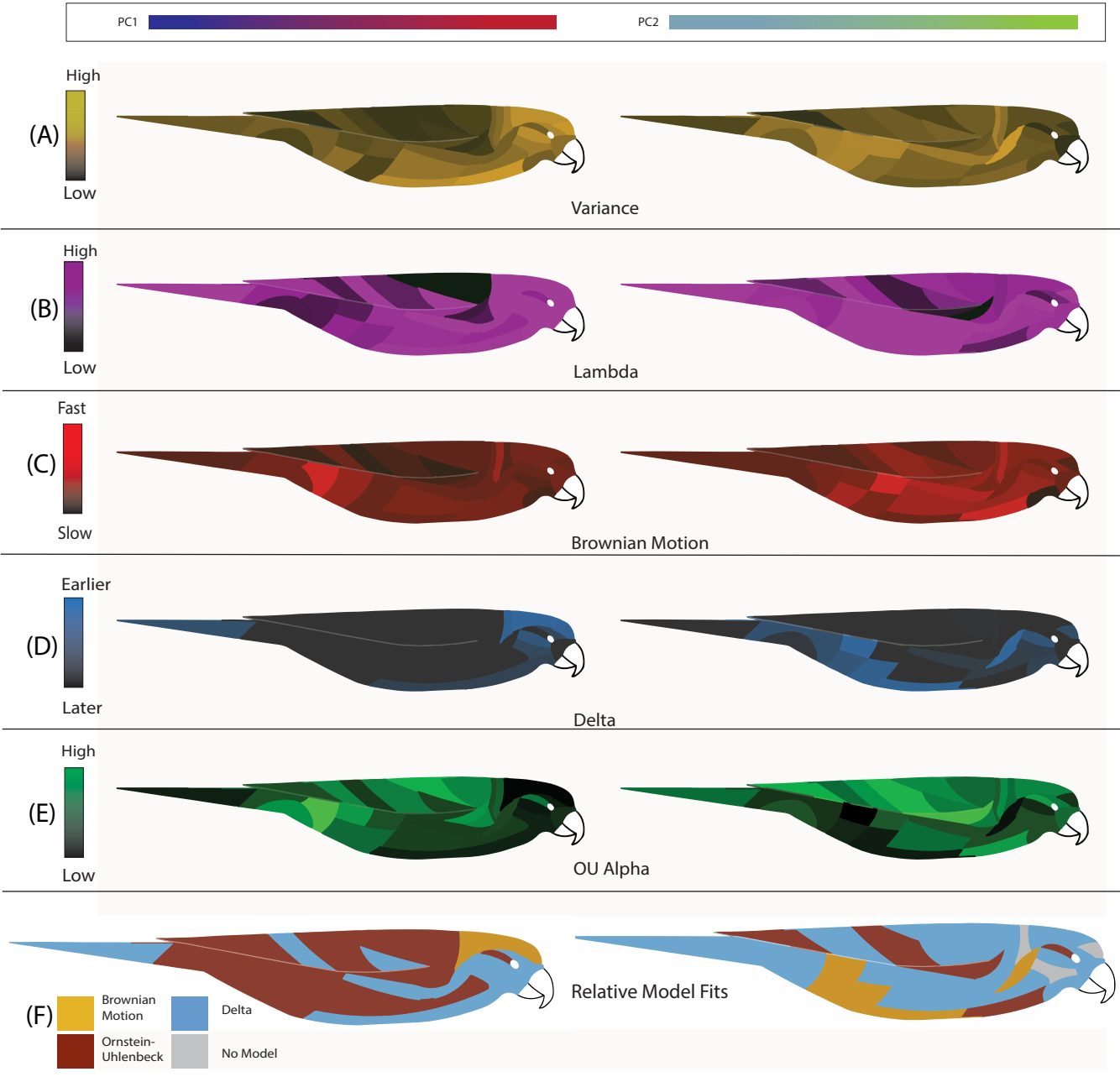
(C)
Wing Length
(D)
Low
High


PC1
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(A)
High
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Variance
(B)
High
Low
Lambda
(C)
Fast
Slow
Brownian Motion
(D)
Earlier
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Delta
(E)
High
Low
OU Alpha
(F)
Brownian Motion
Delta
Ornstein-Uhlenbeck
No Model
Relative Model Fits

Figure 1: Quantifying and plotting plumage color on a phylogeny of the lories and lorikeets. (A) An image of a museum specimen of *Chalcopsitta duivenbodei* (top), a blank patchmap showing the 35 plumage regions measured from images of museum specimens (middle), and the corresponding patchmap for this exemplar taxon (bottom). (B) Patchmaps of all taxa (n = 98) plotted on a phylogeny. The tree was split into three sections and the connecting portions are indicated with corresponding filled in or empty points. (C) The tetrahedral color space of the Loriini, which contains four vertices for the four measured reflectance wavelengths: UV (purple, top), short (blue, left), medium (green/yellow, right), and long (orange/red, center). Each point represents one of the 35 color patch measurements for each taxon. The color space was centered slightly towards the longwave (red) vertex of the tetrahedral color space. While the distribution of colors in the color space skews towards the longwave part of the spectrum, it was most variant in the UV spectrum and also exhibits wide variance in the medium-wave spectrum. colors represent the RGB colors which were mapped onto the real-color patchmaps.

Figure 2: Continuous character mapping shows that color is a more labile trait than body size. Ancestral states were estimated using a Brownian Motion process. Included are exemplar patches and wing length on the phylogeny. The patches correspond to PC1 for the top of the head (A, Crown), lateral view above the legs (B, Lower Flank), area directly next to the bill (C, Lores), and a proxy for body size (D, Wing Length).

Figure 3: Patchmaps show within and among patch variability in PC values, model parameters, and best-fit models. PC scale bars at top show axes of color variance encompassed by each PC. Each patch in a patchmap was colored according to values for principal component variance (A), the modeled parameters lambda (B), Brownian Motion rate (C), delta (D) and OU alpha (E), and the best-fit model, after model adequacy (F). The left and right patchmaps within each panel represent PC1 and PC2, respectively. From top to bottom, the darker patches are less variable across taxa (A), have less phylogenetic signal (B), are evolving slower (C), diversified closer to the tips of the tree (D), or were relatively more constrained (E). Relative model fit shows a mosaic of best-fit models across patches (F), and that most patches were a good absolute fit to the data. Only patches with good absolute fits were plotted.