TITLE: Bursts and constraints generate a rainbow in the lorikeets

Jon T. Merwin[1*2], Glenn F. Seeholzer[1], Brian Tilston Smith[1]

[1]*Department of Ornithology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA*
[2]*Department of Ecology, Evolution and Environmental Biology, Columbia University, New York, NY 10027, USA*

RUNNING HEADER: Mosaic Evolution of Colour in Lorikeets

*Corresponding Author: Jon T. Merwin, *Department of Ornithology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA; Email address: jmerwin@amnh.org*

Keywords — macroevolution, bird, colour, phylogeny, model adequacy, lorikeet, mosaic evolution

Abstract

Bird plumage exhibits a diversity of colours that serve functional roles ranging from signaling to camouflage and thermoregulation. Macroevolutionary research on the evolution of plumage colour has focused on the impact of natural selection, but drift and sexual selection likely play important roles in originating brilliant colours and patterns. One kaleidoscopic group is the lorikeets, or brush-tongued parrots, which have radiated across Australasia. To quantify and characterize plumage colour, we imaged taxa using visible-light and UV-light photography of museum specimens. We measured colour from 35 plumage patches on each specimen and modeled colour across lorikeets' evolutionary history. Lorikeets occupy a third of the avian visual colour space, which is qualitatively similar to the colour space occupied by all birds. We found that the wing and back were less variable than the breast and face. Crown and forehead colour was best explained simply by phylogeny. At a macroevolutionary scale, the evolution of elaborate colours in lorikeets involved an interplay wherein regions likely under natural selection were constrained during the radiation while regions known to be involved in signaling underwent late-burst evolution. Overall, patch-specific modeling showed that plumage diversity in the

lorikeets was likely generated by a mosaic of evolutionary processes.

1. Introduction

Animals and plants express a dazzling range of colours. Colour has a direct impact on fitness through signaling [1–5], camouflage [2–4], and thermoregulation [6–8], and is therefore a key component of biological diversification. For birds in particular, plumage colour is integral to life history and evolution. Many birds have four colour 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 colours exhibited by myriad pigments and keratin structures [9]. While climatic adaptations and crypsis are prominent explanations for the evolution of avian plumage colour [10], sexual selection is clearly important in the evolution of elaborate plumage colour palettes [4]. Sexual selection is often invoked to explain the evolution of extreme ornamentation and colourfulness seen in various groups of birds [4,11,12]. Signaling and mate choice can drive rapid trait divergence among and within species [13,14], but the evolutionary patterns of sexually selected traits over deep phylogenetic scales are unclear for most elaborately coloured groups. Examining the macroevolution trends of traits within brightly coloured clades provides a framework for understanding how the interplay between natural and sexual selection shape the diversification of colour [10,15].

Parrots (Order: Psittaciformes) are among the gaudiest of birds. The evolution of avian colouration can be viewed as the outcome of an interplay between natural selection, sexual selection, and stochasticity [10,15]. Typical avian clades that have ornamental traits often show extreme sexual dimorphism in which males exhibit exaggerated feathers often with fabulous colours compared to the more unornamented and modestly coloured female [12]. In contrast, the brightly coloured parrots are predominately monomorphic [16], indicating that sexual selection alone may not adequately explain their evolution. Parrots harbor one of the largest colour palettes in birds [17] and a unique pigment class called psittacofulvins [18,19]. These pigments, along with melanins and UV-reflective physical feather nano-structures, produce a range of colours that rival flowering plants [9,17]. Psittacofulvin concentration in feathers is linked to antibacterial resistance, and parrot colour can provide anti-predator defense [20,21]. Phylogenetic relationships among all parrots are reasonably well known [22], and some subclades have the dense taxon-sampling [23] necessary for detailed comparative analysis, such as the the brush-tongued parrots or lories and lorikeets (Tribe: Loriini; hereafter lorikeets). In comparison to other parrots, lorikeets are species-rich given their age [24], which may be linked to the evolution of their specialized nectarivorous diet [25]. Lorikeets have radiated into over 100 taxa across the Australasian region [16] since their origin in the mid-Miocene [24] An outcome of this radiation is that lorikeets exhibit extraordinary colours which range from vibrant ultraviolet blue to deep crimson and black, and these colours are organized in discrete “colour patches” which in turn vary in size, colour, and placement among taxa. The macroevolutionary patterns that underlie the radiation of these colour patches in lorikeets can provide context into how diverse and brightly coloured 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 colour metrics (e.g., hue vs. brightness) to balance a tradeoff between eye-catching ornamentation and cryptic background matching [15,21]. The overall colour 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 colour 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 colour evolution was early or late, unconstrained or constrained by phylogeny or constrained by environmental variables.

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

2. Materials and Methods

(a) Specimen imaging

To quantify colour, 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, electronic supplementary information). 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) colour as well as in the UV spectrum [24,26].

We demarcated 35 plumage patches on the images produced for each specimen to fully capture all clade-wise colour variance (figure 1*a*; electronic supplementary material). Using the multispectral imaging package (MSPEC) in ImageJ [27] we linearized and normalized our images to five gray standards placed alongside each bird and extracted RGB and UV reflectance for each patch [27]. Colour measurements were collected using a visual model which transformed the data from the D70s colour space into an objective colour space and then into a tetrachromatic avian visual model [27]. Data were normalized to sum to one and plotted in tetrahedral colour space using the R v. 3.4.3 [28] package Pavo v. 1.3.1 [29]. Using Pavo, we extracted statistics (volume, relative volume, hue angle, and hue angle variance) of colour spaces at varying phylogenetic scales within the Loriini. For each specimen, we also measured proxies for body size, wing length and tarsus length.

(b) Plotting colour on patch maps and traits on trees

To visualize colour data and model output, we created a 2-D schematic of an outline of a generic lory, hereafter referred to as a "patchmap." (figure 1*a*) We inputted the red, blue, and green values of the tetrahedral colour space (figure 1*b*) into the RGB method in the R package grDevices version 3.4.3 to generate hex colours for each of the 35 patches [28,30]. Images were plotted as tip labels on a published phylogeny representing 92% of described taxa in Loriini ([23], figure 1*c*) using ggtree v. 1.10.4 in R [29,31]. To convert the branch lengths of the tree to absolute time, we used a secondary calibration from [24] 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 [32]. Patchmap image sizes were scaled to represent relative taxa sizes measured from museum skin wing lengths. To visualize how colour and body size evolved across the phylogeny, we used the contMap method in the phytools package (version 0.6.44) [33] in R.

(c) Colour and climate

To test for adaptive plumage variation, we examined the relationship between temperature and precipitation variables and colour space. Using shapefiles of taxa ranges [34] we extracted mean bioclim variables within each taxa range. We then used the PGLS method in the R package Caper [35] to test the response of the first principal component of patch colour to the first principal component of the climatic variables. To normalize the data, the climatic dataset was log-transformed and the colour dataset was power transformed.

(d) Phylogenetic model selection and adequacy test

We used a comparative phylogenetic method to select the relative best-fit evolutionary model for each colour patch, and checked the absolute fit of the model using a model adequacy approach. To avoid assumptions about whether colour patches evolve synchronously, we initially modeled each patch independently. First, we condensed colour into one continuous measurement with a centered and scaled principal component analysis using the prcomp function in the base stats package in R [28] (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 [36] (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: colours evolved randomly with respect to phylogeny (Brownian Motion), colour evolved within selective constraints (OU), colour evolved in a random pattern, irrespective of phylogeny (white noise), or colour 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 [37]. To test absolute model fit, we compared our empirical trait values to simulated trait values using the arbutus package [37] (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 (electronic supplementary material, table S2) 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 [37,38]. 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 [33,37]. Because modeling of individual patches may mis-specify models due to separate analysis of correlated traits [39], we tested for non-independence of patches using the R package Phylocurve [40]. 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 [41]. We found best-fit models for PC1, PC2, and full 4-dimensional colour 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 colour (e.g., just wing patches).

3. Results

(a) Colour and its correlation with climate

Lorikeets occupied 33.5% of the colours predicted to be perceived by tetrachromatic birds. The average colour 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 colour 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 (figure 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 (figure S3). Climatic variables poorly predicted colour variation. R^2 values explained between 4% and 0.3%, and none of these relationships were significant.

(b) Patterns of plumage colour evolution

Continuous character mapping of both colour and wing length showed heterogeneity in the distributions of states within and among clades (figure 2*a*-*d*). Patch colour showed a dynamic pattern where colours changed frequently and independently towards similar states (figure 1*b*, 2). We found repeated evolution of patch colours across distantly related genera and high colour

divergence between closely related genera. For comparison, wing length and tarsus length exhibited less heterogeneous evolutionary rates (figure 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 (figure 3*b*). 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 (figure 3*d*). 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 α 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 colour 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) (figure 1, electronic supplementary 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 colour. 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.

4. Discussion

In this study we quantified and characterized the evolution of the colour variance in the lorikeets. Despite representing less than 1% of avian diversity we found that that lorikeets manifest a third of the colours predicted to be perceptible by tetrachromatic birds. In turn, the entire Australian avifauna and all birds together represent a fifth [17] and a third [9], respectively, of the predicted colour space tetrachromatic birds can perceive. This suggests that lorikeets likely contain most of the colour diversity found in birds.

The evolutionary origins of this exceptional colour variation was 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 colour 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 [42,43]. As is the case with many ornamental traits [4], the plumage of lorikeets was not correlated with climatic variation despite the clade occurring across a large geographic area. Instead, the brilliant colours 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 colours in the lorikeets.

(a) Colourful groups have recurring colours

When individual clades radiate across a high percentage of the available colour space, then the repeated evolution of similar colours may be a common feature [10,44]. 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 (figure 2), suggest that similar colours have repeatedly and independently evolved across this radiation. So, despite being exceptionally colourful, the lorikeet radiation was not characterized by constant gain of new colours. Novel colour evolution in birds is modulated by interactions between genes, gene expression patterns, structures, and existing metabolic pathways [45–47]. Biochemical constraints likely played a role in this plumage convergence because parrot feather colour is controlled via regulatory pathways as opposed to dietary pigmentation [48]. Certain trait shifts, such as loss of ancestral yellow/green pigments and gains of red, are common in lorikeets and all parrots [49]. In carotenoid-based colour systems such as in the songbird genus *Icterus*, a relatively small number of colour states rapidly oscillate, leading to convergence in carotenoid and melanosome-based colours [50,51]. A similar process may be occurring in lorikeets despite the unique pigmentation found in Psittaciformes. Regardless of mechanism, architectural constraints on plumage colour or morphological traits, necessarily produce similar looking but distantly-related taxa.

(b) Independent or correlated patches

The developmental architecture that underlies potential concerted evolution among feather regions remains unknown for most birds [46,47]. We found that there were three clusters of correlated patches that correspond to adjacent sections on the wing, breast, and face (figure 3, electronic supplementary 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 [47]. This simple molecular change may explain the evolution of the two brilliant blue taxa in the Loriini; *Vini ultramarina* and *V. peruviana [49]*. However, the evolution of complex feather colour may be due to differential regulation of separate genes across patches (e.g., [52]). Regulatory controls on feather colour may work at patch-level, feather tract-level, or whole bird-level scales [46,47,53], and understanding how these pathways are connected will elucidate how complex plumage colours 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 colouration on some wing patches, demonstrating a clear interplay between region- and patch-level pigment regulation.

(c) 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 colour patches, identified OU as the best fit model, suggesting overarching constraints on plumage colour evolution. While seemingly contrary, these results are reconcilable. Plumage colour evolution may be constrained by natural selection, sexual selection, phylogeny, and the physiological bounds of colour 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 colour axes and among colour patches which will necessarily form distinct macroevolutionary patterns [10,15].

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, (figure 3*a*), 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 (figure 3, electronic 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.

(d) 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 colour 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 [54].

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 colour and climate. In the forest canopy, green body and wing colour may serve the purpose of camouflage against predation [6,55], while brighter plumage colours 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 [21]. Other highly variable and colourful 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 colour variance within any patch evolved relatively recently. Although this pattern can be interpreted as evidence for character displacement [56], the majority of taxa within clades are currently allopatric [16] so colour evolution was presumably unaffected by interactions with congeneric or contribal taxa. Instead, the recent evolution of many colour patches likely reflects the commonly observed pattern of rapid colour evolution at the tips of phylogenies [57]. 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 [16,57], as 90% of parrots have green patches and 85% are primarily green [49]. 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 [21]. The impact of climatic adaptation appears to be limited because we found no evidence that colour was adaptive to climatic conditions. In contrast to monochromatic birds, which may be under strong selection for uniform plumage colour (such as the snow-coloured winter plumage of Rock Ptarmigans [58]), lorikeets may be colourful, in part, because much of

the colour 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 [54]. 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.

(d) Model adequacy

Overall, we found that most best-fit models were also a good absolute fit to the patch colour data with the exception of a few patches (figure 3*f*). 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-colour traits, found that relative best-fit models are frequently a poor absolute fit to empirical trait data [37,38]. 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 colour exhibit rate heterogeneity [38,59,60]. 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.

(d) Challenges in studying plumage colour

Quantifying colour 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 colour 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 [61]. The potential error in our approach pertains mostly to patch delineation, not the overall colour 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 colour and pattern regulation [53], may lead to more objective patch-specific analyses. Delineating high-contrast boundaries would enable patch geometry and boundaries to be objectively quantified [45,53] and provide a clearer means of interpreting patch colours in the context of sexual or social signaling.

5. Conclusion

We found that alternative macroevolutionary models best explained the exceptional colour 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 colours despite the selective costs of conspicuous colouration. Demonstrating that mosaic evolution operates in birds and other animals will clarify how extreme phenotypic diversification occurred under variable evolutionary pressures.

Data accessibility

Dryad link pending

Authors' contributions

JTM, GFS, and BTS designed the study, JTM collected and analyzed the data, and JTM and BTS wrote the first draft, and all authors wrote the final draft.

Competing interests

We have no competing interests.

Funding

None

Acknowledgements

We would like to thank S. Simpson for measuring skins, and we thank W. Mauck, M. Pennell, F. Burbrink, L. Joseph, E. Morrison, R. Maia, J. Troscianko, M. Palmer, D. H. Brainard, K. Provost, L. Moreira, L. Musher, G. Rosen, T. Trombone, and P. Sweet.

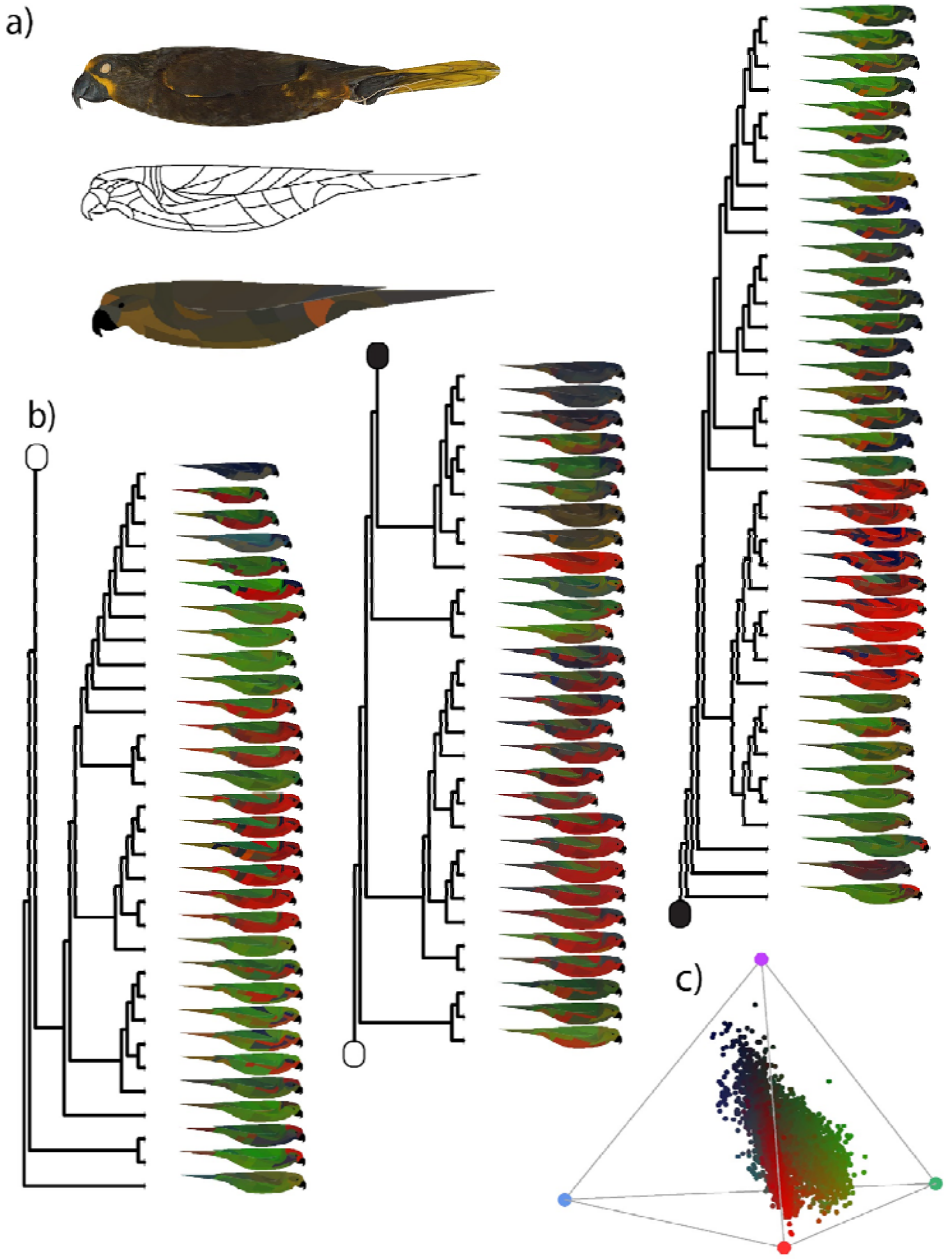

Figure 1. Quantifying and plotting plumage colour on a phylogeny of the lories and lorikeets. (a) An image of a museum specimen of *Chalcopsitta duivenbodei* (top), a blank patchmap showing the 35 plumage regions measured from images of museum specimens (middle), and the corresponding patchmap for this 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 colour 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 colour patch

measurements for each taxon. The colour space was centered slightly towards the longwave (red) vertex of the tetrahedral colour space. While the distribution of colours in the colour 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. Colours represent the RGB colours which were mapped onto the real-colour patchmaps.

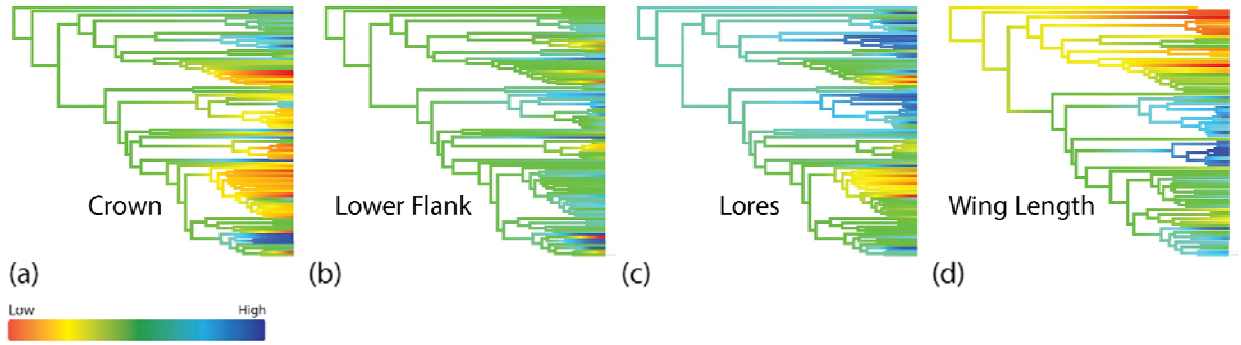

Figure 2. Continuous character mapping shows that colour is a more labile trait than body size. Ancestral states were estimated using a Brownian Motion process, and included are exemplar patches and wing length on the phylogeny. The patches correspond to PC1 for the top of the head (a, Crown), lateral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

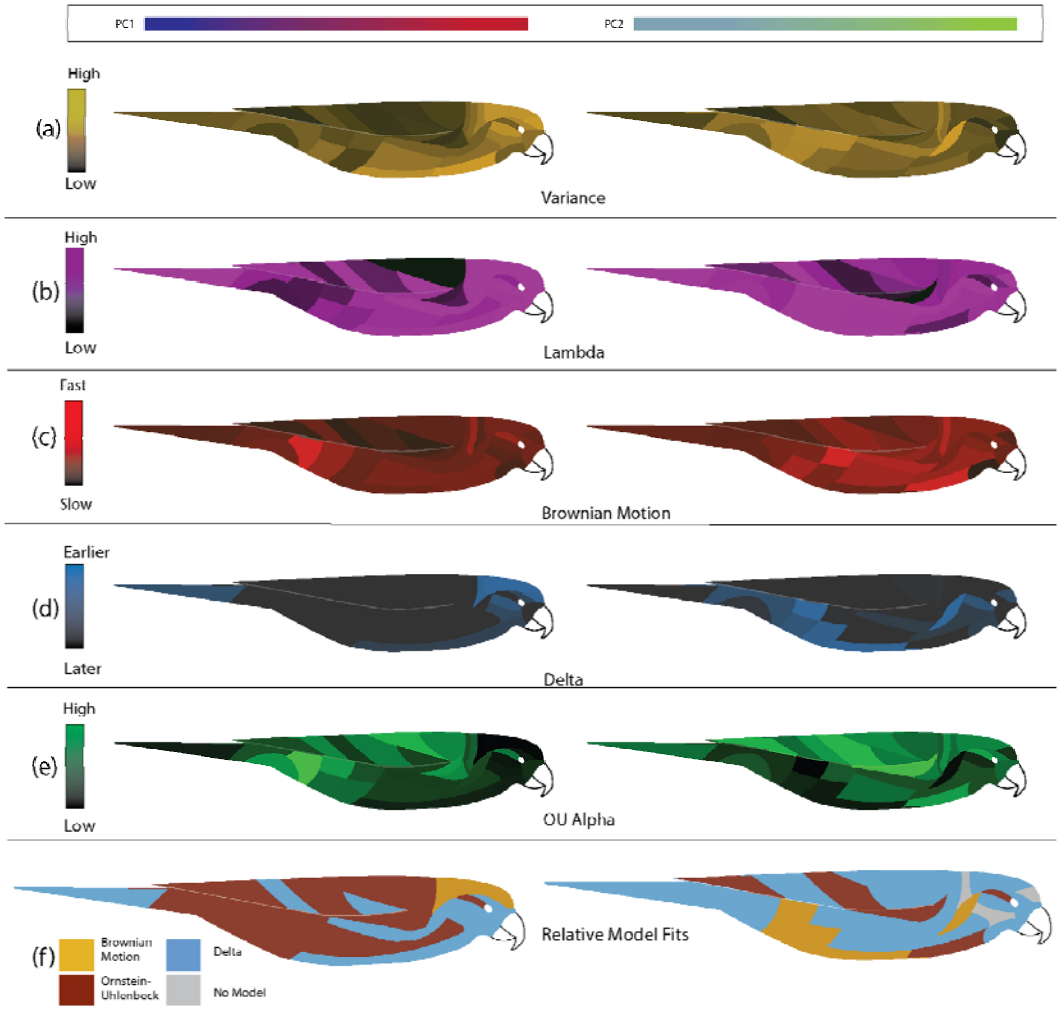

Figure 3. Patchmaps show within and among patch variability in PC values, models parameters, and best-fit models. PC scale bars at top show axes of colour variance encompassed by each PC. Each patch in a patchmap was coloured according to values for principal component variance (a), the modeled parameters 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.

References

1. Edler AU, Friedl TWP. Plumage Colouration, Age, Testosterone and Dominance in Male Red Bishops (Euplectes orix): A Laboratory Experiment. Ethology. 2010.

2. Stevens M, Marshall KLA, Troscianko J, Finlay S, Burnand D, Chadwick SL. Revealed by conspicuousness: distractive markings reduce camouflage. Behav Ecol. 2012;24: 213–222.

3. Gluckman T-L, -L. Gluckman T, Cardoso GC. The dual function of barred plumage in birds: camouflage and communication. J Evol Biol. 2010;23: 2501–2506.

4. Hill GE, Hill GE, McGraw KJ. Bird Coloration: Function and evolution. Harvard University Press; 2006.

5. Bennett AT, Cuthill IC, Partridge JC, Lunau K. Ultraviolet plumage colors predict mate preferences in starlings. Proc Natl Acad Sci USA. 1997;94: 8618–8621.

6. Medina I, Newton E, Kearney MR, Mulder RA, Porter WP, Stuart-Fox D. Reflection of near-infrared light confers thermal protection in birds. Nat Commun. 2018;9: 3610.

7. Beasley BA, Davison Ankney C. The effect of plumage color on the thermoregulatory abilities of Lesser Snow Goose goslings. Can J Zool. 1988;66: 1352–1358.

8. Hill RW, Beaver DL, Veghte JH. Body Surface Temperatures and Thermoregulation in the Black-Capped Chickadee (Parus atricapillus). Physiol Zool. 1980;53: 305–321.

9. Stoddard MC, Prum RO. How colorful are birds? Evolution of the avian plumage color gamut. Behav Ecol. 2011;22: 1042–1052.

10. Nordén KK, Price TD. Historical Contingency and Developmental Constraints in Avian Coloration. Trends Ecol Evol. 2018;33: 574–576.

11. Saranathan V, Hamilton D, Powell GVN, Kroodsma DE, Prum RO. Genetic evidence supports song learning in the three-wattled bellbird Procnias tricarunculata (Cotingidae). Mol Ecol. 2007;16: 3689–3702.

12. Irestedt M, Jønsson KA, Fjeldså J, Christidis L, Ericson PGP. An unexpectedly long history of sexual selection in birds-of-paradise. BMC Evol Biol. 2009;9: 235.

13. Seddon N, Botero CA, Tobias JA, Dunn PO, Macgregor HEA, Rubenstein DR, et al. Sexual selection accelerates signal evolution during speciation in birds. Proc Biol Sci. 2013;280: 20131065.

14. Uy JA, Borgia G. Sexual selection drives rapid divergence in bowerbird display traits. Evolution. 2000;54: 273–278.

15. Dunn PO, Armenta JK, Whittingham LA. Natural and sexual selection act on different axes of variation in avian plumage color. Sci Adv. 2015;1: e1400155.

16. Forshaw JM. Parrots of the World. 2010.

17. Delhey K. The colour of an avifauna: A quantitative analysis of the colour of Australian birds. Sci Rep. 2015;5: 18514.

18. McGraw KJ, Nogare MC. Carotenoid pigments and the selectivity of psittacofulvin-based coloration systems in parrots. Comp Biochem Physiol B Biochem Mol Biol. 2004;138: 229–233.

19. McGraw KJ, Nogare MC. Distribution of unique red feather pigments in parrots. Biol Lett. 2005;1: 38–43.

20. Burtt EH Jr, Schroeder MR, Smith LA, Sroka JE, McGraw KJ. Colourful parrot feathers resist bacterial degradation. Biol Lett. 2011;7: 214–216.

21. Heinsohn R, Legge S, Endler JA. Extreme reversed sexual dichromatism in a bird without sex role reversal. Science. 2005;309: 617–619.

22. Provost KL, Joseph L, Smith BT. Resolving a phylogenetic hypothesis for parrots: implications from systematics to conservation. Emu - Austral Ornithology. 2018;118: 7–21.

23. Smith BT, Mauck WM, Benz B, Andersen MJ. Uneven missing data skews phylogenomic relationships within the lories and lorikeets [Internet]. 2018. doi:10.1101/398297

24. Schweizer M, Wright TF, Peñalba JV, Schirtzinger EE, Joseph L. Molecular phylogenetics suggests a New Guinean origin and frequent episodes of founder-event speciation in the nectarivorous lories and lorikeets (Aves: Psittaciformes). Mol Phylogenet Evol. 2015;90: 34–48.

25. Schweizer M, Güntert M, Seehausen O, Leuenberger C, Hertwig ST. Parallel adaptations to nectarivory in parrots, key innovations and the diversification of the Loriinae. Ecol Evol. 2014;4: 2867–2883.

26. McKay BD. The use of digital photography in systematics. Biol J Linn Soc Lond. 2013;110: 1–13.

27. Troscianko J, Stevens M. Image calibration and analysis toolbox - a free software suite for objectively measuring reflectance, colour and pattern. Methods Ecol Evol. 2015;6: 1320–1331.

28. R Core Team. R: A language and Environment for Statistical Computing [Internet]. R Foundation for Statistical Computing, Vienna, Austria; 2017. Available: https://www.R-project.org

29. Maia R, Eliason CM, Bitton P-P, Doucet SM, Shawkey MD. pavo : an R package for the analysis, visualization and organization of spectral data. Tatem A, editor. Methods Ecol Evol. 2013;I. doi:10.1111/2041-210X.12069

30. Dale J, Dey CJ, Delhey K, Kempenaers B, Valcu M. The effects of life history and sexual selection on male and female plumage colouration. Nature. 2015;527: 367–370.

31. Yu G, Smith DK, Zhu H, Guan Y, Lam TT-Y. ggtree : an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. McInerny G, editor. Methods Ecol Evol. 2017;8: 28–36.

32. Smith SA, O'Meara BC. treePL: divergence time estimation using penalized likelihood for large phylogenies. Bioinformatics. 2012;28: 2689–2690.

33. Revell LJ. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol Evol. 2011;3: 217–223.

34. Birdlife International. Bird Species Distribution maps of the World. Cambridge, United Kingdom and Arlington, United States: Birdlife International and NatureServe; 2011.

35. Orme D. The caper package: comparative analysis of phylogenetics and evolution in R. 2018.

36. Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W. GEIGER: investigating evolutionary radiations. Bioinformatics. 2008;24: 129–131.

37. Pennell MW, FitzJohn RG, Cornwell WK, Harmon LJ. Model Adequacy and the Macroevolution of Angiosperm Functional Traits. Am Nat. 2015;186: E33–50.

38. Seeholzer GF, Claramunt S, Brumfield RT. Niche evolution and diversification in a Neotropical radiation of birds (Aves: Furnariidae). Evolution. 2017;71: 702–715.

39. Adams DC, Collyer ML. Multivariate Phylogenetic Comparative Methods: Evaluations, Comparisons, and Recommendations. Syst Biol. 2017;67: 14–31.

40. Goolsby EW. Likelihood-Based Parameter Estimation for High-Dimensional Phylogenetic Comparative Models: Overcoming the Limitations of "Distance-Based" Methods. Syst Biol. 2016;65: 852–870.

41. Taiyun Wei VS. corrplot: Visualization of a correlation matrix. 10/2013.

42. Felice RN, Randau M, Goswami A. A fly in a tube: Macroevolutionary expectations for integrated phenotypes. Evolution. 2018; doi:10.1111/evo.13608

43. Cooper N, Thomas GH, Venditti C, Meade A, Freckleton RP. A cautionary note on the use of Ornstein Uhlenbeck models in macroevolutionary studies. Biol J Linn Soc Lond. 2016;118: 64–77.

44. Hofmann CM, Cronin TW, Omland KE. Using spectral data to reconstruct evolutionary changes in coloration: carotenoid color evolution in new world orioles. Evolution. 2006;60: 1680–1691.

45. Endler JA, Cole GL, Kranz X. Boundary Strength Analysis: Combining colour pattern geometry and coloured patch visual properties for use in predicting behaviour and fitness. 2018. doi:10.1101/342063

46. Morrison ES, Badyaev AV. The Landscape of Evolution: Reconciling Structural and Dynamic Properties of Metabolic Networks in Adaptive Diversifications. Integr Comp Biol. 2016;56: 235–246.

47. Cooke TF, Fischer CR, Wu P, Jiang T-X, Xie KT, Kuo J, et al. Genetic Mapping and Biochemical Basis of Yellow Feather Pigmentation in Budgerigars. Cell. 2017;171: 427–439.e21.

48. Berg ML, Bennett ATD. The evolution of plumage colouration in parrots: a review. Emu - Austral Ornithology. 2010;110: 10–20.

49. Nemeśio A. Colour production and evolution in parrots. International Journal of Ornithology. 2001;4: 75–102.

50. Omland KE, Lanyon SM. Reconstructing plumage evolution in orioles (Icterus): repeated convergence and reversal in patterns. Evolution. 2000;54: 2119–2133.

51. Morrison ES, Badyaev AV. Structure versus time in the evolutionary diversification of avian carotenoid metabolic networks. J Evol Biol. 2018;31: 764–772.

52. Abolins-Abols M, Kornobis E, Ribeca P, Wakamatsu K, Peterson MP, Ketterson E, et al. A role for differential gene regulation in the rapid diversification of melanic plumage coloration in the dark-eyed junco (Junco hyemalis). 2018. doi:10.1101/315762

53. Schwochow-Thalmann D. Molecular Identification of Colour Pattern Genes in Birds. 2018.

54. Serpell J. Visual displays and taxonomic affinities in the parrot genus Trichoglossus. Biol J Linn Soc Lond. Oxford University Press; 1989;36: 193–211.

55. Soma M, Garamszegi LZ. Evolution of patterned plumage as a sexual signal in estrildid finches. Behav Ecol. 2018;29: 676–685.

56. Schluter, Schluter. Ecological Character Displacement in Adaptive Radiation. Am Nat. 2000;156: S4.

57. Stoddard MC, Prum RO. Evolution of avian plumage color in a tetrahedral color space: a phylogenetic analysis of new world buntings. Am Nat. 2008;171: 755–776.

58. Montgomerie R. Dirty ptarmigan: behavioral modification of conspicuous male plumage. Behav Ecol. 2001;12: 429–438.

59. Chira AM, Thomas GH. The impact of rate heterogeneity on inference of phylogenetic models of trait evolution. J Evol Biol. 2016;29: 2502–2518.

60. Felice RN, Goswami A. Developmental origins of mosaic evolution in the avian cranium. Proc Natl Acad Sci U S A. 2018;115: 555–560.

61. Miller ET, Leighton GM, Freeman BG, Lees AC, Ligon RA. Climate, habitat, and geographic range overlap drive plumage evolution. bioRxiv. 2018. p. 375261. doi:10.1101/375261