

1 **Title: Visual signal evolution along complementary color axes in**  
2 **four bird lineages**

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26 **Abstract**

27 Animal color patterns function in varied behavioral contexts including recognition,  
28 camouflage and even thermoregulation. The diversity of visual signals may be  
29 constrained by various factors, for example, dietary factors, and the composition of  
30 ambient environmental light (sensory drive). How have high-contrast and diverse signals  
31 evolved within these constraints? In four bird lineages, we present evidence that  
32 plumage colors cluster along a line in tetrachromatic color space. Additionally, we  
33 present evidence that this line represents complementary colors, which are defined as  
34 opposite sides of a line passing through the achromatic point (putatively for higher  
35 chromatic contrast). Finally, we present evidence that interspecific color variation over at  
36 least some regions of the body is not constrained by phylogenetic relatedness. Thus, we  
37 hypothesize that species-specific plumage patterns within these bird lineages evolve by  
38 swapping the distributions of a complementary color pair (or dark and light patches in  
39 one group, putatively representing an achromatic complementary axis). The relative role  
40 of chromatic and achromatic contrasts in discrimination may depend on the environment  
41 that each species inhabits.

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## 52 **Introduction**

53 The diverse colors of birds are important communication signals for advertisement and  
54 species recognition (Baker and Parker 1979; Alatalo et al. 1994; Greene et al. 2000;  
55 Bleiweiss 2004; Uy et al. 2009; Seddon et al. 2013). A number of ecological factors may  
56 constrain the diversification of plumage color, including diet (Hill et al. 2002; McGraw  
57 and Nogare 2004), the sensory systems of species and their predators (Gotmark 1993;  
58 Marchetti 1993; Gomez and Théry 2007), the composition of ambient light in different  
59 habitats (Endler 1992; Boughman 2002), and the additional constraint of phylogenetic  
60 relatedness (the tendency of related species to resemble each other). These constraints  
61 may operate both on what colors a bird may exhibit, and in where they are placed on the  
62 body (Endler 1992; Gomez and Théry 2007). Multiple studies have shown that plumage  
63 (and egg) colors in birds do not occupy the entire available color space (Endler et al.  
64 2005; Cassey et al. 2008; Stoddard and Prum 2011). This constrained diversity of color  
65 has important consequences for signal perception and discrimination by receiver visual  
66 systems (Endler 1992; Endler and Mielke 2005; Cole and Endler 2016). In spite of these  
67 constraints, many bird groups have evolved highly diverse colors and patterns (Hill and  
68 McGraw 2006a); understanding signal evolution remains a focus of research (Stoddard  
69 and Prum 2008; Mason et al. 2014; Doutrelant et al. 2016).

70 The evolution of color signals may be influenced by how they are perceived by receivers,  
71 and this perception involves two neural processes: 1) photoreceptors (cones) sensitive  
72 to different wavelengths of light, and 2) downstream neural mechanisms comparing  
73 photoreceptor output, both spectrally and spatially (Endler and Mielke 2005; Endler et al.  
74 2005; Kelber 2016). Neurons performing these comparisons possess a distinct spatial  
75 receptive field (Ventura et al. 2001), and thus likely respond differently to different  
76 patterns of the same pair of colors. Birds possess a tetrachromatic visual system, with  
77 ultraviolet-sensitive UVS (or violet-sensitive VS) (Ödeen and Håstad 2013), short (blue,

78 SWS), medium (green, MWS) and long-wavelength (red, LWS) sensitive  
79 cones (Vorobyev et al. 1998; Vorobyev 2003; Osorio and Vorobyev 2008). Opponent-  
80 color mechanisms in birds support color discrimination, and multiple studies have  
81 investigated the behavioral abilities of birds to discriminate colors (Yazulla and Granda  
82 1973; Wright 1975; Goldsmith and Goldsmith 1979; Osorio et al. 1999a,b; Vorobyev  
83 2003; Goldsmith and Butler 2005; Ham and Osorio 2007).

84 How do visual signals diversify within avian lineages, considering the constraints on  
85 color, location and processing of visual signals? The visual signals of some species are  
86 known to exhibit complementary colors, which have little spectral overlap, and tend to  
87 excite distinct sets of photoreceptors. **Complementary colors** represent a continuum  
88 lying on opposite sides of a line passing through the achromatic point (where all color  
89 receptors are equally stimulated) in color space (Endler 1992; Endler et al. 2005; Ham  
90 and Osorio 2007). The extremes of this continuum represent the most complementary  
91 colors, offering high contrast and discriminability when combined together, particularly  
92 over adjacent body regions (Endler 1992; Osorio et al. 1999a; Endler and Mielke 2005;  
93 Hill and McGraw 2006a). For example, in forest canopy birds, dwelling against a  
94 primarily green background, blue colors serve to increase the contrast of red colors  
95 against the background (Endler 1992). If each species within a lineage possesses a  
96 similar pair of complementary colors (as defined by the distribution in color space  
97 mentioned above), diverse patterns may evolve by redistributing these colors over the  
98 body. Thus, diverse yet high-contrast visual signals may evolve, supporting species  
99 discrimination. In addition to chromatic signals, luminance signals (achromatic or black-  
100 and-white variance) are also important to consider as they offer high contrast (Marchetti  
101 1993; Mennill et al. 2003; Griggio et al. 2011), and may be prioritized for discrimination in  
102 certain circumstances (Schaefer et al. 2006). Thus, if we quantify color (and luminance)  
103 across all species within a lineage, we would predict 1) that plumage colors within a

104 lineage would lie along a line in perceptual tetrachromatic color space (Goldsmith 1990;  
105 Endler and Mielke 2005), and 2) each species should possess colors that lie on opposite  
106 sides of the achromatic point within this continuum of complementary colors (Endler  
107 1992) (Figure 1). As a corollary to this second, the presence of both complementary  
108 colors across species could alternatively indicate phylogenetic constraints due to shared  
109 ancestry. Therefore, if we perform phylogenetic comparative analyses of color and  
110 luminance scores (to account for phylogenetic non-independence), we also predict that  
111 we should uncover evidence of a departure from phylogenetic Brownian motion models  
112 of trait evolution over at least some regions of the body. This, together with the previous  
113 predictions, would be consistent with complementary colors being redistributed across  
114 body regions during diversification.

115 Here, we describe the color space and interspecific color variation in four ecologically  
116 and phylogenetically diverse bird lineages, using ultraviolet-visible light reflectance  
117 spectrometry (Hill and McGraw 2006a). They are: 1) Pittas (Pittidae), understory  
118 invertebrate-eaters occurring from Africa to Australasia (Erritzoe and Erritzoe 1998), 2)  
119 Asian barbets (Megalaimidae), tropical forest-canopy frugivores (Short and Horne 2001),  
120 3) Afro-Asiatic *Psittacula* parakeets (Psittacidae), fruit and seed-eaters inhabiting  
121 deciduous forests and woodland (Forshaw and Cooper 1989), and 4) Sandgrouse  
122 (Pteroclididae), arid-country ground-dwelling granivores (Maclean 1996) (Figure 1A-E,  
123 Supplementary Appendix). These families putatively represent both UVS- and VS-type  
124 avian visual systems (see Supplementary Figure 1 and Appendix for further discussion)  
125 (Stoddard and Prum 2011; Ödeen and Håstad 2013). We first test the prediction that  
126 plumage colors in each lineage largely lie along a single line in color space (Prediction  
127 1). Secondly, we examine interspecific patterns in color or luminance scores across  
128 body regions and species, to address whether this linear distribution represents  
129 complementary colors that are redistributed over the body during diversification

130 (Prediction 2) (Figure 1F). By identifying common interspecific patterns across these  
131 families with diverse life-histories and habitats, our study aims for insight into general  
132 processes underlying the evolution and perception of visual signals.

133

## 134 **Materials and Methods**

### 135 *Museum specimens*

136 We measured museum specimens of four avian lineages (Number of specimens,  
137 Number of species measured: Pittas: 80,28; Barbets: 81,30; Parakeets: 55,12;  
138 Sandgrouse: 57,16)(del Hoyo et al. 2014), held in the collections of the Division of Birds,  
139 Smithsonian National Museum of Natural History (USNM), Washington, D.C., USA (total  
140 273 specimens, Supplementary Dataset). Where possible, we measured specimens  
141 collected relatively recently (Armenta et al. 2008), male and female specimens of  
142 sexually dichromatic species, and distinct subspecies (also see Supplementary  
143 Appendix) to obtain a comprehensive estimate of the color space occupied by each  
144 family.

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### 146 *Reflectance spectrometry and photon catch of color cones*

147 We measured plumage reflectance of museum skins, using an S2000 UV-visible fiber-  
148 optic reflectance spectrophotometer (Ocean Optics, Inc.) with a DT1000 deuterium-  
149 tungsten halogen light source. Measurements were referenced to a CIE D65 (white  
150 under average daylight illumination) white standard (Milton Roy Color Products), and  
151 dark referenced to a black surface. We first moved the probe over each region of the  
152 body, looking at the computer display to ensure that (to the best of our ability) we did not  
153 miss any patches that are not visible to the human eye (particularly cryptic UV sexual  
154 dimorphism). We then measured one reflectance spectrum for each color patch on each  
155 specimen using the Overture software. Although our dataset did not take into account

156 within-patch variation as a result, we generally observed that intraspecific variation (and  
157 qualitatively, within-patch variation observed by moving around the probe) for the same  
158 patch was lower than interspecific variation, and is thus unlikely to alter the patterns we  
159 observe.

160 We used the MATLAB (MathWorks, Inc.) program TetraColorSpace(Stoddard and Prum  
161 2008) and the R (R Core Team 2013) package PAVO(Maia et al. 2013) to analyze  
162 reflectance spectra. These algorithms incorporate cone sensitivities for averaged VS and  
163 UVS avian visual systems, to calculate theoretical photon catch for each cone (this  
164 representing the signaler phenotype, or visual signal under idealized light conditions  
165 (Stoddard and Prum 2008). Although the use of averaged visual systems does not  
166 directly model perception for each species, photon catch provides an objective way to  
167 quantify spectral signal in different portions of the avian-visible spectrum(Burkhardt  
168 1989; Goldsmith 1990; Endler and Mielke 2005). We calculated photon catch of the four  
169 color cones using both programs (the values were concordant across both), performing  
170 the von Kries correction (Vorobyev and Osorio 1998) using a uniform white light (or  
171 idealized light) spectrum. Birds process luminance information separately from color  
172 information(Vorobyev and Osorio 1998; Endler and Mielke 2005), using the double  
173 cones(Goldsmith and Butler 2005). Thus, we also used PAVO to calculate the photon  
174 catch of the double cones as a measure of luminance, using known sensitivities for the  
175 double cone of the blue tit (*Cyanistes caeruleus*)(Hart et al. 2000). Again, although this  
176 does not directly represent luminance perception by each species, it provides an  
177 objective comparison of luminance differences in plumage. Using the relative photon  
178 catch values for each cone, we visualized plumage colors of each bird family in  
179 Goldsmith's tetrahedral color space(Burkhardt 1989; Goldsmith 1990).

180

181 *Analyses*

182 After obtaining raw photon catch values for each cone, we transformed these values into  
183 a three-dimensional XYZ color space representing the receptor-noise limited model of  
184 tetrachromatic color vision(Vorobyev and Osorio 1998; Vorobyev et al. 1998; Vorobyev  
185 2003; Siddiqi et al. 2004). This was accomplished using the Weber fraction of each  
186 cone, which is calculated using the signal:noise ratio and the relative abundance of each  
187 cone in the retina. We incorporated published Weber fractions of the four cones for  
188 *Leiothrix lutea*(Vorobyev et al. 1998) as described in the literature(Cassey et al. 2008;  
189 Delhey et al. 2015), to transform photon catch values for each color patch into XYZ  
190 coordinates using a custom-written MATLAB code. The advantage of this color space is  
191 that distances between points are expressed in just noticeable differences (JND), an  
192 indication of the perceptual distance between them (Vorobyev and Osorio 1998; Siddiqi  
193 et al. 2004; Cassey et al. 2008; Pike 2012), thus providing a better approximation of how  
194 differences in color are perceived by the avian visual system. We also plotted color  
195 distributions for each family in this color space using the RGL package(Adler et al. 2003)  
196 in R sensu (Delhey et al. 2015).

197 To test Prediction 1, that plumage colors should largely distribute along a single  
198 line(Endler et al. 2005), we estimated the proportion of variation in coordinate space  
199 explained by the first major axis using principal components analysis (PCA) on the XYZ  
200 coordinates obtained above, following published studies(Cassey et al. 2008; Delhey et  
201 al. 2015). In order to test prediction 2, that this line represents a complementary color  
202 axis, and that the positions of these colors may be redistributed across the body during  
203 the diversification of these avian lineages, we required a metric that included not only the  
204 distance of each color from the origin (indicating “how complementary” a color is along  
205 the continuum), but which distinguished colors lying on opposite sides of the achromatic  
206 point (information which is lost in Euclidean distance measures). To achieve this, we  
207 transformed the XYZ coordinates into a spherical coordinate space in MATLAB, with the



208 achromatic point at the origin. We used this coordinate (in radians) as a “color score” in  
209 subsequent analyses (using a species average, also see Results). By the definition of a  
210 complementary color pair as detailed above, they should therefore exhibit color scores  
211 with opposite signs. This is because they occur on opposite sides of the achromatic  
212 point(Endler 1992; Endler and Mielke 2005), and exhibit little spectral overlap (Ham and  
213 Osorio 2007). Additionally, this enabled us to transform complex measurements of color  
214 space into a ‘trait’ that could be compared using comparative phylogenetic analyses,  
215 while simultaneously testing hypotheses about the evolution of complementary colors.  
216 To examine whether each species possessed both colors in a complementary color pair,  
217 we constructed histograms of the maximum and minimum color score for each species  
218 within a family. Finally, we used phylogenetic comparative analyses to investigate  
219 whether color and luminance scores across each body region exhibited phylogenetic  
220 signal. We first sorted all the patches measured in each of the four avian lineages into  
221 crown, cheek, throat, back, wing, tail, and underpart patches (except the parakeets,  
222 where we measured crown, cheek, back, wings, underparts and both upper and  
223 undertail, see Supplementary Appendix). Next, we calculated the average color score  
224 and luminance index (double cone photon catch) for each region of the body for the  
225 male plumage of each species (to account for some species possessing more color  
226 patches than others, and thus enable direct comparisons). Using published phylogenetic  
227 information for each family (Groombridge et al. 2004; Irestedt et al. 2006; Jetz et al.  
228 2012; Kundu et al. 2012; Den Tex and Leonard 2013) and the ape and phytools  
229 packages (Paradis et al. 2004; Revell 2012) in R, we calculated Pagel’s  $\lambda$ , a measure of  
230 phylogenetic signal, for color and luminance scores of each body region. This index  
231 measures whether trait evolution (in this case, color and luminance scores) follows a  
232 Brownian motion model of evolution, where phylogenetic effects drive trait evolution. In  
233 this scenario, the  $\lambda$  value is 1, whereas departures from Brownian motion result in a

234 value lower than 1 (Pagel 1999; Münkemüller et al. 2012). To estimate the significance  
235 of the measured statistic, we compared this value to 1000 randomized values obtained  
236 using the inbuilt functions of the phytools package. To further verify these results, we  
237 additionally performed a second analysis. Using a phylogenetic distance matrix derived  
238 from the ape package, we calculated Mantel correlations between this matrix and an  
239 interspecific trait distance matrix derived for color and luminance for each body region  
240 (see Supplementary Data). This test provided additional quantification on the effects of  
241 phylogenetic relatedness on interspecific color variation.

242

## 243 **Results**

### 244 *Prediction 1: Plumage colors lie along a line in tetrachromatic color space*

245 Across the four avian lineages, we find that plumage colors distribute between two  
246 points in tetrahedral color space. The color signals of pittas lie between red (LWS) and  
247 violet (VS) color vertices (indicating highly-saturated colors) (Figure 2A). Barbets largely  
248 distribute between the green (MWS) and red (LWS) vertices, with a few blue-violet  
249 patches (Figure 2C). Plumage colors of *Psittacula* parakeets lie between the middle of  
250 red-green space and the middle of blue-uv space (Figure 2E), with a few patches near  
251 the LWS and MWS vertices. Finally, the plumage colors of sandgrouse are restricted to  
252 a region between the black achromatic point (the centroid)(Stoddard and Prum 2008)  
253 and the LWS (red) vertex (Figure 2G). The XYZ color space using the noise-limited  
254 model also recovers a linear axis of color variation, suggesting that this “axis” is genuine,  
255 and not an artifact of the tetrahedral color space. The results of PCA to quantify the  
256 proportion of variation explained by this line are summarized below for each avian  
257 lineage, and also in Supplementary Table 1 (see Supplementary Dataset):

258 **Pittas:** PC1 (the major axis of variation) of the XYZ coordinates in color space explains  
259 85% of chromatic variation (Figure 2B). PC1 loads weakly negatively on X (-0.15), and  
260 exhibits strong positive loadings (0.6 and 0.78) on Y and Z, respectively.

261 **Barbets:** PC1 explains almost 74% of chromatic variation (Figure 2D), loading weakly  
262 negatively on X (-0.03), moderately positively on Y (0.465), and strongly positively on Z  
263 (0.884).

264 **Parakeets:** PC1 explains 75% of variation in color (Figure 2F), loading weakly  
265 negatively on X (-0.2), moderately positively on Y (0.57) and strongly positively on Z  
266 (0.8).

267 **Sandgrouse:** PC1 explains about 70% of color variation, loading weakly negatively on X  
268 (-0.32) and strongly positively on Y and Z (0.64 and 0.7).

269 Across all four lineages, the Z coordinate loads most strongly on PC1, thus suggesting  
270 that most variation in perceptual coordinate space occurs along the elevational rather  
271 than azimuthal direction along the PC1 line. Therefore, in subsequent analyses, we used  
272 the elevational coordinate  $\Phi$  and the sign of this coordinate as an indicator of where  
273 different colors lie along this line. Although this does not take variation in the azimuthal  
274 plane into account, the results of our analysis suggest that this variation is negligible  
275 compared to variation along the elevational axis in all four families. Thus, colors with  
276 opposite signs of  $\Phi$  in this dataset lie on opposite sides of the achromatic point (as is  
277 evident from the spread of the data in Figure 2). We used PCA only to estimate the  
278 proportion of variance along this line, and not in any subsequent analysis.

279

280 *Prediction 2: Color space axes represent complementary colors, lying on opposite sides*  
281 *of the achromatic point*

282 We next test if color pattern diversity is achieved by redistributing complementary colors  
283 across certain body regions using species averages for color and luminance scores

284 across body regions. We predict a) that species within families exhibit colors lying on  
285 both sides of the achromatic point, and b) that measures of phylogenetic signal indicate  
286 departure from a Brownian motion model of evolution, consistent with changing positions  
287 of a complementary color pair during diversification. We summarize the results of these  
288 analyses below:

289 **Pittas:** After transforming into a spherical coordinate space, elevation coordinates span  
290 between -1.54 and +1.57 across the family, i.e. on opposite sides of the achromatic point  
291 and at roughly equal distances from it along the elevation axis, consistent with the  
292 interpretation of a complementary color axis. For example, the deep-blue (to human  
293 eyes) crown of the male *Hydrornis baudii* has, on average, a color score of -1.15, and  
294 the deep-red crown of the sympatric (Erritzoe and Erritzoe 1998) *Erythropitta granatina*  
295 scores +1.12. This is also consistent with a hypothesis of complementary colors, in that  
296 these colors also represent opposite ends of the avian-visible light spectrum. Histograms  
297 of maximum and minimum color scores of each species within the family show that most  
298 of these species possess both complementary colors, the peaks of these distributions  
299 lying on opposite sides of the achromatic point (Figure 3A). Phylogenetic comparative  
300 analyses (Table 1) reveal that patterns of plumage evolution are heterogeneous across  
301 the body regions of pittas. Color scores are consistent with a Brownian motion model of  
302 evolution on the cheek, wing and tail, and exhibit weak and non-significant phylogenetic  
303 signal across other body regions. Luminance scores exhibit significant phylogenetic  
304 signal only on the crown and wing. Mantel tests for correlation between phylogenetic and  
305 trait distance broadly corroborate these results: luminance distance correlates  
306 significantly with phylogenetic distance only on the wing, whereas color correlates on the  
307 cheek, wing and tail (Supplementary Data). In addition, the regions with non-significant  
308 phylogenetic signal all possess relatively high coefficients of variation in color scores  
309 (Table 1). Thus, plumage patterns in the pittas are consistent with species possessing

310 colors on opposite sides of a continuum of complementary colors, and with the body  
311 distributions of these colors being swapped across certain body regions during  
312 diversification.

313 **Barbets:** Color scores span between -1.4 and +1.57, also consistent with a  
314 complementary color axis. For example, the red throat of *Psilopogon mystacophanos*,  
315 exhibits, on average, a color score of +1.1, and the turquoise throat of the  
316 sympatric (Short and Horne 2001) *P. rafflesii* a score of -1.21, which, like pitta colors, lie  
317 on opposite sides of the achromatic point and on a line through it. Again, histograms of  
318 color distribution (Figure 3B) demonstrate that most barbet species exhibit colors lying  
319 on opposite sides of the achromatic point (i.e. complementary colors). Color and  
320 luminance scores (Table 1) exhibit significant  $\lambda$  values across all regions (except  
321 luminance scores on the wing and tail), but values for head patches (particularly the  
322 cheek and the throat) are much lower than 1 (0.61 and 0.57), indicating a departure from  
323 a Brownian motion model of trait evolution. Phylogenetic and color distance are  
324 correlated on all body regions, but not on the head regions (Supplementary Data),  
325 corroborating the results from phylogenetic signal. In addition, all head regions possess  
326 relatively high CVs for color scores, but not body regions (except the wing, which does,  
327 however, exhibit phylogenetic signal suggesting that this variation has a phylogenetic  
328 component). Taken together, these results are also consistent with body colors being a  
329 constrained feature within this lineage, but colors being swapped around on the head.

330 **Parakeets:** Color scores span between -1.47 and +1.57, again consistent with a  
331 complementary color axis. For example, the wing of the male *Psittacula longicauda*  
332 *nicobarica* (-1.38) exhibits the opposite sign to the red shoulder patch of male *P.*  
333 *cycnocephala* (+1.32). Color histograms again indicate the presence of both  
334 complementary colors on all species within the family, with maximum and minimum  
335 scores for each species lying on opposite sides of the achromatic point (Figure 3C).

336 Neither color nor luminance scores exhibit significant phylogenetic signal across any  
337 body regions (Table 1) when compared to a Brownian motion model of trait evolution,  
338 and additionally do not exhibit significant correlations with phylogenetic distance  
339 (Supplementary Data). Color scores exhibit higher CVs than luminance scores (Table 1),  
340 which is also consistent with signal diversification along a chromatic complementary  
341 axis.

342 **Sandgrouse:** Color scores span between 0 (the achromatic point) and +1.33. This  
343 suggests that sandgrouse are clustered in chromatic space to one side of the achromatic  
344 point, further supported by color histograms (Figure 3D). However, aside from luminance  
345 scores on the wing (Table 1), neither color nor luminance scores exhibit significant  
346 phylogenetic signal on any body region. In addition, other than color scores on the crown  
347 (Supplementary Data), interspecific color and luminance distances are not significantly  
348 correlated with phylogenetic distance. Coefficients of variation of color scores (Table 1)  
349 are generally lower than those for luminance across body regions, unlike the other three  
350 lineages. Taken together with the apparent lack of chromatic complementarity in  
351 sandgrouse, interspecific luminance variation may putatively play a greater role in signal  
352 diversification. The sympatric *Pterocles alchata* and *P.orientalis*(Benítez-López et al.  
353 2014) represent a noteworthy example of such divergence. The black belly of the male  
354 *P.orientalis* exhibits an average luminance score  $<0.0001$ , whereas the white belly of the  
355 male *P.alchata* exhibits an average luminance score of 0.34.

356

## 357 **Discussion**

358 Across families, we thus uncover consistent evidence that complementary colors (or  
359 putatively patches of different luminance in sandgrouse, representing black-and-white  
360 variation, which are also inverses of each other) underlie the evolution of contrasting  
361 visual signals. Most species within each lineage possess both colors of a

362 complementary pair, and phylogenetic comparative analyses indicate generally low or  
363 insignificant phylogenetic signal (except the barbets, where, however, the head regions  
364 diverge from a Brownian motion model of trait evolution). Taken together with the  
365 constrained distribution of color scores (largely along a single line in color space), we  
366 hypothesize that evolutionary diversification of color patterns occurs by redistributing a  
367 complementary color pair across body regions. We discuss this further below.

368

369 *Visual signals and complementary colors*

370 To summarize, we find that plumage colors in each of the four bird lineages distribute  
371 along an axis between two colors (or regions of the avian-visible spectrum) which are  
372 complementary (spanning either side of the achromatic point), except the arid-country  
373 sandgrouse whose colors are found to only one side of the achromatic point.  
374 Additionally, based on phylogenetic comparative analyses, we hypothesize that signals  
375 diversify by redistribution of complementary colors. In the case of sandgrouse, the lack  
376 of phylogenetic signal and chromatic complementarity, together with the larger variance  
377 in luminance scores across body regions compared to color scores, leads us to  
378 tentatively hypothesize that signal evolution in this family has occurred along an  
379 achromatic (black-white) complementary axis rather than a chromatic one. Although not  
380 directly confirmed in our study systems, it is important to note here that tetrachromatic  
381 visual systems (avian and reptilian) possess a number of opponent color processes to  
382 compare cone outputs (Yazulla and Granda 1973; Osorio et al. 1999b; Ventura et al.  
383 2001; Smith et al. 2002; Goldsmith and Butler 2005; Rocha et al. 2008). In human  
384 trichromatic visual systems, red-green, yellow-blue and luminance (black-white)  
385 opponent comparisons result in all perceived hues occupying a continuum between  
386 these perceptually distinct opponent colors (Hurvich and Jameson 1957). Different  
387 opponent mechanisms (or color axes) dominate at various wavelengths and intensities

388 of ambient light, accordingly shifting the perceived color space (the Bezold–Brücke  
389 phenomenon)(Boynton and Gordon 1965). This may represent a putative mechanism  
390 enabling discrimination of complementary colors across light environments, although we  
391 note that we do not possess the evidence at present (i.e. physiological data) to explicitly  
392 test this.

393 We uncover evidence both that most species within a lineage possess complementary  
394 colors in their plumage (Figure 3), and also that phylogenetic patterns of trait evolution  
395 depart from Brownian motion over at least some body regions in all families (Table 1).  
396 Taken together, this is consistent with complementary colors in their plumage, putatively  
397 for high chromatic contrast (Endler 1992) under constraints, and with signal  
398 diversification by redistributing these complementary colors over certain regions of the  
399 body. In the case of pittas, these regions appear to be the crown, throat, back and  
400 underparts. For example, *Hydrornis baudii* possesses a blue crown and underparts, and  
401 a reddish-brown back, whereas the sympatric *Erythropitta granatina* possesses a deep  
402 blue-violet back and a bright red crown patch and belly. For Asian barbets, this  
403 redistribution of colors appears to occur primarily on the cheek and throat, and an  
404 examination of their color patterns supports this. Most members of the family possess  
405 largely green bodies, and bright colors are confined to the head regions. For parakeets,  
406 a lack of phylogenetic signal across the body suggests that diversification may occur by  
407 redistribution of colors across all body regions. This is consistent with the fact that  
408 different species possess both short-and long-wavelength colors on the head, wing,  
409 underparts and tail. Within all four lineages, multiple species occur in sympatric  
410 assemblages(Forshaw and Cooper 1989; Maclean 1996; Erritzoe and Erritzoe 1998;  
411 Short and Horne 2001; Groombridge et al. 2004; del Hoyo et al. 2014; Krishnan and  
412 Tamma 2016). Complementary colors may thus support pattern discrimination between  
413 species (or sexes of dichromatic species)(Hill and McGraw 2006b; Osorio and Vorobyev



414 2008). Future studies using image analysis techniques (such as those used to study egg  
415 patterns)(Stoddard et al. 2014), will aim to quantify and obtain further insight into how  
416 patterns of complementary colors have evolved.

417

418 *Ecological processes may constrain plumage diversity to complementary color axes*

419 How do ecological constraints influence plumage colors, and how might these explain  
420 patterns observed in our study? Lineages descended from a common ancestor may be  
421 predicted to resemble each other; alternatively, sexual selection and species recognition  
422 may accelerate signal diversification (Seddon et al. 2013; Mason et al. 2014). Signals  
423 may also be constrained by ecological factors to a specific complementary color axis.  
424 For example, the red colors of pittas and barbets are due to carotenoids(Thomas et al.  
425 2014), putatively derived from dietary sources(Hill et al. 2002), in contrast to structural  
426 short-wavelength colors (Saranathan et al. 2012). Parakeet pigment colors are due to  
427 psittacofulvins (McGraw and Nogare 2004). Finally, sandgrouse do not possess  
428 plumage carotenoids(Thomas et al. 2014), and pigmentation is thus likely to be primarily  
429 melanin-based (brown-black). This may constrain plumage diversification to an  
430 achromatic axis (or to changes in barring and speckling, which our study did not  
431 investigate), albeit with the caveat that luminance variation is difficult to compare using  
432 museum specimens. However, a comparison of plumage patterns in sandgrouse (Figure  
433 1) reveals that many species possess conspicuous black and white patches, whose  
434 distributions differ between species. Some possess these patches on the face, others on  
435 the wings and belly. Similar patterns of evolution along an achromatic axis may have  
436 also putatively occurred in other melanin-pigmented bird groups, such as larks, bustards,  
437 and coursers, as well as many raptors(del Hoyo et al. 2014), and merit further  
438 investigation.

439 Ecological pressures of sensory drive (for example, crypsis from predators and  
440 conspicuousness to intended receivers) may additionally constrain plumage diversity in  
441 bird lineages where all members exhibit relatively similar ecological preferences. All four  
442 families studied here experience predation, and possess both cryptic colors, and colors  
443 that offer maximal contrast in their preferred habitats. For example, blue-violet and  
444 saturated reds are very conspicuous against a forest understory background(Siddiqi et  
445 al. 2004), and reds also against the green forest canopy, where blue serves to increase  
446 within-pattern contrast(Endler 1992; Gomez and Théry 2007); these are the colors  
447 exhibited by pittas and barbets, which typically occupy these habitats(Erritzoe and  
448 Erritzoe 1998; Short and Horne 2001) (Figure 2). Cryptic colors, defined as matching the  
449 background in a habitat(Endler 1992; Gomez and Théry 2007) (green in tree-dwelling  
450 barbets and parakeets, reddish-brown in ground-dwelling pittas and sandgrouse), also  
451 occur across all four families, which are additionally noted in the literature as being  
452 unobtrusive, camouflaged or difficult to locate within their habitats (see discussion in  
453 Supplementary Online Appendix). It is possible that the composition of ambient light  
454 exerts constraints on plumage colors by shifting the perceived color space (Boynton and  
455 Gordon 1965; Wright 1975), although birds are known to exhibit color constancy, and the  
456 effects of this shift may be minor (Olsson et al. 2016). Alternatively, microhabitat  
457 variation in light composition may influence which colors are the most conspicuous  
458 (Endler and Thery 1996; Uy and Endler 2004), as well as whether birds use chromatic or  
459 achromatic contrasts in pattern discrimination (Endler and Thery 1996; Schaefer et al.  
460 2006). However, we have not directly measured the light environments inhabited by  
461 these species. Additionally, our use of theoretical models makes the assumption that all  
462 species within a lineage perceive color the same way, whereas some differences  
463 between species are likely to exist. The effects of sensory drive must thus be treated as

464 a tentative hypothesis, and field data are needed to further understand both the  
465 predation these birds experience, and the light microhabitats they use.  
466 Color vision is challenging to study comparatively in speciose bird lineages containing  
467 rare or range-restricted species, and many of the species we examine are poorly known.  
468 Thus, although our study design does not permit us to conclusively identify the  
469 ecological driver of these patterns, we do find consistent evidence of overall patterns of  
470 evolution along complementary color (or achromatic) axes in a manner that does not  
471 depend on their phylogenetic relatedness. Based on this, we hypothesize that color  
472 patterns may have diversified by redistribution or replacement of these complementary  
473 colors between species. We speculate that this mechanism supports the evolution of  
474 highly contrasting, yet diverse recognition signals under constraints (dietary and  
475 sensory, among others), and further research will focus on the ecological processes  
476 underlying these constraints. Bird lineages that have undergone multiple niche shifts  
477 would provide a suitable crux to address ecology and plumage diversification.

478

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496  
497 **Authors' Contributions**

498 Conceived and designed the study: AK, Collected data: AK AS, Analyzed and  
499 interpreted the data: AK KT, Wrote the first draft of the manuscript: AK, Contributed to  
500 editing and revision of the manuscript: AK AS KT. All authors approved submission of  
501 the manuscript.

502

503 **Competing Interests**

504 We have no competing interests.

505

506 **References**

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708

709 **Figure legends**

710 **Figure 1:** (A-D) Representative museum specimens of the four bird lineages examined  
711 in this study, the pittas (A), barbets (B), parakeets (C), and sandgrouse (D), from the  
712 collections of the Division of Birds, Smithsonian National Museum of Natural History,

713 Washington D.C. (E) Workflow of analyses. Using museum specimens from all four  
714 lineages (left; the regions of the body are demarcated by lines), we measured  
715 reflectance spectra (examples in centre), and analyzed them using theoretical models of  
716 avian color vision including Goldsmith's tetrahedron (right), where each vertex  
717 represents maximal relative excitation of one of the four cones (and therefore saturated  
718 colors). (F) Diagrammatic depiction of predictions. The color schemes here are  
719 illustrative, and are not meant to represent the real bird. We predict that plumage colors  
720 lie along a line representing putative complementary colors, which lie on opposite sides  
721 of the achromatic point on this line. We additionally predict that each species  
722 incorporates complementary colors for maximum contrast, and hypothesize that signal  
723 diversity evolves by redistributing a complementary color pair over the body.

724

725 **Figure 2:** Color space occupancy and analyses of signal variation in pittas (A,B),  
726 barbets (C,D), parakeets (E,F) and sandgrouse (G,H). Left-hand side figures represent  
727 color space occupied by each family (1 point/color patch measured), as visualized using  
728 Goldsmith's tetrahedron. Each vertex represents relative photon catch of a particular  
729 cone (see Figure 1). Right hand side plots represent the same data points transformed  
730 into a three-dimensional XYZ color space using a noise-limited model of avian  
731 tetrachromatic vision. The black lines through the points represent the first major axis  
732 (PC1) of chromatic variation.

733

734 **Figure 3:** Linear axes of plumage variation represent complementary colors. Shown  
735 here are histogram distributions of maximum (red) and minimum (blue) color scores (the  
736 maximum and minimum phi-coordinate in radians) of each species within four bird  
737 lineages, the pittas (A), Asian barbets (B), Afro-Asiatic parakeets (C), and sandgrouse

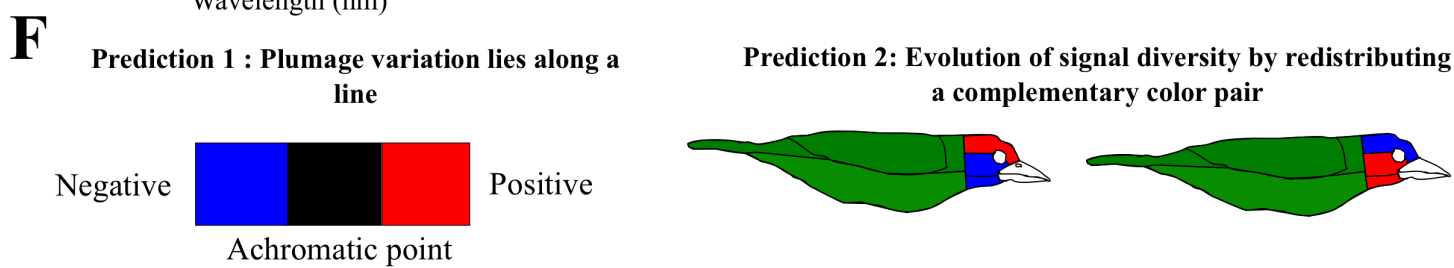
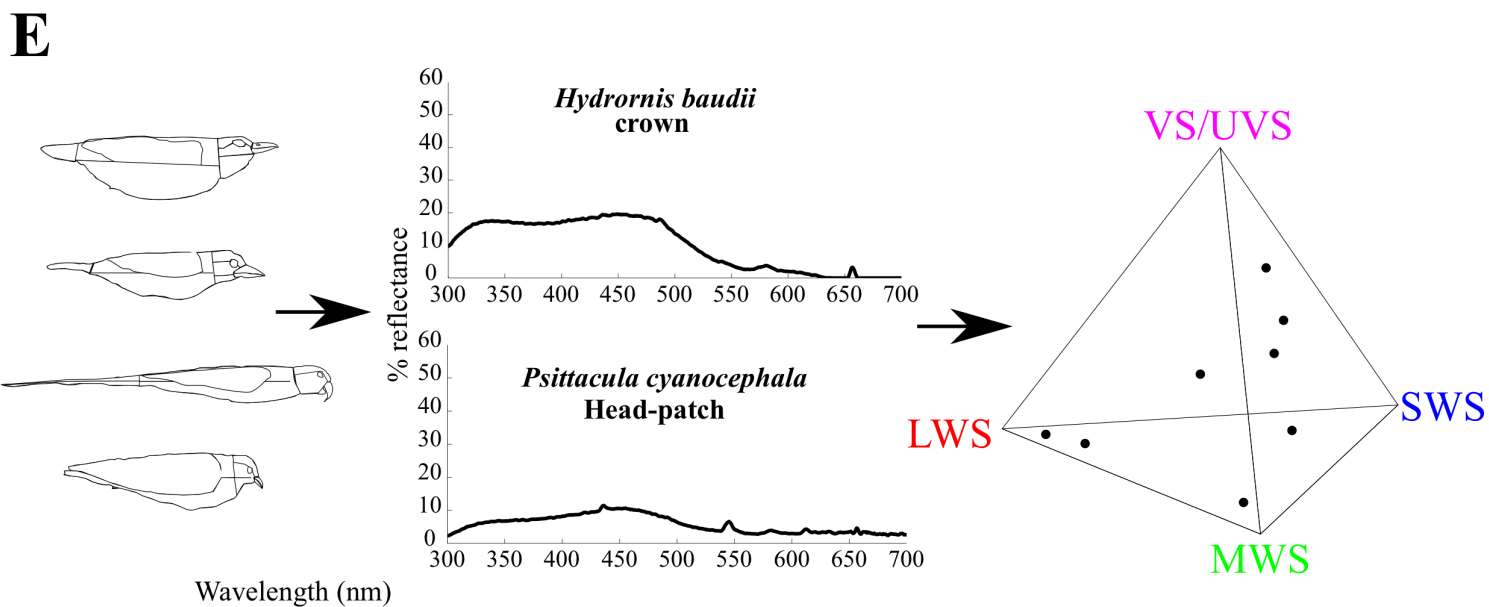
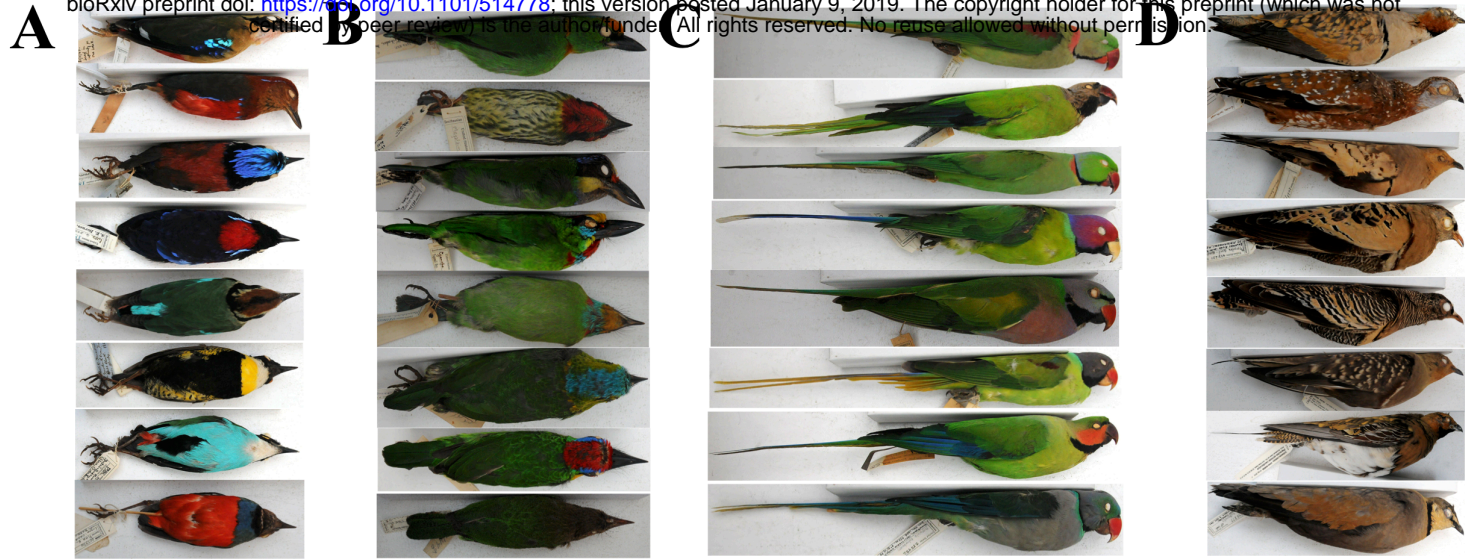


<b>Pittas (<math>\lambda</math>)</b>	<b>0.88</b>	0.00006	0.00006	0.25	<b>0.71</b>	0.38	0.00006	
<b>Mean</b>	0.03	0.02	0.10	0.06	0.09	0.03	0.07	
<b>CV(%)</b>	113.93	156.62	79.46	101.64	84.59	117.61	61.69	
<b>Asian Barbets (<math>\lambda</math>)</b>	<b>0.88</b>	<b>0.38</b>	<b>0.68</b>	<b>0.92</b>	0.00006	0.00006	<b>0.92</b>	
<b>Mean</b>	0.03	0.05	0.06	0.01	0.01	0.01	0.08	
<b>CV(%)</b>	76.28	63.50	54.78	60.56	69.97	63.53	62.25	
<b>Afro-Asiatic Parakeets (<math>\lambda</math>)</b>	0.00006	0.13		0.00006	0.087	0.00006	0.00006	0.00006
<b>Mean</b>	0.11	0.08		0.09	0.04	0.08	0.11	0.13
<b>CV(%)</b>	38.89	60.08		65.06	43.77	48.45	40.64	36.59
<b>Sandgrouse (<math>\lambda</math>)</b>	0.21	0.00007	0.00005	0.39	0.82	0.00007	0.12	
<b>Mean</b>	0.07	0.13	0.12	0.03	0.06	0.06	0.06	
<b>CV(%)</b>	69.08	56.07	65.18	123.17	83.93	115.84	93.06	

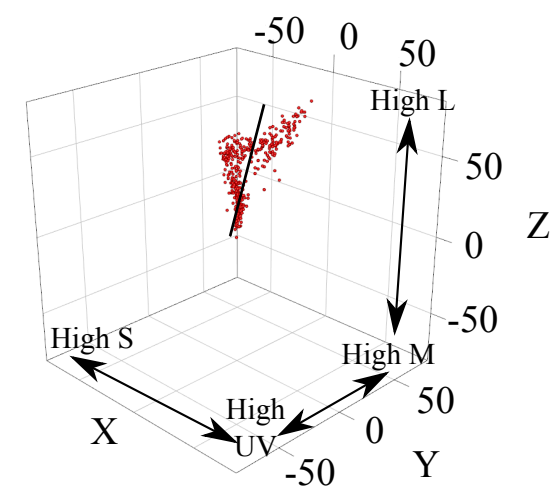
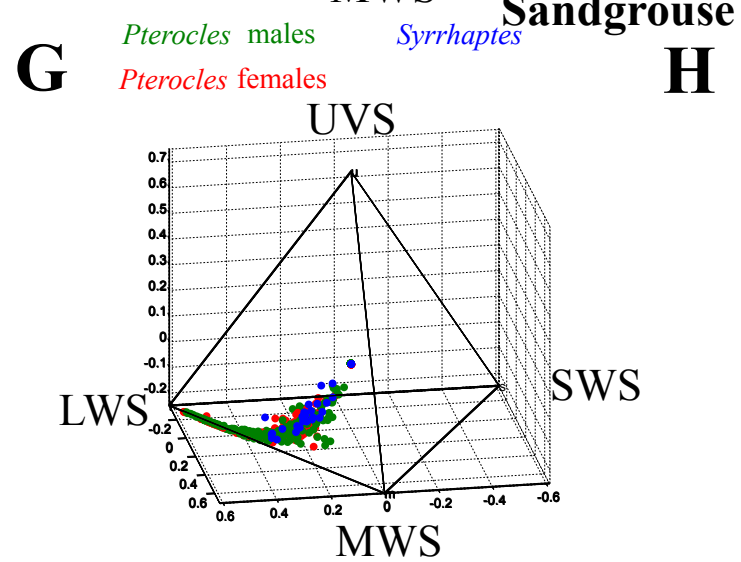
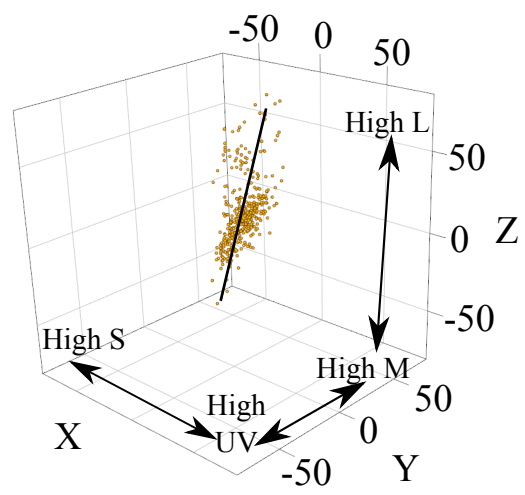
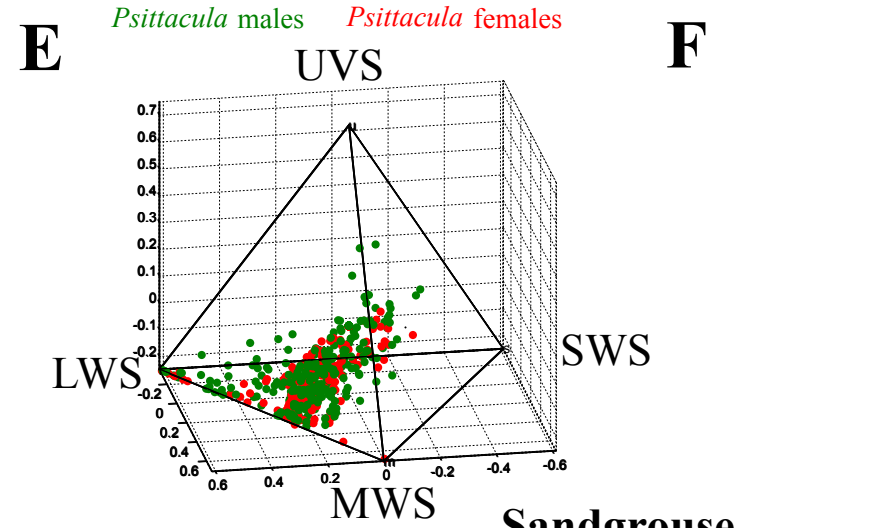
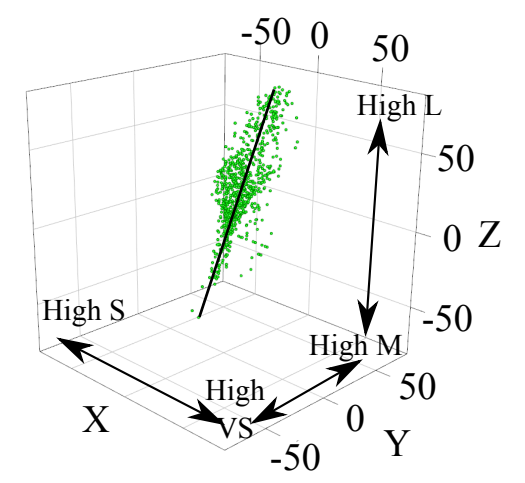
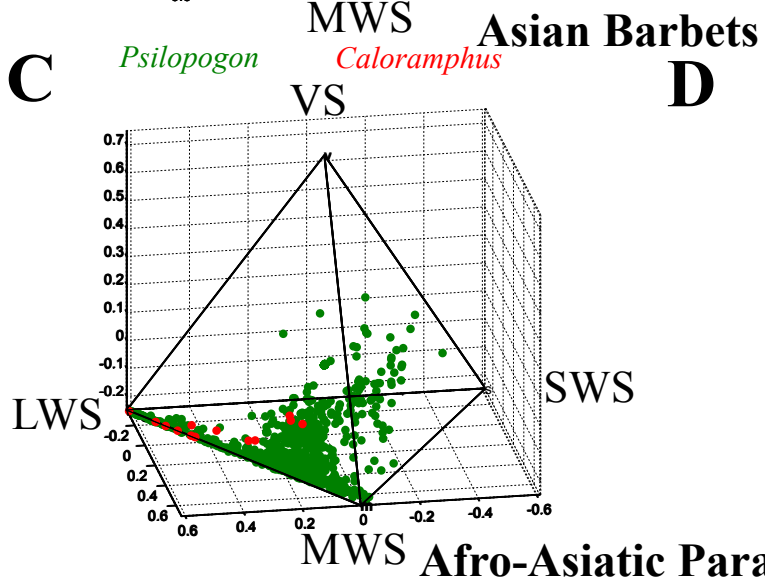
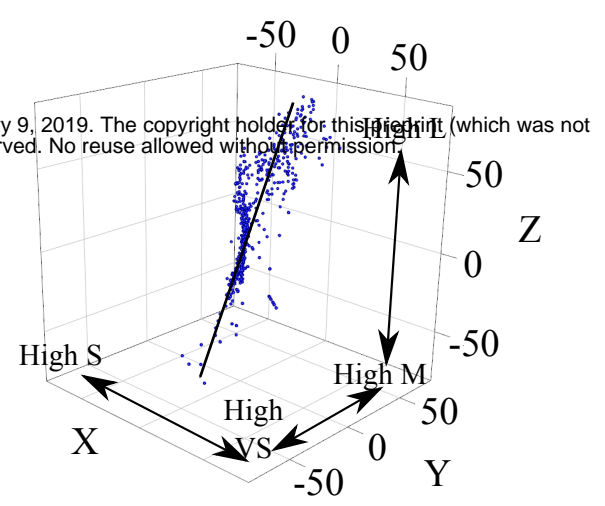
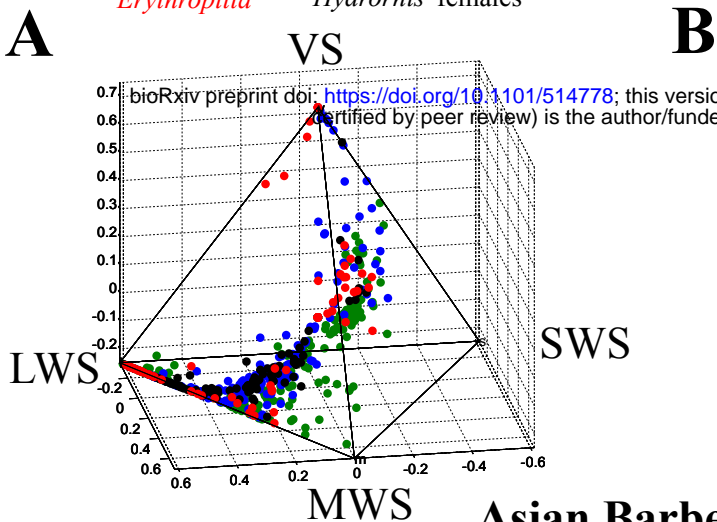
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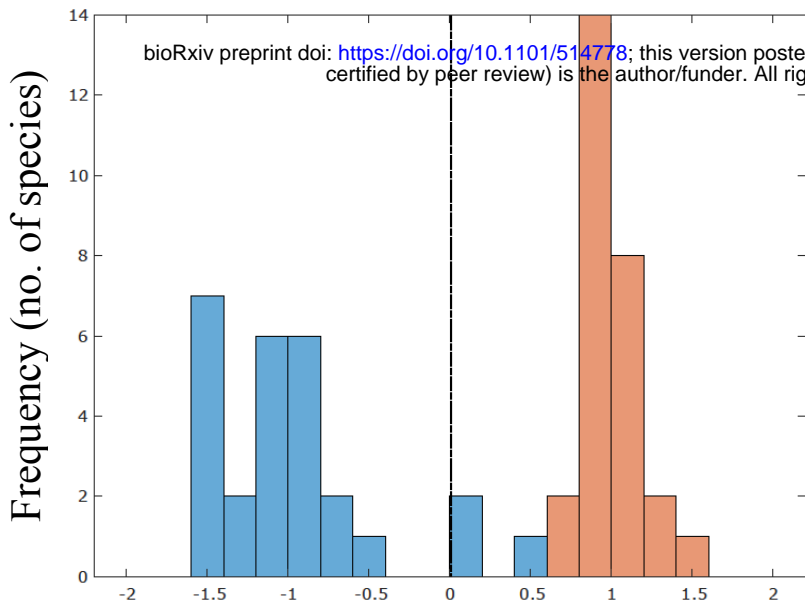
748 **Table 1:** Pagel's  $\lambda$ , a measure of phylogenetic signal (i.e. fit to a Brownian motion model  
749 of trait evolution) for different body regions for each of the four bird lineages. Values  
750 given represent  $\lambda$  for color scores (top) and luminance below. Values that are in bold are  
751 significant at a P-value of 0.05 (P-value obtained by comparison to 1000 randomized  
752 phylogenetic trees for each region). For each family, below the  $\lambda$  value are two rows  
753 indicating the means and coefficient of variation (as a percentage) for each body region  
754 for both color and luminance scores.



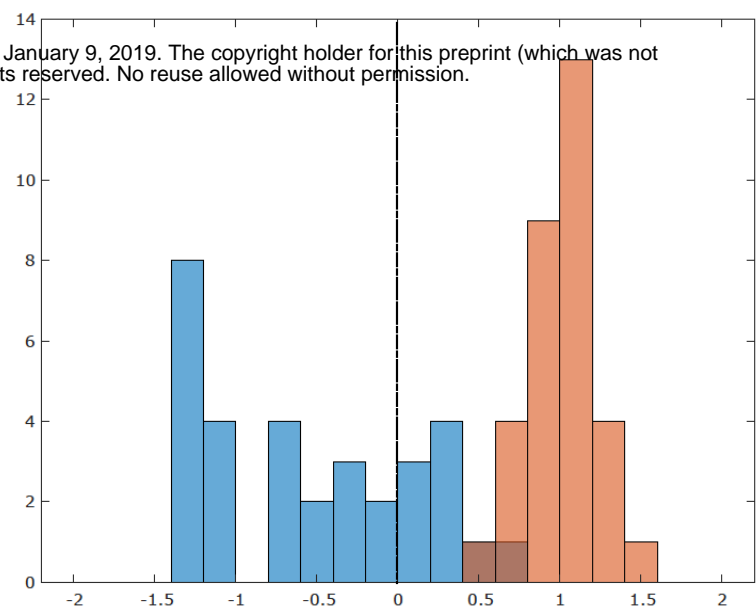
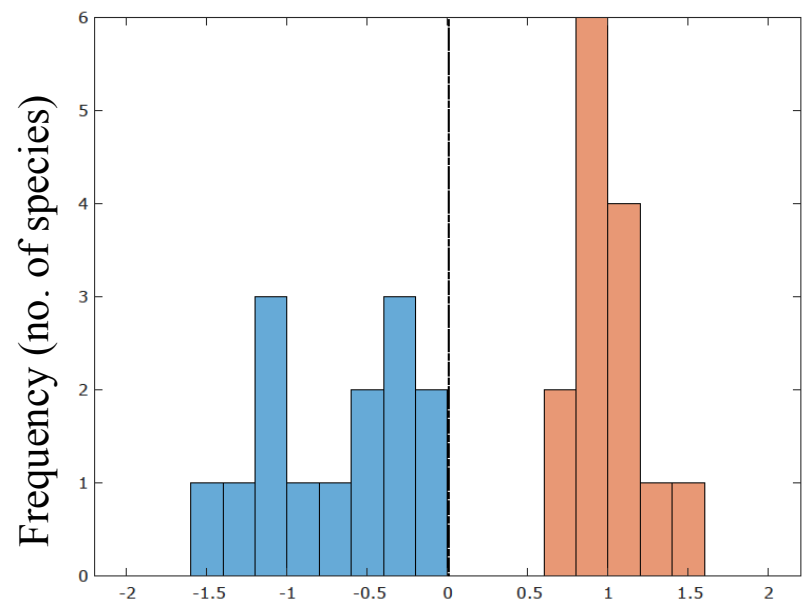
*Pitta* *Hydrornis* males **Pittas**  
*Erythropitta* *Hydrornis* females



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**A****Pittas**

Phi coordinate of color patches (radians)

**B****Asian Barbets****C****Afro-Asiatic Parakeets**

Phi coordinate of color patches (radians)

**D****Sandgrouse**