Latitudinal gradients in avian colourfulness

Authors: Christopher R. Cooney[1]*, Yichen He[1], Zoë K. Varley[1,6], Lara O. Nouri[1], Christopher J. A. Moody[1], Michael D. Jardine[1], András Liker[2,3], Tamás Székely[4,5], Gavin H. Thomas[1,6]

Affiliations:

[1] Department of Animal and Plant Sciences, University of Sheffield; Alfred Denny Building, University of Sheffield, Western Bank, Sheffield S10 2TN, UK.

[2] MTA-PE Evolutionary Ecology Research Group, University of Pannonia, Veszprém H-8210, Hungary

[3] Behavioral Ecology Research Group, Center for Natural Sciences, University of Pannonia, Veszprém H-8210, Hungary

[4] Milner Centre for Evolution, Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, United Kingdom

[5] *Department of Evolutionary Zoology and Human Biology, University of Debrecen, Debrecen H-4032, Hungary*

[6] Bird Group, Department of Life Sciences, The Natural History Museum at Tring; Akeman Street, Tring, HP23 6AP, UK.

*Corresponding author. Email: c.cooney@sheffield.ac.uk

It has long been suggested that tropical species are generally more colourful than temperate species, but whether latitudinal gradients in organismal colourfulness exist remains controversial. Here, we quantify global latitudinal trends in colourfulness (i.e. within-individual colour diversity) by analysing a novel photographic dataset of whole-body plumage reflectance information for >4,500 species of passerine birds. We show that tropical passerine species are generally more colourful than their temperate counterparts, both on average and in the extreme, and that these patterns are consistent for males and females. We also show that these geographic gradients can be explained in part by the effect of several latitude-related factors related to classic hypotheses for climatic and ecological determinants of organismal colourfulness. Taken together, our results reveal that species' colourfulness peaks in the tropics for passerine birds, confirming the existence of a long-suspected yet hitherto elusive trend in the distribution of global biodiversity.

Is life generally more colourful in the tropics? The possible existence of global-scale trends in organismal colourfulness was first suggested by 19th century European naturalists such as von Humboldt, Darwin and Wallace, who upon being afforded the opportunity to travel extensively in the tropics, remarked on the 'rich variety' and 'mixtures of colors' they encountered during their travels[1-3]. Since then, a variety of explanations focused on latitude-associated gradients in biotic and abiotic factors have been proposed to account for the assumed increases in tropical species' colourfulness, including positive effects of more benign climatic conditions and particular ecological strategies that are more prevalent at low latitudes[2,4-8]. However, in the centuries following these early anecdotal observations, biologists have struggled to conclusively test for the existence of global-scale latitudinal gradients in species colourfulness, calling into question whether this long-assumed biogeographic 'rule' really exists at all[5,7,9,10].

Part of the challenge in resolving this controversy revolves around the difficulty of obtaining accurate, meaningful measurements of organismal colouration, and doing so on a scale that permits a global-scale test of these ideas. Due to practical constraints, previous studies have been limited to addressing this question using subjective and/or incomplete measures of colourfulness (e.g. human scoring) or else by studying radiations of species that span only a limited fraction of the Earth's latitudinal gradient[5-12] (see Supplementary Table 1). While some of these studies have found patterns consistent with a latitudinal colourfulness gradient in birds and other taxa, other studies have found no such effect, and an explicit, broad-scale test of the latitudinal colourfulness hypothesis is currently lacking. Fortunately, recent advances in cost-effective imaging technology, combined with the increasing availability of accurate geographic information for many taxa, now make it possible to test for the existence of latitudinal gradients in species' colourfulness on a truly global scale.

We tested whether a latitudinal gradient in species' colourfulness exists for the global radiation of passerine birds (Order: Passeriformes)—the largest avian order comprising ~60% of the ~10,000 bird species. Our approach is centred around a novel dataset of plumage colouration based on >140,000 calibrated visible and ultraviolet light photographs of male and female museum specimens for 4,527 species (~76% of passerine diversity; Supplementary Fig. 1a,b). For each included specimen, we took photographs from three different angles (dorsal, lateral, ventral), extracted calibrated pixel values from each image using machine learning approaches, and mapped these into avian tetrahedral colour space (see Methods). We then sampled 500 points from each view to give a total of 1,500 measurements capturing whole-body plumage reflectance for each specimen (Supplementary Fig. 1c). This process resulted in a dataset consisting of >24,000 photographed specimens and >36 million unique measurements of passerine plumage colouration (Fig. 1a), which together occupy a colour space that is comparable to that estimated for all birds[13].

Colourfulness can be considered in multiple ways. Here we follow the sensory ecology literature by defining colourfulness in terms of within-individual colour diversity; that is, the overall colour contrast of a multi-coloured pattern[14]. Defined in this way, colourfulness arises when an organism displays a range of colours (potentially produced by different colour producing mechanisms) that are perceptually different from one another, and can be quantified using metrics that measure the spread of colour traits in colour space[14]. Importantly, this characterisation of colourfulness is distinct from other notions of colourfulness that include uniform plumage colouration of a particular (often conspicuous) hue and also differs from approaches used by other broad-scale bird colouration studies, which have variously quantified plumage colouration in terms of brightness and hue[12] and degree of elaboration (e.g. the 'maleness' metric introduced by Dale et al.)[11], rather than colourfulness (i.e. colour diversity) *per se*. Here we use two established metrics of colourfulness: convex hull volume[13,15] and the number of occupied colour loci[16]. The former reliably captures the breadth of colours in a sample but is heavily influenced by extreme values, whereas the latter reflects that species will generally not occupy all areas of colour space within the extent of occurrence (Fig. 1b). Values of the two metrics are strongly correlated across specimens in our dataset (r = 0.85, P < 0.001, n = 24,345; Supplementary Figure 1d) but because the colour loci metric is generally less sensitive to noise, outliers and large 'gaps' in colour space occupation that can bias estimates of colourfulness[14,16] (Fig. 1b), we focus in the main text on results based on colour loci scores, with colour volume results provided as Supplementary Information.

We separately mapped mean male and mean female colourfulness scores for grid cell assemblages and find evidence for a strong latitudinal gradient in species' colourfulness in passerine birds (Fig. 2). Analysis of per-cell mean species' colour loci values revealed a pronounced tropical peak in species' colourfulness with respect to latitude that was evident for both male and female birds (Fig. 2a,b). For example, mean male and mean

female colour loci scores for species in tropical cell assemblages are 92 and 86, respectively (<23.5^{o}; n = 16,997 cells), compared to corresponding values of 76 and 70 for high-latitude assemblages (>45^{o}; n = 22,412 cells). Similar patterns were evident when using colour volume scores (Supplementary Fig. 2) and when cell averages are calculated by downweighting the influence of geographically widespread species (Supplementary Fig. 3; for methods see 'Statistical analyses'). To formally test the relationship between latitudinal position and species' colourfulness while minimising the impact of spatial autocorrelation, we calculated mean colourfulness scores across species present in unique terrestrial ecoregions[17] rather than individual grid cells (Fig. 2c) and used spatial simultaneous autoregressive (SAR) models with absolute ecoregion latitude as a predictor[18]. Regardless of how ecoregion colourfulness averages are calculated (see Methods), all models had a highly significant effect of latitude on both male and female colourfulness (P < 0.001 in all cases; Supplementary Table 2) corresponding to steep declines in the average colourfulness of species within ecoregions moving from the equator towards the poles (Fig. 2c and Supplementary Fig. 2c).

One common assertation is that equatorial regions may be perceived as being more colourful simply because they contain more species overall[2,9,10]. In other words, even if the proportion of colourful species per assemblage is approximately constant across latitudes, tropical communities are regarded as being more colourful simply because they contain a greater absolute number of colourful species. Although our analyses based on average colourfulness scores per assemblage suggest this is unlikely, we addressed this assertion directly by analysing the geographical distribution of the top 25% most colourful species in our dataset as a proportion of assemblage species richness. These analyses confirm that not only are tropical passerine taxa generally more colourful than temperate-zone taxa, but that the tropical zones also harbour a substantially higher than expected proportion of the world's most colourful passerine bird species (Supplementary Fig. 4 and Supplementary Table 2).

To corroborate our grid cell-based results, we also tested the relationship between species' colourfulness and midpoint latitude using species-level phylogenetic comparative analyses (see Methods). Consistent with our previous findings, equatorial-zone species (midpoint latitude <23.5^{o}) are generally characterised by higher colourfulness scores than extra-tropical species (Fig. 3a) and as expected, there is a strong relationship between midpoint latitude and colourfulness across species for both sexes (Fig. 3b,c). However, an important consideration is that both midpoint latitude and the degree of male and female colourfulness exhibit considerable phylogenetic conservatism, with mean phylogenetic heritability[19] values of 0.83 [95% credible interval (CI): 0.80, 0.86] for latitude and 0.90 (95% CI: 0.87, 0.91) and 0.88 (95% CI: 0.86, 0.90) for male and female colourfulness, respectively. It is therefore possible that the latitudinal gradient in colourfulness we observe is the result of phylogenetic non-independence between tropical residency and elevated colourfulness—for example, if

the ancestors of speciose tropically-restricted passerine clades happened to be colourful, and both traits have simply been retained by descendent lineages over evolutionary time[2]. However, testing the relationship between colourfulness and species' absolute latitudinal position while controlling for phylogenetic history, we find significant negative correlations with latitude for both male [standardised slope coefficient: -0.04 (95% CI: -0.07, -0.02)] and female colourfulness scores [-0.12 (95% CI: -0.14, -0.09)] (Supplementary Table 3). This indicates that the observed gradient in species' colourfulness cannot be explained solely by phylogenetic conservatism of both latitudinal position and degree of colourfulness.

Our finding of a clear latitudinal increase in passerine bird colourfulness towards the equator is in line with related findings of other broad scale studies of avian colouration[11,12] which together support the notion that tropical zone species tend to be generally more colourful than those in the temperate zone[1-3]. Although unambiguous empirical support for this belief has so far remained elusive (see Supplementary Table 1), several explanations for tropical peaks in species' colourfulness have nonetheless been proposed. These explanations broadly fall into hypotheses focused on latitudinal variation in climatic conditions, (e.g. energy, temperature, precipitation, or productivity), species' behavioural and/or ecological traits, or biotic interactions (particularly inter-specific competition and signalling). For example, early explanations focused on 'the direct action of heat and light from the sun'[2] in promoting tropical colourfulness, but the importance of climatic factors such as temperature, precipitation and solar radiation have been hotly debated[2,3,5,20]. Another broad class of hypotheses emphasises the role of species' ecological and behavioural traits in promoting colourfulness. This includes dark, closed habitat types (e.g. forests) selecting for increased reflectivity[21,22] and the positive effects of particular foraging (e.g. frugivorous, nectarivorous) and life-history strategies (e.g. sedentary, territorial breeding) that are common in the tropics and may deterministically promote the evolution of colourful plumages[2,11]. Furthermore, the strength of biotic interactions has long been argued to increase towards the equator[23,24], due in part to greater numbers of co-existing species in tropical systems[25]. In theory, elevated levels of colourfulness in tropical taxa may also emerge as a response to increased selection for more distinguishable visual signals for recognising conspecifics in diverse tropical communities[2,5,6].

To explore the factors promoting passerine colourfulness and to illuminate potential explanations for the latitudinal gradients in colourfulness we observe, we used multi-predictor Bayesian phylogenetic mixed models[26] to assess the relative importance of variables capturing relevant environmental and ecological axes of variation among species (n = 4,415) (see Methods). Importantly, the majority of these predictor variables (11 of 13) were significantly correlated with species' midpoint latitude ($P < 0.001$ in all cases; for correlation coefficients see Fig. 4), indicating that they indeed represent viable explanations for the observed latitudinal gradients. The only exceptions to this were species' mean body mass (P = 0.535) and degree of sexual dichromatism (P =

0.802) – the latter representing a useful proxy for sexual selection acting on visual signalling traits[27,28].

We find that species' colourfulness is significantly predicted by several factors (Fig. 4 and Supplementary Tables 4 and 5). Across all analyses, the strongest correlate of colourfulness we identified is sexual dichromatism: males of highly dichromatic species are far more colourful on average than males of less dichromatic species. This supports the view that bright male colouration often evolves in response to increases in sexual selection intensity[12,29]. The lack of a similar, or negative, effect in females also implies that dichromatism primarily indexes the intensity of sexual selection acting on males[30] and that variation in female colourfulness across species cannot be explained simply as a correlated response of selection acting on males[12,31].

In addition to dichromatism, we also find a strong negative effect of body mass on colourfulness in both sexes, with larger birds being less colourful than smaller birds. Body size has been proposed as an important constraint for the evolution of colourful plumage, due to physiological limits on both the relative number of body feathers and circulating carotenoid levels in larger birds[32]. This hypothesis has received little prior support, particularly considering that other broad-scale bird studies have found positive rather than negative effects of body size on axes of plumage colour elaboration[11,12]. However, these results are difficult to compare due to differences in the taxonomic scope and metrics of colouration used among studies (see Supplementary Table 1). Here, using colourfulness metrics that are closely aligned with the concept of 'plumage colour heterogeneity' forming the basis of the original hypothesis[32], we find a strong inverse relationship between body size and colourfulness across passerine species. This negative relationship is therefore consistent with large birds experiencing greater physiological constraints on colourfulness than smaller birds[32], and argues against the idea that increased predation risks associated with being small strongly constrain the evolution of plumage colourfulness[11].

While these associations provide insight in the factors contributing to variation in passerine colourfulness (i.e. Fig 3a), they are unable to account for a tropical colourfulness peak as neither dichromatism nor body mass is correlated with latitude. However, our analyses also identified significant effects of several latitude-related climatic and ecological variables that evidently contribute to generating latitudinal gradients in passerine colourfulness (Fig. 4 and Supplementary Tables 4 and 5). First, it has long been suggested that more benign environmental conditions promote elevated colourfulness in the tropics, due to lower evolutionary constraints on elaborate plumage colouration imposed by the types of harsh environmental conditions often found towards the poles and in deserts[2]. In support of this, we find that male and female colourfulness scores are consistently and positively associated with precipitation and net primary productivity (NPP), such that species are on average more colourful in wetter, more productive areas. Second, we also find that species' occupying closed (i.e. forested)

habitat types and foraging niches associated with a high degree of resource defence and carotenoid intake (i.e. frugivores and nectarivores) generally have increased levels of colourfulness, supporting hypotheses linking signalling conditions[21,22,33] and dietary factors[2,34] to interspecific differences in colourfulness. Third, a strong and consistent positive association between colourfulness and community diversity (i.e. the average number of co-occurring passerine species) supports the suggestion that latitudinal gradients in species' colourfulness emerge at least in part due to selection on both sexes for accurate conspecific recognition in species-rich tropical communities[2,5,6,9]. Finally, for female birds we find a strong negative effect of migration on colourfulness that is absent in males. An association between migration and reduced female colourfulness across passerines generalises earlier findings[35] (though see [12]) and reinforces the idea that in migratory passerine taxa at least, changes in selection acting on females may play an important role in generating sex-differences in colouration (cf. above)[2,34]. As many high-latitude breeding passerines are migratory, this female-specific reduction in colourfulness in migratory taxa may also help to explain a general pattern emerging from our analysis: that latitudinal gradients in colourfulness tend to be more pronounced in females than in males (e.g. Fig. 3b; Supplementary Table 6).

Together, our results support the existence of a strong latitudinal gradient in species' colourfulness for passerine birds. This gradient exists for both male and female colouration and is consistent across major tropical realms. We demonstrate that this pronounced tropical-zone peak in colourfulness can be explained, in part, by latitude-associated gradients in climatic conditions and species' ecological traits that facilitate the evolution of increased colour diversity of tropical passerine species. However, we note that many potentially important factors remain to be investigated, including latitudinal gradients in predation pressure[24] and the intensity of social selection[31], particularly acting on females. More broadly we note that conceptions of the latitudinal colourfulness gradient are not limited to the plumages of birds, with early naturalists such as Alexander von Humboldt remarking on the apparent colourfulness of many tropical taxa, including plants, insects, fish and 'even crayfish'[36]. Thus, while our results provide clear support for broad-scale latitudinal gradients in colourfulness for passerine birds, the extent to which other global radiations follow the 'rule' that life in the tropics is generally more colourful than in the temperate zones remains to be seen.

Methods

Specimen selection. We based our data collection on the taxonomic framework of Jetz *et al.* [37], which currently represents the only integrated species-level taxonomic and phylogenetic dataset for all (passerine) birds. We collected data on plumage colouration using study skins housed at the Natural History Museum, Tring, UK. We focused on species with representatives of both sexes and for which geographic range data were available (see below). Where possible, we sampled specimens of three males and three females, taking care to select only mature individuals in breeding plumage with no

obvious signs of moult. Based on these criteria, we were able to sample specimens of both sexes for 4,527 (76%) of the 5,966 taxa represented in the Jetz *et al.* taxonomy, with a mean sampling of 2.77 male and 2.61 female specimens per species and 24,345 specimens in total.

Digital photography. Whole-specimen plumage colouration was measured using calibrated ultraviolet (UV) and visible (Vis) light photography[27]. To do this we used a modified Nikon D7000 digital single-lens reflex camera with a Nikon 105mm f/4.5 UV Nikkor lens combined with two Baader photographic lens filters: one permitting human visible wavelengths (400–680 nm; Baader UV/IR Cut filter / L filter) and another permitting UV wavelengths (320–380 nm; Baader U-Venus-Filter). Specimens were illuminated using two Bronocolor Pulso G 1600 J lamps (with UV filters removed) connected to a single Broncolor Scoro 1600 S Power Pack. The same camera settings were used for all photographs (1/250 sec, f/16.0, ISO 100, 'Daylight' white balance, RAW photo format), with the exception that a higher ISO sensitivity (2000) was used for UV images to achieve correct exposure. Each image also contained five Labsphere Spectralon Diffuse Reflectance standards of known relative reflectance (2%, 40%, 60%, 80% and 99%). All specimens were photographed through each filter (UV, Vis) from three different angles (dorsal, lateral, ventral), resulting in six images per specimen. Therefore our digital photography dataset of consisted of 6 x 24,345 = 146,070 images.

Image segmentation using deep learning. To facilitate the extraction of pixel information from our photography dataset, we applied an automated image segmentation protocol based on convolutional neural networks. Full details of this approach and a comprehensive analysis of its performance, particularly in relation to other methods, can be found in He *et al.* [38]. A brief account is also provided below.

We used DeepLabv3+, which is a deep convolutional neural network architecture based on fully convolutional networks[39], to build a pixel-wise semantic segmentation network capable of accurately identifying specimen pixels in each of our images. DeepLabv3+ has been shown to outperform both classical computer vision techniques (e.g. thresholding) and other neural network architectures for sematic segmentation on benchmark tasks[39]. It has also previously been shown to perform well when applied to a similar task as ours, involving the segmentation of biological specimens from photographic images of herbarium specimens[40].

To generate a dataset of expert labelled images for use in network training and evaluation, we placed polygons on a diverse sample of bird specimen images using Project Plumage (www.projectplumage.org), an online citizen science project with bespoke image labelling protocols. These polygons were used to manually identify plumage areas and to exclude non-plumage areas obscuring the specimen, such as eye holes, feet, specimen labels and string. The images labelled as part of developing this protocol form part of a broader effort to measure avian colouration. As such, across the three views (dorsal, lateral ventral) a total of 5,094 images were labelled for 1,698

species distributed across the entire avian radiation (i.e. passerines and non-passerines), encompassing representatives of more than 81% of all bird genera and 27 bird orders.

We used this 5,094-image dataset to train and validate the network and then to generate specimen segmentation predictions for each of the >140,000 images in our dataset[38], which took ~72 hours to complete on a desktop computer. Finally, each of the resulting image masks was individually checked by eye and manually refined where necessary using bespoke software (https://github.com/EchanHe/PhenoLearn).

Image processing. All raw (.NEF) images of specimens were linearised and exported as linear TIFF files using DCRAW[41]. Pixel values were then normalised using mean pixel intensity values from the five grey standards included in each image in order to control for variation in lighting conditions, following established approaches[27,42]. Finally, the image was segmented using the image masks described above to leave only pixel values corresponding to the specimen in each image. As individual pixel measurements can be noisy—and because different specimens were represented by different numbers of pixels in our raw dataset—we downsampled each specimen image to a comparable resolution (see Supplementary Fig. 1c), prior to calculating cone catch values and colourfulness metrics (see below). To do this, we treated each specimen image as a raster and, using the aggregate() function in the R package 'raster' (version 3.4-5)[43], we found the smallest aggregation factor in the range 100 to 1 that resulted in at least 500 aggregated cells (pixels) being returned, with aggregated cell values calculated as the mean of relevant neighbouring values. We then randomly sampled 500 measurements from this aggregated dataset to represent the plumage colouration of that specimen view in all further analyses. Samples from the each of the three specimen views (dorsal, lateral, ventral) were pooled to give a final set of 1,500 whole-body plumage colour measurements per specimen.

As well as helping to reduce the impact of measurement noise on our colour measurements, another benefit of this procedure of downsampling each image to a comparable resolution is that it ensures that a comparable proportion of the surface of each specimen view is sampled when taking a sample of a set number of measurements. For example, taking a random sample of 500 pixels from raw specimen datasets represented by 1,000 and 5,000 pixel values, respectively, would result in 50% coverage of the former and only 10% coverage of the latter. However, by first aggregating pixel values to an approximately equal resolution—in our case, to a resolution resulting in approximately, but no less than, 500 points per view—then subsequent random sampling of 500 values for each specimen view results in approximately equal coverage (~100%) in each case.

Visual modelling. We used established methods[42] to generate mapping functions to convert sampled specimen RGB pixel values into avian cone-catch values. This approach works by first estimating camera responses and visual system cone-catch

values to a library of natural spectra under a specific illuminate, and then uses multiple regression to create mapping functions for each receptor channel from the camera's responses. Using tools available in the IMAGEJ Multispectral Image Calibration and Analysis Toolbox (version 2.2)[42], we generated mapping functions for each photoreceptor using equations containing second-order polynomial terms and three-way interactions between channels. Note that this approach does not incorporate information on camera responses in the UV G channel due to typically low sensitivities of the G channel in the UV range[42]. We fit these equations to our data incorporating information on the estimated spectral sensitivities of our camera set up and the irradiance spectrum of our illuminant (i.e. flash units), both of which had estimated previously[27]. For modelling receptor responses, we assumed idealised illumination conditions[13,15] and receptor sensitivities corresponding either to an 'average' violet-sensitive (VS) or 'average' ultraviolet-sensitive (UVS) avian visual system, both extracted from the R package pavo (version 2.6.1)[44]. We used this information to generate mapping functions for each cone class, and the resulting models were all characterised by a high degree of mapping accuracy (R^2 values > 0.99). These mapping functions were then used to convert linearised and normalised image RGB values into cone catch values (*u*/*v*, *s*, *m*, *l*) for use in downstream analyses. We have previously shown that cone catch values generated by this photography-based approach are highly correlated (r > 0.92) with corresponding values calculated from spectrophotometric measurements[27].

Following previous studies, we represented chromatic (i.e. colour) variation among measurements using a standard avian colour space model in which raw cone catch values are converted to relative cone catch values and projected in a tetrahedron[13,15]. This tetrahedron—in which the luminance (i.e. achromatic) dimension is removed and each vertex represents one of the four cones characterising avian colour vision (i.e. *u*/*v*, *s*, *m*, *l*)—is the sensory equivalent of a morphospace, where similar colours fall in close proximity in the colour space and disparate colours are far apart[13,15,45]. As quantifying the colour of patches with very low overall reflectance can be problematic[46], pixels exhibiting a mean normalised reflectance value of <1% across all channels (uR, uB, vR, vG, vB) were re-cited to the achromatic centre.

Colourfulness metrics. We quantified colourfulness using two simple and intuitive metrics for quantifying variation in organismal colourfulness[14] (Fig. 1b). First, we calculated the volume of the minimum convex polygon containing all colour measurements for a given specimen, which represents the standard and most widely-used metric of (avian) colourfulness employed in the literature[13,15]. However, convex hull polygon volume can strongly depend on extreme values[14] and can generate overinflated volume estimates when highly disparate colours are separated by large areas of unoccupied colour space[47]. Therefore we also employed a second metric of colour space occupation that is less sensitive to these issues. This approach[16] is based on sub-dividing (rasterising) tetrahedral colour space into a series of equally-sized 3D cells termed 'colour loci'. This is done by defining two 2D grid systems in the XY and YZ

axes of colour space that, when intersected, define a 3D grid system covering the entirety of colour space. Each 3D cell (dimensions: 0.022 × 0.022 × 0.022) therefore represents a 'chromatic locus' and provides a way of partitioning the continuous variation in colour space into discrete units. The strength of this approach is that the colour diversity (i.e. colourfulness) of a particular set of measurements can then be assessed by simply counting the number of colour loci occupied [16], with these estimates being less impacted by outlier values and intermediate areas of unoccupied colour space.

We calculated estimates of convex hull volume and number of colour loci occupied for each specimen separately, and then calculated species-level values for each sex as the mean of $\log_{10}$-transformed specimen-level values.

Phylogenetic framework. To provide a phylogenetic framework for the species included in our analysis (*n* = 4,527), we downloaded 100 trees from the posterior distribution of complete trees produced by Jetz *et al.* [37] from http://www.birdtree.org, which were then pruned to generate a distribution of trees containing only the focal species set. All of our analyses incorporating phylogenetic information were run over this distribution of 100 trees to incorporate phylogenetic uncertainty into our parameter estimates. For plotting purposes, we identified a maximum clade credibility (MCC) tree from this posterior distribution of trees using the maxCladeCred() function in the R package 'phangorn' (version 2.5.5)[48].

Geographic data. We base our geographic analyses on the comprehensive dataset of bird species' geographic range maps produced by BirdLife International (http://datazone.birdlife.org/). We resolved taxonomic differences between the BirdLife and Jetz *et al.* datasets as far as possible, manually editing (i.e. combining or splitting) range maps for BirdLife taxa where necessary. We focused on species' breeding geographic ranges only (seasonality = 1 or 2) and regions where species are known to be native or reintroduced (origin = 1 or 2) and extant or probably extant (presence = 1 or 2). To map and test the predictors of species' colourfulness, we extracted polygon range maps onto an equal area grid (Behrmann projection) at 0.5° resolution (~50 km at the equator). Species' latitudinal midpoints were calculated as the mean latitude of occupied grid cells. The same projection and grid system was also used to extract range-wide values for species' environmental variables (see below).

Predictor variables. To test the role of factors hypothesised to influence passerine species' colourfulness and its possible co-variation with latitude, we collected data for 13 key environmental and ecological variables.

Global spatial information on temperature, precipitation, solar radiation, ultraviolet-B (UV-B) radiation and net primary productivity (NPP) were extracted from various sources and then reprojected and resampled to match the resolution of our range dataset. Annual mean temperature (bio1) and annual precipitation (bio12) data were

437 downloaded from the WorldClim (version 2.1) database[49] at 2.5 arc-minute resolution.
438 Monthly information on solar radiation was downloaded at 30 arc-second resolution and
439 monthly totals were summed to give a measure of total annual solar radiation.
440 Information on annual mean UV-B radiation was extracted from Beckmann *et al.* [50] at 15
441 arc-minute resolution. Information on NPP at 1 km was extracted from datasets
442 produced by Running *et al.* [51]. In all cases, species' values represent averages across
443 their geographic range.

444 Information on species-level ecological and behavioural traits were extracted from
445 several sources, specifically BirdLife International's Data Zone
446 (http://www.datazone.birdlife.org) (forest dependency) and Tobias and Pigot [52] (foraging
447 niche, migration, nest placement, territoriality, body mass). To reduce the complexity of
448 the categorical variables included in these datasets, and to facilitate effect size
449 comparison in our multipredictor models, we re-coded variation in forest dependency,
450 foraging niche, migration, nest placement, and territoriality as binary variables that
451 aligned with our hypotheses (see main text). Specifically, species were coded as forest
452 dependent ('low', 'medium' or 'high' dependency) or not ('does not usually occur in
453 forest'), frugivorous/nectarivorous or not (all other dietary niches), migratory ('migratory'
454 or 'partially migratory') or not ('sedentary'), ground nesting ('exposed ground') or not
455 ('cavity' or 'exposed elevated'), and territorial ('strong' or 'weak') or not ('none'). Sexual
456 dichromatism was scored from handbook plates as the mean value of plumage
457 dimorphism estimated from five body regions (head, back, belly, wings and tail) using
458 the following scheme: -2, the female was substantially brighter and/or more patterned
459 than the male; -1, the female was brighter and/or more patterned than the male; 0, there
460 was no sex difference in the body region or there was a difference but neither could be
461 considered brighter than the other; 1, the male was brighter and/or more patterned than
462 the female; 2, the male was substantially brighter and/or more patterned than the
463 female. Thus these scores are independent of the data used in this study to quantify
464 colourfulness and positive values represent male-biased ornamentation, zero represent
465 unbiased ornamentation, and negative values represent female-biased ornamentation.
466 To assess the effect of variation in community diversity on species' colourfulness, we
467 generated a variable capturing the average richness of passerine species co-occurring
468 with each species in our dataset. To do this we used range data for all passerine
469 species (i.e. not just those sampled in our dataset) to calculate for each grid cell the
470 number of co-occurring passerine species. We then calculated the mean value of this
471 variable across species' geographic ranges to provide a measure of average community
472 diversity for each species, analogous to the community diversity metric generated by
473 Dalrymple *et al.* [5]. Overall, we were able to collect complete data on these variables for
474 4,415 of the 4,527 species in our dataset.

475 Finally, social mating system has been shown to correlate with various aspects of avian
476 colouration, including dichromatism, brightness/hue and extent of elaboration[11,12,53]. To
477 assess the importance of social mating system relative to the factors outlined above, we

used available data from Dunn *et al.* [12] (*n* = 608 species) to run a parallel set of models including mating system as a factor alongside the other predictors. As above, mating system variation was re-coded as a binary variable contrasting mating systems associated with relatively low social polygyny rates ['monogamy' (*n* = 469), 'cooperative' (*n* = 79), 'polyandry' (*n* = 1)] versus those with higher rates ['mostly polygyny' (*n* = 31), 'lekking or promiscuous' (n = 28)]. Based on this dataset, we found no evidence that variation in social mating system correlates with male or female colourfulness (Supplementary Table 7). We explored the sensitivity of these findings using alternative classification strategies (e.g. monogamy yes/no, cooperative yes/no) but results were similar in all cases and so only results based on the classification scheme outlined above (and on UVS colour scores) are presented. The lack of a clear effect of social mating system on colourfulness using this dataset suggests that mating system variation across species cannot account for any of the patterns we report in our main analysis, particularly the effect of dichromatism, which remained significant even in this reduced dataset (Supplementary Table 7).

Statistical analyses. *Grid-cell based analyses.* We calculated mean sex-specific colourfulness scores (volume, loci) for local assemblages of passerine species that are presumed to occur together at the scale of 50 × 50 km grid cells. We calculated the mean colourfulness score for individual grid cells in two different ways. First, we simply calculated the mean (log_{10}-transformed) colourfulness score for all species present in a particular cell (Fig. 2). Next, to reduce the impact of spatial and taxonomic pseudoreplication across cells, we followed previous studies[18,37] by calculating weighted (arithmetic) means of colourfulness scores to reduce the contribution of geographically widespread taxa to the overall mean of a given cell. Weights for each species were calculated as the inverse of the number of grid cells in which the species was found (i.e. their range size)[18,37].

To formally assess the relationship between latitude and assemblage-level colourfulness, we followed the approach of Rabosky *et al.* [18] by testing this relationship at the scale of ecoregions rather than individual grid cells. We chose to do this to reduce the computation burden of analysing the full 59,102 grid cell dataset and, more importantly, to minimise levels of autocorrelation between assemblages, which is far higher between adjacent grid cells than between adjacent ecoregions[18]. We therefore calculated mean colourfulness scores for all cells within terrestrial ecoregions of the world[17] containing passerine species in our dataset (*n* = 800) and related this to the absolute latitude of ecoregions' centroid position. To account for spatial autocorrelation between ecoregions, we used simultaneous autoregressive error (SAR) models implemented using the function spautolm() in the R package 'spdep' (version 1.1-5)[54]. For these models, neighbours were defined as those ecoregions with contiguous boundaries and we then selected the appropriate weighting style using Akaike information criterion (AIC) model selection based on code provided by Rabosky *et al.* [18]. We used Moran's *I* to test for spatial autocorrelation in the residuals of SAR regressions

to determine the extent to which SAR models successfully accounted for spatial non-independence in the data. These results showed that all models retained some evidence of residual spatial autocorrelation, but to lesser degree in models based on richness-corrected ecoregion colourfulness scores (see below) than raw ecoregion scores (Supplementary Table 1).

An important consideration when analysing aggregated species-level variables in a spatial context is that underlying species richness gradients can generate strong spatial patterns in aggregated data[55]. To address the extent to which the latitudinal colourfulness gradients we observe in our spatial analyses are independent of underlying species richness differences, we used a randomisation approach to calculate colourfulness standardised effect size (SES) values for each ecoregion, which corrects for the effect of species richness differences on aggregated trait scores[55]. To do this we generated 200 null communities for each ecoregion by randomising species' colourfulness scores with respect to species' identity across our dataset. These null communities were then used to generate a null distribution of mean colourfulness scores for each ecoregion, against which observed colourfulness scores were compared. The resulting SES scores, in which the effects of species richness on mean colourfulness values have been factored out, were then analysed using the same SAR modelling approach described above.

Species-level analyses. To test the relationship between species' absolute midpoint latitude and colourfulness, and between species' colourfulness scores and variation in the 13 predictor variables described above, we used multi-predictor Bayesian phylogenetic mixed models implemented in the R package 'MCMCglmm'[26,56]. All models included a phylogenetic random effects term and were run over a posterior distribution of 100 trees to incorporate phylogenetic uncertainty and posterior distributions of parameter estimates associated with different trees were pooled to give model estimates that incorporate phylogenetic error[57]. In all cases, models were run for 55,000 iterations (sampled every 25th iteration) with a 5,000 iteration burn-in, and we used standard non-informative priors [i.e. list(R=list(V=1, nu=0.002), G=list(G1=list(V=1, nu=0.002)))]. All variables were standardised (mean = 0, standard deviation = 1) prior to model fitting to facilitate effect size comparison. Before running models we also checked for evidence of multi-collinearity among predictors in our multi-predictor models using variance inflation factors (VIFs) and found no evidence of severe (VIF > 10) or even moderate (VIF > 4) multi-collinearity in our models (median VIF = 1.60; range = 1.05 – 3.89).

Finally, phylogenetic heritability (H^2) values[19] were estimated by fitting intercept-only models for each variable of interest and then calculating the proportion of the total variance explained by phylogenetic effects across the posterior distribution of parameter estimates.

References:

1 Darwin, C. R. *On the origin of species, or the preservation of favoured races in the struggle for life*. (John Murray, 1859).

2 Wallace, A. R. *Natural selection and tropical nature: essays on descriptive and theoretical biology. 2nd edition*. (Macmillan, 1895).

3 Darwin, C. R. *A naturalist's voyage round the world*. (John Murray, 1913).

4 Wallace, A. R. Colour in nature. *Nature* **19**, 580-581 (1879).

5 Dalrymple, R. L. *et al.* Abiotic and biotic predictors of macroecological patterns in bird and butterfly coloration. *Ecol. Monogr.* **88**, 204-224 (2018).

6 Adams, J. M., Kang, C. & June-Wells, M. Are tropical butterflies more colorful? *Ecol. Res.* **29**, 685-691, doi:10.1007/s11284-014-1154-1 (2014).

7 Bailey, S. F. Latitudinal gradients in colors and patterns of passerine birds. *Condor* **80**, 372-381 (1978).

8 Wilson, M. F. & Von Neaumann, R. A. Why are Neotropical birds more colourful than North American birds? *Avicultural Magazine* **78**, 141-147 (1972).

9 Dalrymple, R. L. *et al.* Birds, butterflies and flowers in the tropics are not more colourful than those at higher latitudes. *Global Ecol. Biogeogr.* **24**, 1424-1432, doi:10.1111/geb.12368 (2015).

10 Friedman, N. R. & Remeš, V. Ecogeographical gradients in plumage coloration among Australasian songbird clades. *Global Ecol. Biogeogr.* **26**, 261-274, doi:10.1111/geb.12522 (2017).

11 Dale, J., Dey, C. J., Delhey, K., Kempenaers, B. & Valcu, M. The effects of life history and sexual selection on male and female plumage colouration. *Nature* **527**, 367-370, doi:10.1038/nature15509 (2015).

12 Dunn, P. O., Armenta, J. K. & Whittingham, L. A. Natural and sexual selection act on different axes of variation in avian plumage color. *Science Advances* **1**, e1400155 (2015).

13 Stoddard, M. C. & Prum, R. O. How colorful are birds? Evolution of the avian plumage color gamut. *Behav. Ecol.* **22**, 1042-1052, doi:10.1093/beheco/arr088 (2011).

14 Renoult, J. P., Kelber, A. & Schaefer, H. M. Colour spaces in ecology and evolutionary biology. *Biological Reviews* **92**, 292-315, doi:10.1111/brv.12230 (2017).

15 Stoddard, M. C. & Prum, R. O. Evolution of avian plumage color in a tetrahedral color space: a phylogenetic analysis of New World buntings. *Am. Nat.* **171**, 755-776, doi:10.1086/587526 (2008).

16 Delhey, K. The colour of an avifauna: a quantitative analysis of the colour of Australian birds. *Scientific Reports* **5**, 18514, doi:10.1038/srep18514 (2015).

17 Olson, D. M. *et al.* Terrestrial ecoregions of the world: a new map of life on earth. *Bioscience* **51**, 933-938 (2001).

18 Rabosky, D. L. *et al.* An inverse latitudinal gradient in speciation rate for marine fishes. *Nature* **559**, 392-395, doi:10.1038/s41586-018-0273-1 (2018).

19 Lynch, M. Methods for the analysis of comparative data in evolutionary biology. *Evolution* **45**, 1065-1080 (1991).

20 Delhey, K. A review of Gloger's rule, an ecogeographical rule of colour: definitions, interpretations and evidence. *Biol. Rev. Camb. Philos. Soc.* **94**, 1294-1316, doi:10.1111/brv.12503 (2019).

21 Marchetti, K. Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature* **362**, 149-152 (1993).

22 Endler, J. A. The color of light in forests and its implications. *Ecol. Monogr.* **63**, 1-27 (1993).
23 Schemske, D. W. in *Speciation and Patterns of Diversity* (eds R. Butlin, J. Bridle, & D. Schluter) Ch. 12, 219-239 (2001).
24 Schemske, D. W., Mittelbach, G. G., Cornell, H. V., Sobel, J. M. & Roy, K. Is there a latitudinal gradient in the importance of biotic interactions? *Annu. Rev. Ecol. Evol. Syst.* **40**, 245-269, doi:10.1146/annurev.ecolsys.39.110707.173430 (2009).
25 MacArthur, R. H. Patterns of communities in the tropics. *Biol. J. Linn. Soc.* **1**, 19-30 (1969).
26 Hadfield, J. D. & Nakagawa, S. General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. *J. Evol. Biol.* **23**, 494-508, doi:10.1111/j.1420-9101.2009.01915.x (2010).
27 Cooney, C. R. *et al.* Sexual selection predicts the rate and direction of colour divergence in a large avian radiation. *Nature communications* **10**, 1773, doi:10.1038/s41467-019-09859-7 (2019).
28 Cooney, C. R., MacGregor, H. E. A., Seddon, N. & Tobias, J. A. Multi-modal signal evolution in birds: re-assessing a standard proxy for sexual selection. *Proc. R. Soc. London Ser. B* **285**, 20181557, doi:10.1098/rspb.2018.1557 (2018).
29 van der Bijl, W. *et al.* Butterfly dichromatism primarily evolved via Darwin's, not Wallace's, model. *Evolution Letters* **4**, 545-555, doi:10.1002/evl3.199 (2020).
30 Darwin, C. R. *The descent of man, and selection in relation to sex*. (John Murray, 1871).
31 Tobias, J. A., Montgomerie, R. & Lyon, B. E. The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Philos. Trans. R. Soc. London Ser. B* **367**, 2274-2293, doi:10.1098/rstb.2011.0280 (2012).
32 Galván, I., Negro, J. J., Rodríguez, A. & Carrascal, L. M. On showy dwarfs and sober giants: body size as a constraint for the evolution of bird plumage colouration. *Acta Ornithologica* **48**, 65-80, doi:10.3161/000164513x670007 (2013).
33 Zahavi, A. & Zahavi, A. *The Handicap Principle*. (Oxford University Press, 1997).
34 Badyaev, A. V. & Hill, G. E. Avian sexual dichromatism in relation to phylogeny and ecology. *Annu. Rev. Ecol. Evol. Syst.* **34**, 27-49, doi:10.1146/annurev.ecolsys.34.011802.132441 (2003).
35 Simpson, R. K., Johnson, M. A. & Murphy, T. G. Migration and the evolution of sexual dichromatism: evolutionary loss of female coloration with migration among wood-warblers. *Proc. R. Soc. London Ser. B* **282**, 20150375, doi:10.1098/rspb.2015.0375 (2015).
36 Helferich, G. *Humboldt's Cosmos*. (Tantor eBooks, 2011).
37 Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. & Mooers, A. O. The global diversity of birds in space and time. *Nature* **491**, 444-448, doi:10.1038/nature11631 (2012).
38 He, Y. *et al.* Segmenting biological specimens from photos to understand the evolution of UV plumage in passerine birds. *bioRxiv*, https://doi.org/10.1101/2021.1107.1122.453339, doi:https://doi.org/10.1101/2021.07.22.453339 (2021).
39 Chen, L. C., Zhu, Y., Papandreou, G., Schroff, F. & Adam, H. Encoder-decoder with atrous separable convolution for semantic image segmentation. *arXiv* **1802**, 02611 (2018).
40 Hussein, B. R., Malik, O. A., Ong, W.-H. & Slik, J. W. F. in *Computational Science and Technology Lecture Notes in Electrical Engineering* Ch. Chapter 31, 321-330 (2020).
41 Coffin, D. DCRAW V. 9.27 https://www.cybercom.net/~dcoffin/dcraw/. (2016).

42 Troscianko, J. & Stevens, M. Image calibration and analysis toolbox – a free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution* **6**, 1320-1331, doi:10.1111/2041-210x.12439 (2015).
43 Hijmans, R. J. raster: geographic data analysis and modeling. R package version 3.4-5. https://CRAN.R-project.org/package=raster. (2020).
44 Maia, R., Gruson, H., Endler, J. A., White, T. E. & O'Hara, R. B. pavo 2: new tools for the spectral and spatial analysis of colour in R. *Methods in Ecology and Evolution* **10**, 1097-1107, doi:10.1111/2041-210x.13174 (2019).
45 Stoddard, M. C. *et al.* Wild hummingbirds discriminate nonspectral colors. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 15112-15122, doi:10.1073/pnas.1919377117 (2020).
46 Gomez, D. & Théry, M. Simultaneous crypsis and conspicuousness in color patterns: comparative analysis of a Neotropical rainforest bird community. *Am. Nat.* **169**, S42-S61 (2007).
47 Blonder, B. Do hypervolumes have holes? *Am. Nat.* **187**, E93-E105, doi:10.1086/685444 (2016).
48 Schliep, K. P. phangorn: phylogenetic analysis in R. *Bioinformatics* **27**, 592-593 (2011).
49 Fick, S. E. & Hijmans, R. J. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* **37**, 4302-4315, doi:10.1002/joc.5086 (2017).
50 Beckmann, M. *et al.* glUV: a global UV-B radiation data set for macroecological studies. *Methods in Ecology and Evolution* **5**, 372-383, doi:10.1111/2041-210x.12168 (2014).
51 Running, S. W. *et al.* A continuous satellite-derived measure of global terrestrial primary production. *Bioscience* **54**, 547-560 (2004).
52 Tobias, J. A. & Pigot, A. L. Integrating behaviour and ecology into global biodiversity conservation strategies. *Philos. Trans. R. Soc. London Ser. B* **374**, 20190012, doi:10.1098/rstb.2019.0012 (2019).
53 Dunn, P. O., Whittingham, L. A. & Pitcher, T. E. Mating systems, sperm competition, and the evolution of sexual dimorphism in birds. *Evolution* **55**, 161-175 (2001).
54 Bivand, R. S., Pebesma, E. & Gómez-Rubio, V. *Applied Spatial Data Analysis with R, Second Edition*. (Springer, 2013).
55 Hawkins, B. A. *et al.* Structural bias in aggregated species-level variables driven by repeated species co-occurrences: a pervasive problem in community and assemblage data. *J. Biogeogr.* **44**, 1199-1211, doi:10.1111/jbi.12953 (2017).
56 Hadfield, J. D. MCMC methods for multi-response generalised linear mixed models: the MCMCglmm R package. *Journal of Statistical Software* **33**, 1-22 (2010).
57 Healy, K. *et al.* Ecology and mode-of-life explain lifespan variation in birds and mammals. *Proc. R. Soc. London Ser. B* **281**, 20140298, doi:10.1098/rspb.2014.0298 (2014).

Acknowledgements: We thank M. Adams, H. van Grouw, R. Prys-Jones, and A. Bond from the Bird Group at NHM Tring for providing access to and expertise in the collection, Natalie Brett for assistance with data collection, and Frane Babarovic, Jonathan Kennedy, Louie Rombault and Alex Slavenko for helpful discussion and feedback. This work was funded by a Leverhulme Early Career Fellowship (ECF-2018-101) and Natural Environment Research Council Independent Research Fellowship (NE/T01105X/1) to C.R.C, a Leverhulme Centre for Advanced Biological Modelling PhD studentship to Y.H., a Royal Society Wolfson Merit Award (WM170050, APEX APX\R1\191045) and National Research, Development and Innovation Office of Hungary grant (ÉLVONAL KKP-126949, K-116310) to T.S., and a European Research Council grant (615709, Project 'ToLERates') and Royal Society University Research Fellowship (UF120016, URF\R\180006) to G.H.T.

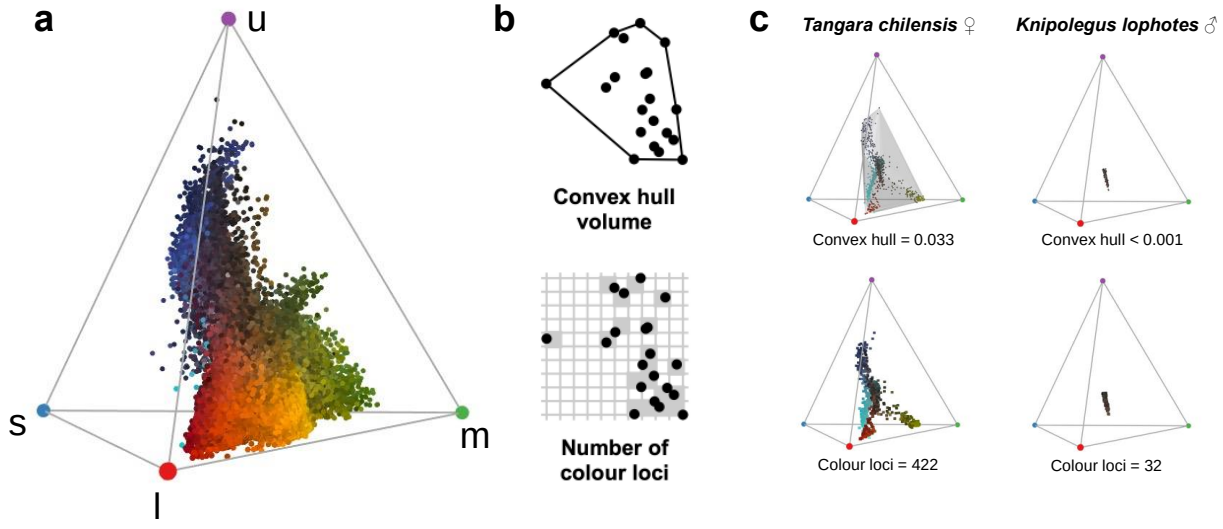
Authors contributions: C.R.C. and G.H.T designed the research; C.R.C., Z.K.V., L.O.N., C.J.A.M., M.D.J., A.L. and T.S. collected data; C.R.C. and Y.H. conducted the analyses. C.R.C. wrote the manuscript, with input from all authors.

Competing interests: All authors have no competing interests.

Materials and correspondence: Correspondence and material requests should be addressed to the corresponding author**.**

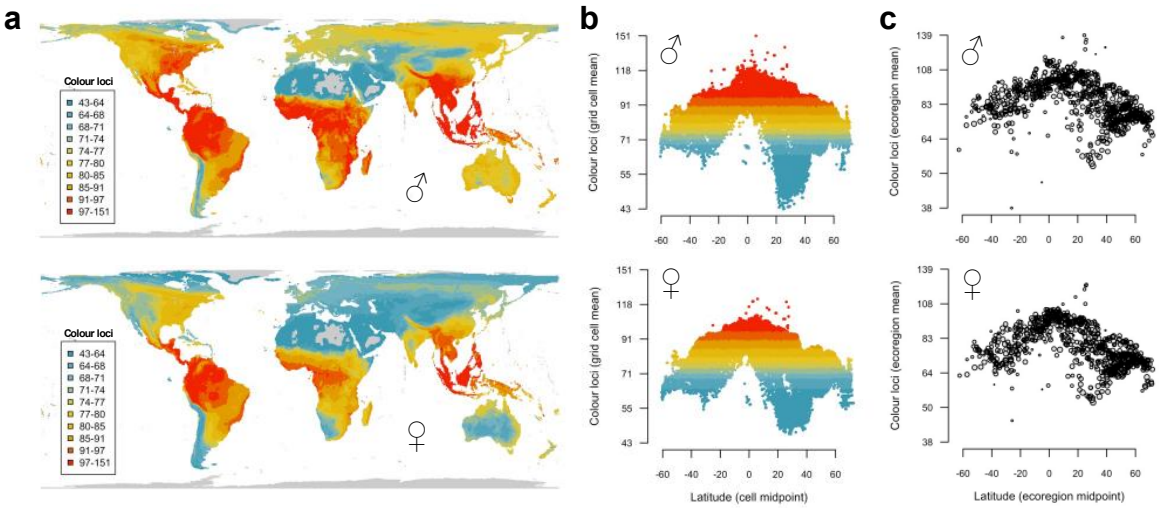
Data and materials availability: All analysis data is available in the supplementary materials. Analysis code is available from the corresponding author upon request.

Supplementary information: An Excel file (Cooney_etal_data_S1.xlsx) containing the dataset analysed in this study.



720

721 **Figure 1. The diversity of passerine plumage colours in avian tetrahedral**
722 **colourspace. a**, A sample of one million passerine plumage colours analysed in this
723 study, visualised in avian ultraviolet-sensitive (UVS) tetrahedral colour space where
724 each vertex represents one of the four colour cone types sensitive to long (*l*), medium
725 (*m*), short (*s*), and ultraviolet (*u*) wavelengths. Measurements are derived from
726 calibrated digital images of male and female museum specimens for 4,527 species. The
727 total number of measurements from which this sample is drawn is >36 million. The
728 vertices of the colourspace **b**, An illustration of the two colour diversity metrics used in
729 this study: convex hull volume (top) and number of colour loci (bottom). For simplicity,
730 the example is based on 2-dimensional simulated data. **c**, Plots and metric values for
731 the species with the largest (*Tangara chilensis* ♀, left) and smallest (*Knipolegus*
732 *lophotes* ♂, right) convex hull volume score, respectively. In all plots, points are
733 coloured according to their approximate appearance as perceived by a human observer
734 by mapping raw pixel reflectance values to CIE 1931 XYZ colour space.



735

736 **Figure 2. Latitudinal gradients in male and female colourfulness in passerine**
737 **birds. a**, Mean colour loci scores for grid cell assemblages, separately for males (top)
738 and females (bottom). **b**, **c**, Distributions of mean species' colour loci scores for grid
739 cells (**b**) and ecoregions (**c**) with respect to latitude, separately for males (top) and
740 females (bottom). Grid cell size is 50 x 50 km for all panels (Behrman projection) and
741 only cells containing at least 5 sampled species are plotted. Colour loci scores are
742 based on an ultraviolet-sensitive (UVS) visual system.

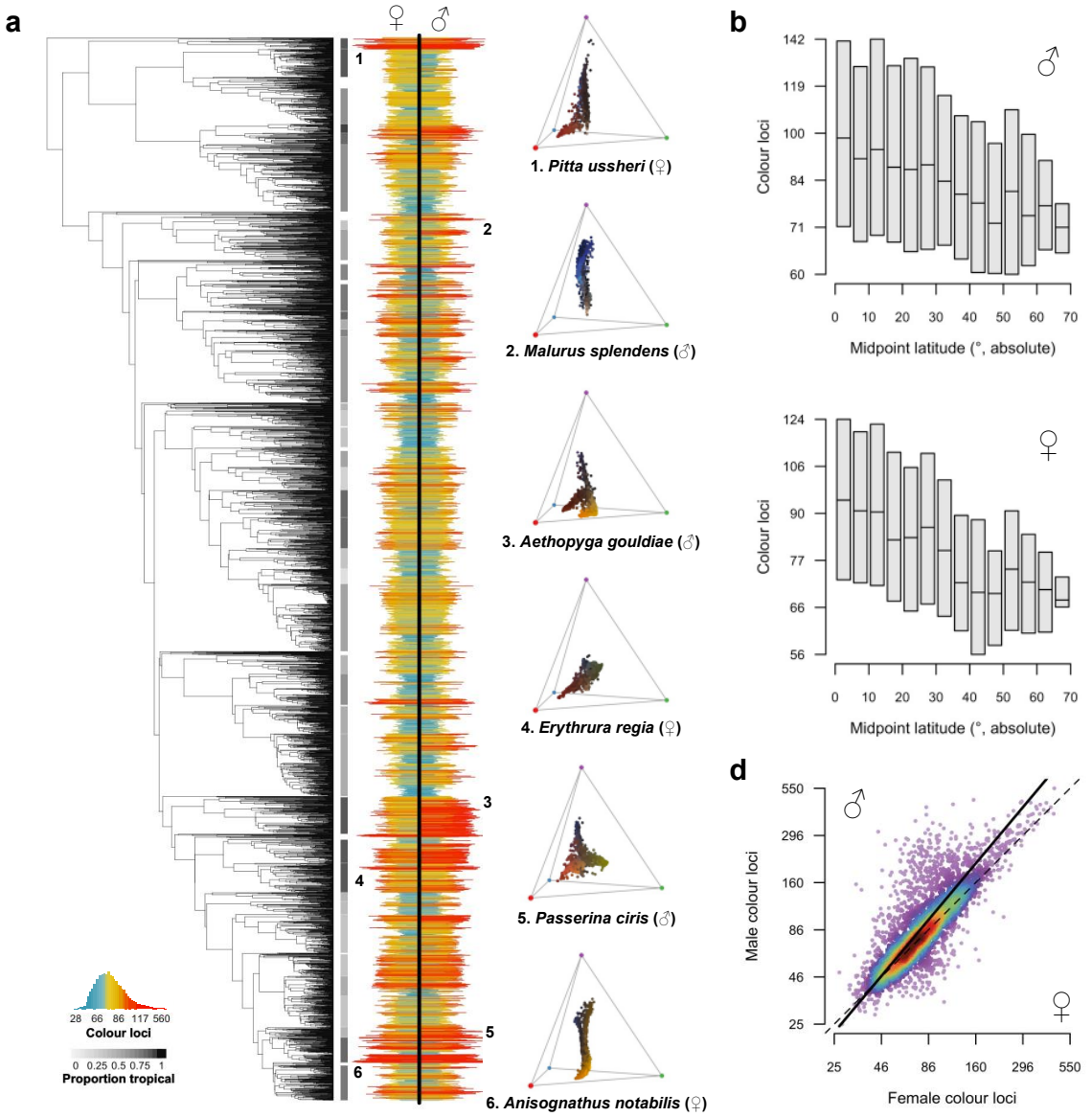


Figure 3. The phylogenetic distribution of male and female colourfulness and its relation to species' midpoint latitude in passerine birds. a, Coloured bars indicate male and female colour loci scores for 4,527 passerine species. Grey segments indicate the proportion of tropical species (i.e. |midpoint latitude| < 23.5º) within major clades. **b**, Box plots showing the median and interquartile range of the distribution of species' colour loci scores with respect to latitude, separately for males (top) and females (bottom), with species binned into 5º increments. **c**, Scatterplot showing the relationship between male and female colour loci scores across species, with points coloured according to point density in the plot. The solid line indicates the relationship between variables estimated using phylogenetic reduced major axis (pRMA) regression, which differs significantly ($P < 0.001$) from a one-to-one relationship (dashed line). Colour loci scores are based on an ultraviolet- sensitive (UVS) visual system.

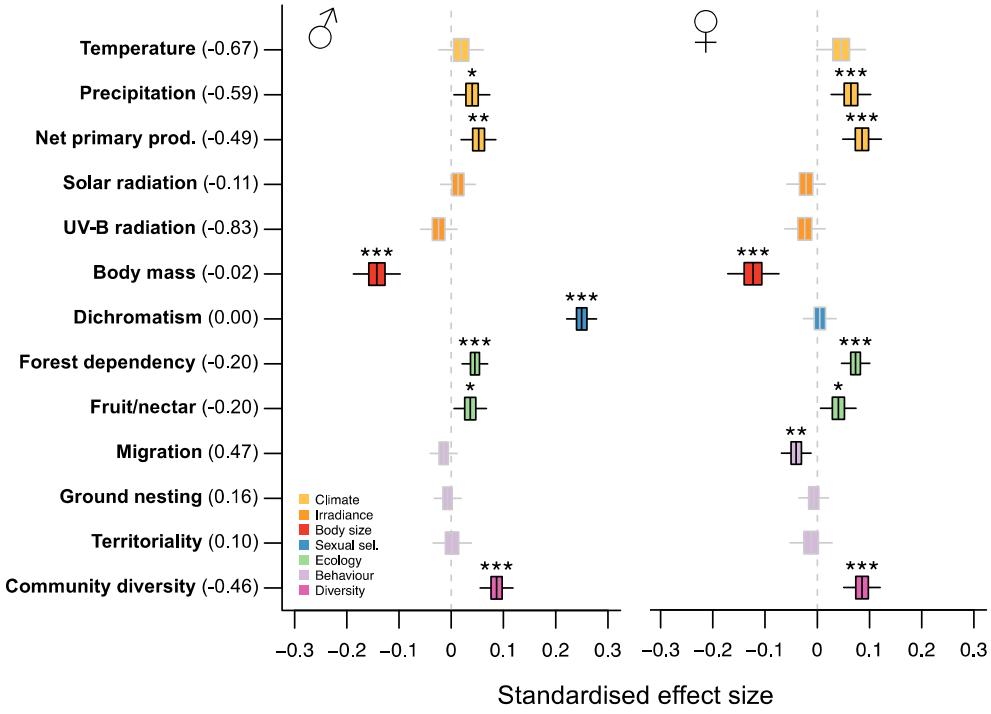
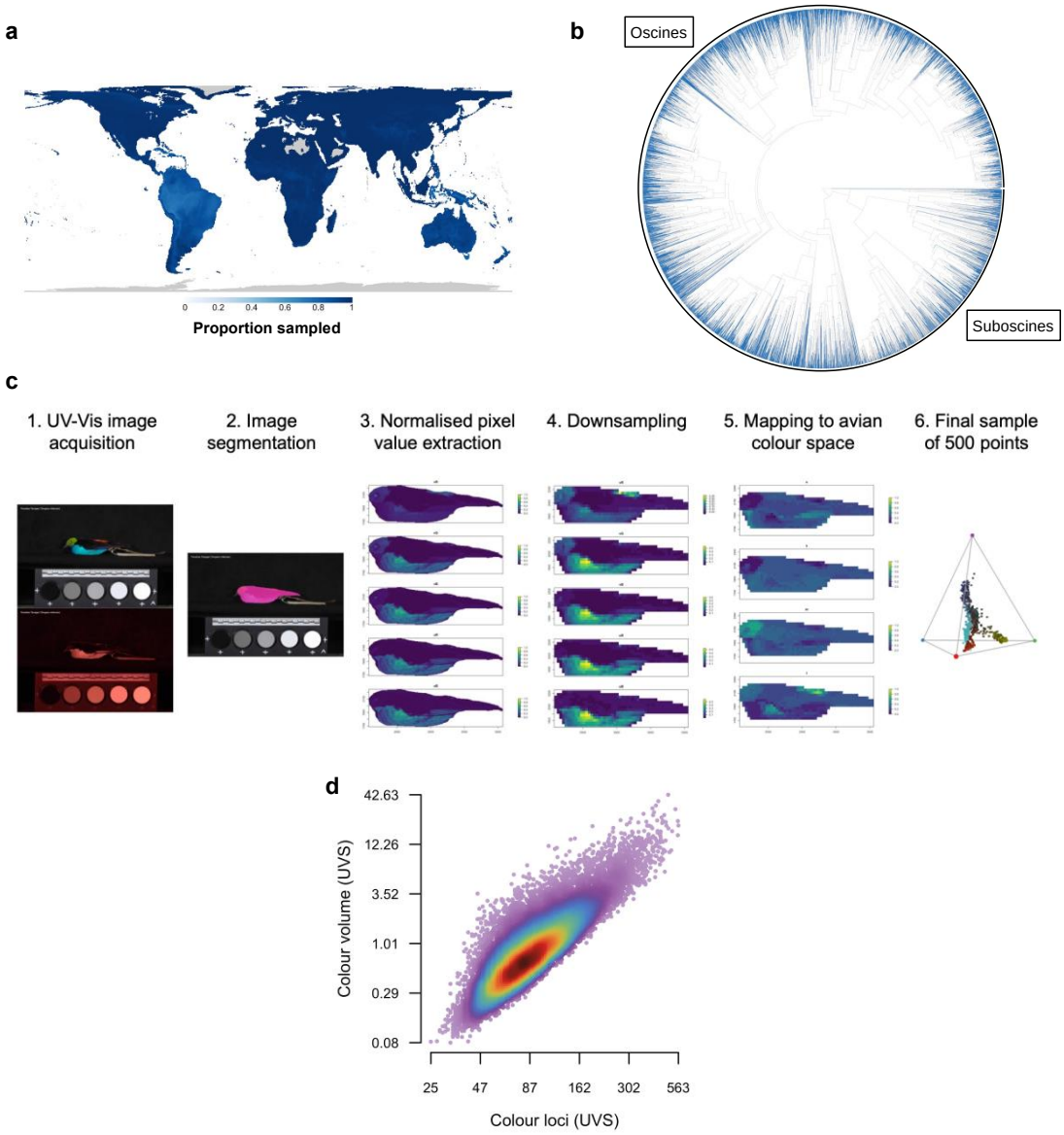
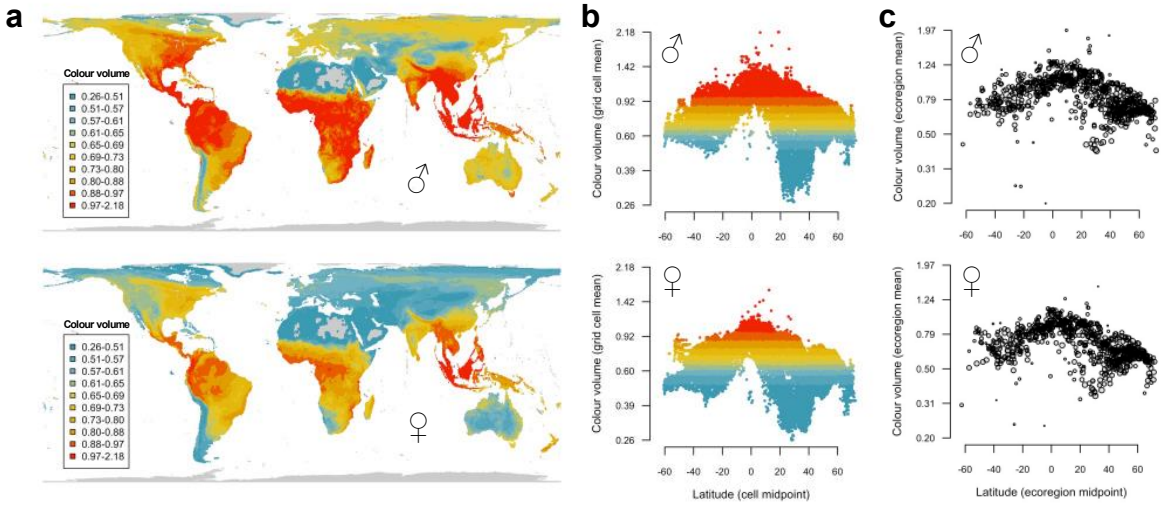


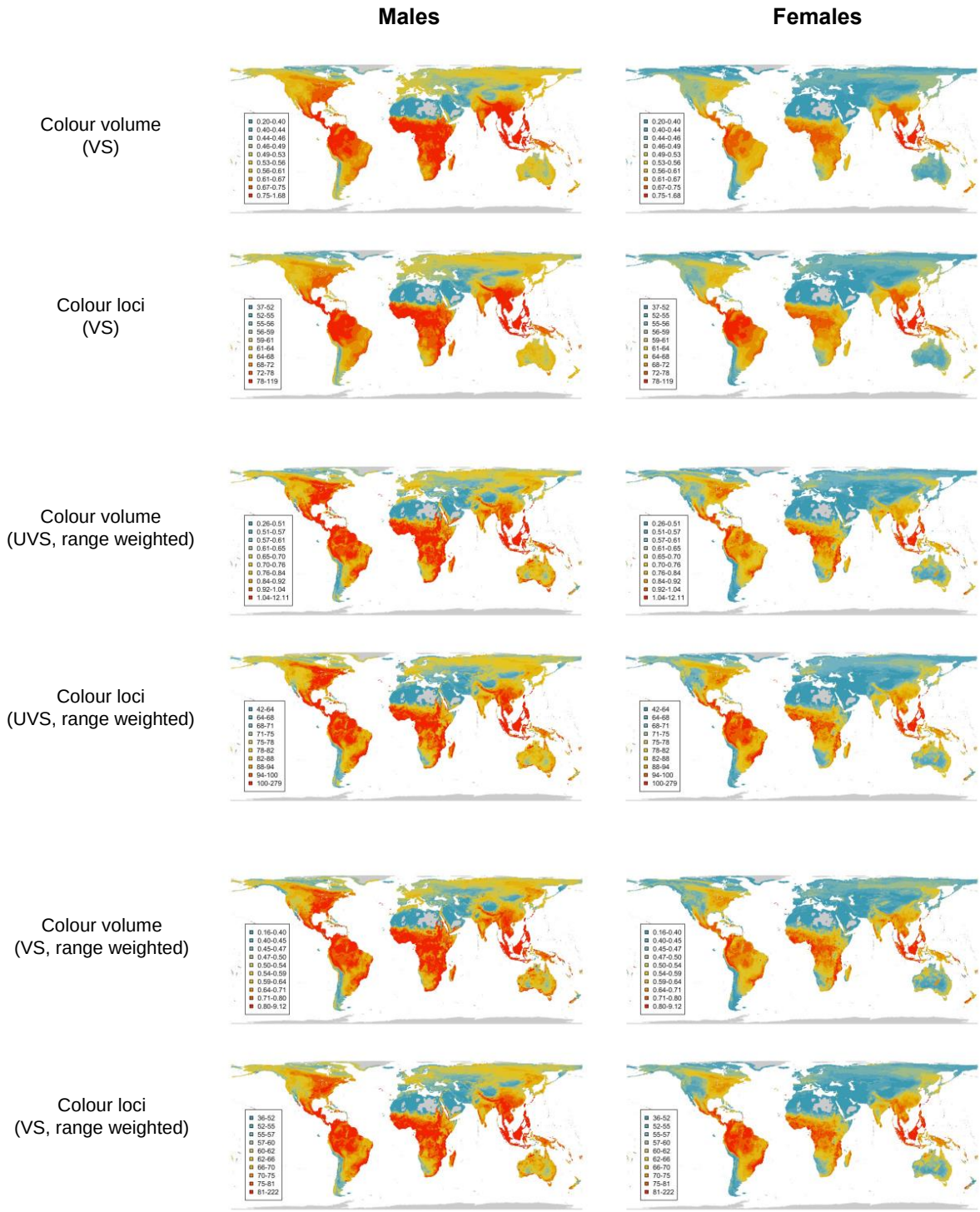
Figure 4. Predictors of male and female colourfulness in passerine birds. Box plots summarise the posterior marginal distributions for all fixed-effects from Bayesian phylogenetic mixed models applied over a sample of 100 phylogenetic trees predicting male (left) and female (right) colour loci scores across 4,415 species. Box widths represent the interquartile range, the median is shown as a vertical line within each box, and whiskers denote the 95% credibility interval of the distribution. Colours indicate the fixed-effect category, with black outlines and asterisks indicating evidence for a non-zero effect of the relevant variable. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Values in parentheses next to each predictor give the correlation coefficient (Spearman's *rho*) for the relationship between each predictor and species' absolute midpoint latitude. Results shown are for colour loci scores calculated assuming an ultraviolet-sensitive (UVS) visual system.



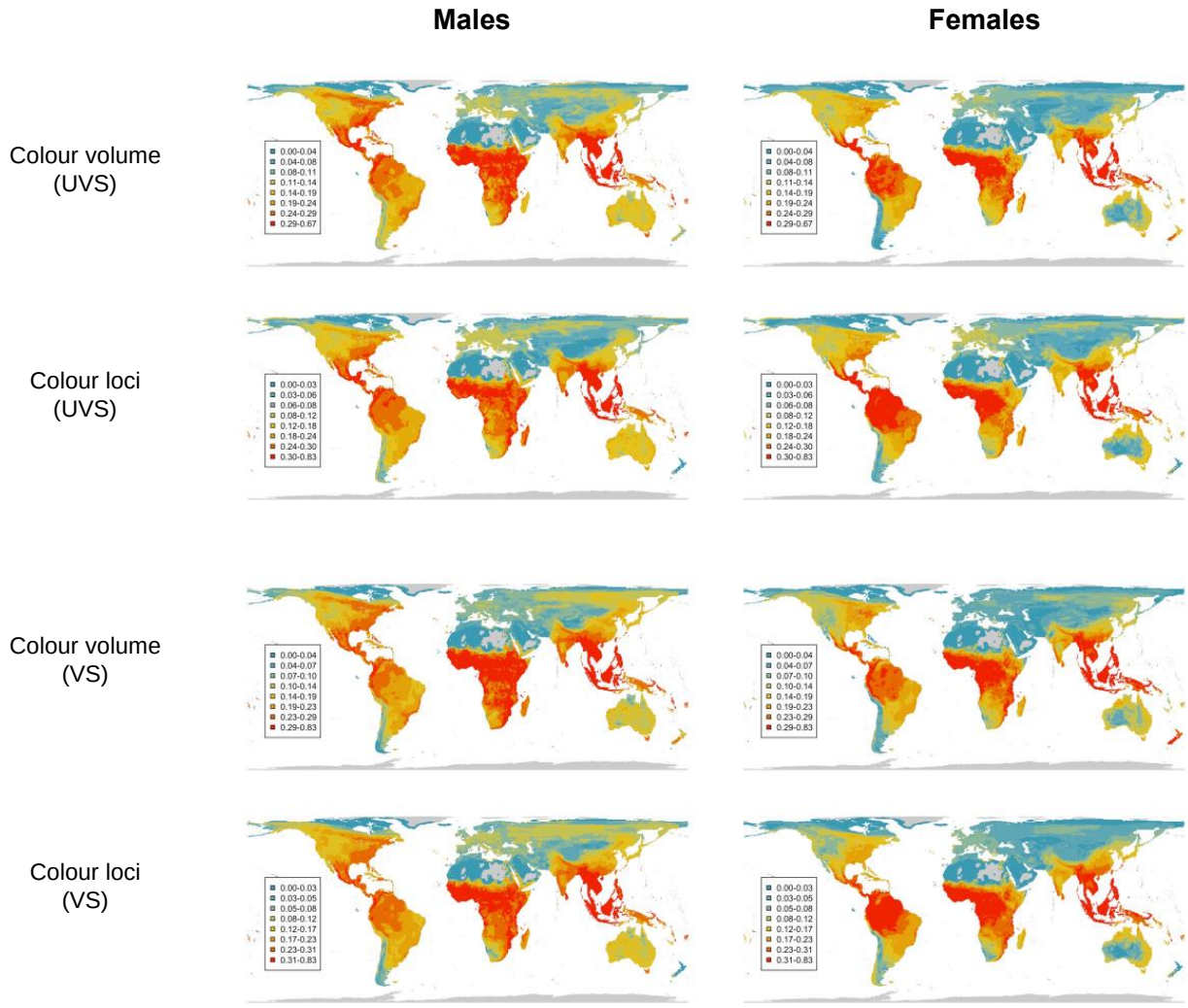
Supplementary Figure 1. The distribution of sampled species and illustrations of the colouration data workflow and colour diversity metrics used in this study. a, The proportion of passerine species sampled per grid cell. Grid cell size is 50 x 50 km (Behrman projection) and only cells containing at least 5 passerine species are plotted. **b**, The phylogenetic distribution of sampled species (blue, *n* = 4,527) relative to the whole passerine radiation (*n* = 5,966). **c**, An example showing the workflow used to extract whole-body reflectance data from specimen images, as applied to lateral (side) view images. An analogous workflow is applied to the other two views of the specimen (dorsal and ventral). The resulting sets of measurements for each view are then combined into a final dataset of 1,500 measurements for each specimen, capturing whole-body plumage colouration. **d**, The relationship between specimen-level scores of colour loci and colour volume based on a ultraviolet-sensitive (UVS) visual system.



Supplementary Figure 2. Latitudinal gradients in male and female colourfulness in passerine birds using colour volume. a, Mean colour volume scores for grid cell assemblages, separately for males (top) and females (bottom). **b**, **c**, Distributions of mean species' colour volume scores for grid cells (**b**) and ecoregions (**c**) with respect to latitude, separately for males (top) and females (bottom). Grid cell size is 50 x 50 km for all panels (Behrman projection) and only cells containing at least 5 sampled species are plotted. Colour volumes are based on an ultraviolet- sensitive (UVS) visual system. Note: colour volume values are multiplied by 1000.



Supplementary Figure 3. Geographic distributions of male and female colourfulness in passerine birds using different datasets. Grid cell size is 50 x 50 km for all panels (Behrman projection) and only cells containing at least 5 sampled species are plotted. UVS, ultraviolet sensitive; VS, violet sensitive. Note: colour volume values are multiplied by 1000.



Supplementary Figure 4. Geographic distribution of the proportion of species in passerine assemblages in the top colour diversity quartile using different datasets. Grid cell size is 50 x 50 km for all panels (Behrman projection) and only cells containing at least 5 sampled species are plotted. UVS, ultraviolet sensitive; VS, violet sensitive.

803

Study	Taxa	Geographic extent	Colour data	Conclusions	Notes
Adams et al. (2014) [6]	Butterflies (n = 247)	New World (Ecuador, Florida, Maine)	Digital photographs of museum specimens (VIS only)	Gradient present – Ecuadorian species more variable in colour intensity, saturation, and hue than species of other regions	Incomplete colour measurements (no UV), limited geographical extent.
Bailey (1978) [7]	Passerine birds (n = 764)	North and Central America (Alaska/Canada to Panama)	Human scores of field guide illustrations	No gradient present – Some significant differences between regions but overall weak support for enhanced tropical colorfulness	Qualitative, subjective scores of colourfulness, limited geographic extent
Dale et al. (2015) [11]	Passerine birds (n = 2,471)	Global	'Maleness' scores based on RGB data for front-facing body regions measured from handbook illustrations	Gradient present – Species with tropical life histories have more elaborate plumages (higher 'maleness' scores)	Incomplete colour measurements (no UV), colour elaboration rather than colourfulness per se, composite predictor variable (latitude + clutch size + environmental stability)
Dalrymple et al. (2015, 2018) [9, 5]	Birds (n = 570), butterflies (n = 424), flowers (n = 339)	Eastern Australia	Reflectance spectrometry (300-700 nm; birds, flowers) and UV/VIS digital photography (butterflies)	No gradient present – High rather than low latitude regions tend to contain the most colourful species	Limited geographical extent
Dunn et al. (2015) [12]	Birds (n = 977)	Global	Reflectance spectrometry (320-700 nm)	Equivocal – In monomorphic species (n = 489), subtropical taxa were brighter but tropical taxa were duller than non-tropical taxa	Colour quantified in terms of brightness and hue; colourfulness per se not measured
Friedman & Remeš (2017) [10]	Australian passerines (Meliphagidae, n = 97; Acanthizidae, n = 40)	Australia, New Guinea	Reflectance spectrometry (300-700 nm)	No gradient present – birds living close to the equator were not more colourful	Limited taxonomic and geographic extent
Wilson & Von Neumann (1972) [8]	Birds (n = 1,678)	N and S America, Europe	Binary categorisation of species as 'colourful' or 'not so' based on pictures and written descriptions	Gradient present – Birds of the South American lowland tropics were more frequently colourful than those of South American extra-tropics, North America and Europe	Qualitative, subjective classification of colourfulness based on photos and/or written descriptions, somewhat geographically limited

804 **Supplementary Table 1. Summaries of studies addressing latitudinal gradients in**
805 **organismal colourfulness.** Numbers in square brackets in the 'Study' column indicate
806 the corresponding reference number in the main text.

		Mean colourfulness		SES colourfulness	
Variable	**Sex**	**Slope**	**Moran's *I***	**Slope**	**Moran's *I***
Volume (UVS)	M	-0.462***	0.047*	-0.231***	0.032
	F	-0.472***	0.061**	-0.226***	0.050*
Loci (UVS)	M	-0.461***	0.059**	-0.220***	0.046*
	F	-0.490***	0.063**	-0.211***	0.058*
Volume (VS)	M	-0.438***	0.043*	-0.217***	0.030
	F	-0.517***	0.054*	-0.237***	0.048*
Loci (VS)	M	-0.486***	0.054*	-0.233***	0.045*
	F	-0.509***	0.051*	-0.211***	0.052*
Volume (UVS, RW)	M	-0.383***	0.042*	-0.260***	0.026
	F	-0.437***	0.061**	-0.319***	0.048*
Loci (UVS, RW)	M	-0.353***	0.036	-0.228***	0.021
	F	-0.400***	0.048*	-0.247***	0.031
Volume (VS, RW)	M	-0.410***	0.041	-0.275***	0.026
	F	-0.464***	0.056*	-0.333***	0.047*
Loci (VS, RW)	M	-0.380***	0.034	-0.244***	0.023
	F	-0.429***	0.037	-0.258***	0.025
Volume (UVS, PTQ)	M	-0.551***	0.054*	-0.267***	0.031
	F	-0.570***	0.053*	-0.266***	0.041
Loci (UVS, PTQ)	M	-0.647***	0.066**	-0.275***	0.036
	F	-0.662***	0.055*	-0.267***	0.058*
Volume (VS, PTQ)	M	-0.650***	0.062**	-0.314***	0.030
	F	-0.582***	0.068**	-0.290***	0.056*
Loci (VS, PTQ)	M	-0.666***	0.054*	-0.292***	0.033
	F	-0.711***	0.061**	-0.298***	0.065*

Supplementary Table 2. Models for the effect of absolute latitude on male and female colourfulness across 800 terrestrial ecoregions using different datasets. Shown are results from spatial simultaneous autoregressive (SAR) models. Slope refers to the estimated slope of the relationship between colourfulness score (loci or volume) and absolute latitude in each model, with asterisks indicating significant effects. Moran's *I* refers to the global Moran's *I* estimate for each model assessing the presence of residual spatial autocorrelation in the model residuals, with asterisks indicating associated significance level. All variables were standardised (mean = 0, sd = 1) prior to model fitting. SES, standardised effect size; UVS, ultraviolet sensitive; VS, violet sensitive; RW, range weighted; PTQ, proportion of species in the top (25%) colour diversity quartile. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Variable	Sex	Estimate (95% CI)	P_{MCMC}
Volume (UVS)	M	-0.034 (-0.059, -0.008)	0.010**
	F	-0.107 (-0.136, -0.078)	<0.001***
Loci (UVS)	M	-0.043 (-0.068, -0.017)	0.001***
	F	-0.118 (-0.144, -0.090)	<0.001***
Volume (VS)	M	-0.038 (-0.064, -0.012)	0.004**
	F	-0.121 (-0.151, -0.092)	<0.001***
Loci (VS)	M	-0.049 (-0.075, -0.024)	<0.001***
	F	-0.140 (-0.167, -0.113)	<0.001***

Supplementary Table 3. Bayesian phylogenetic mixed model results for the effect of absolute latitude on male and female colourfulness across passerine species (*n* = 4,527). All variables were standardised (mean = 0, sd = 1) prior to model fitting. UVS, ultraviolet sensitive; VS, violet sensitive. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. All models were run over 100 posterior phylogenetic trees.

		Males		Females	
Variable	**Term**	**Estimate (95% CI)**	P_{MCMC}	**Estimate (95% CI)**	P_{MCMC}
Loci (UVS)	(Intercept)	0.140 (-0.691, 0.971)	0.739	0.228 (-0.643, 1.115)	0.609
	Temperature	0.019 (-0.024, 0.061)	0.379	0.046 (-0.001, 0.092)	0.055
	Precipitation	**0.040 (0.005, 0.074)**	**0.022***	**0.065 (0.027, 0.102)**	**0.001****
	Net primary productivity	**0.052 (0.019, 0.085)**	**0.002****	**0.086 (0.049, 0.123)**	**<0.001****
	Solar radiation	0.013 (-0.021, 0.046)	0.445	-0.021 (-0.059, 0.016)	0.257
	UV-B radiation	-0.024 (-0.059, 0.011)	0.175	-0.024 (-0.062, 0.015)	0.227
	Body mass	**-0.143 (-0.188, -0.098)**	**<0.001****	**-0.123 (-0.172, -0.073)**	**<0.001****
	Sexual dichromatism	**0.250 (0.221, 0.279)**	**<0.001****	0.005 (-0.027, 0.036)	0.753
	Forest dependency	**0.045 (0.021, 0.070)**	**<0.001****	**0.073 (0.046, 0.101)**	**<0.001****
	Frugivore-nectarivore	**0.036 (0.006, 0.068)**	**0.021***	**0.041 (0.006, 0.074)**	**0.019***
	Migratory	-0.015 (-0.041, 0.011)	0.272	**-0.040 (-0.069, -0.012)**	**0.006****
	Ground nesting	-0.007 (-0.033, 0.019)	0.592	-0.007 (-0.035, 0.021)	0.642
	Territoriality	0.002 (-0.035, 0.039)	0.932	-0.012 (-0.052, 0.028)	0.572
	Community diversity	**0.087 (0.055, 0.118)**	**<0.001****	**0.086 (0.051, 0.121)**	**<0.001****
Loci (VS)	(Intercept)	0.159 (-0.666, 0.982)	0.703	0.279 (-0.599, 1.150)	0.527
	Temperature	0.010 (-0.031, 0.053)	0.628	0.045 (-0.004, 0.091)	0.064
	Precipitation	**0.056 (0.021, 0.090)**	**0.001****	**0.089 (0.051, 0.127)**	**<0.001****
	Net primary productivity	**0.051 (0.018, 0.085)**	**0.002****	**0.084 (0.047, 0.122)**	**<0.001****
	Solar radiation	0.022 (-0.011, 0.056)	0.194	-0.019 (-0.056, 0.019)	0.330
	UV-B radiation	-0.019 (-0.054, 0.015)	0.285	-0.017 (-0.056, 0.022)	0.405
	Body mass	**-0.141 (-0.185, -0.096)**	**<0.001****	**-0.123 (-0.171, -0.074)**	**<0.001****
	Sexual dichromatism	**0.258 (0.229, 0.287)**	**<0.001****	-0.006 (-0.038, 0.026)	0.713
	Forest dependency	**0.040 (0.016, 0.065)**	**0.002****	**0.060 (0.032, 0.087)**	**<0.001****
	Frugivore-nectarivore	0.026 (-0.004, 0.057)	0.093	**0.037 (0.003, 0.072)**	**0.032***
	Migratory	-0.009 (-0.035, 0.017)	0.486	**-0.039 (-0.068, -0.010)**	**0.008****
	Ground nesting	-0.006 (-0.032, 0.020)	0.638	-0.004 (-0.033, 0.024)	0.788
	Territoriality	0.003 (-0.034, 0.039)	0.872	0.006 (-0.034, 0.046)	0.756
	Community diversity	**0.086 (0.055, 0.118)**	**<0.001****	**0.090 (0.054, 0.125)**	**<0.001****

Supplementary Table 4. Bayesian phylogenetic mixed model results for the effect of predictor variables on male and female colour loci scores across passerine species (*n* = 4,415). All variables were standardised (mean = 0, sd = 1) prior to model fitting. UVS, ultraviolet sensitive; VS, violet sensitive. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. All models were run over 100 posterior phylogenetic trees.

		Males		Females	
Variable	**Term**	**Estimate (95% CI)**	**P_{MCMC}**	**Estimate (95% CI)**	**P_{MCMC}**
Volume (UVS)	(Intercept)	0.181 (-0.576, 0.931)	0.635	0.221 (-0.569, 1.012)	0.581
	Temperature	-0.001 (-0.045, 0.042)	0.951	-0.002 (-0.053, 0.049)	0.948
	Precipitation	**0.054 (0.019, 0.090)**	**0.003****	**0.086 (0.044, 0.127)**	**<0.001*****
	Net primary productivity	**0.035 (0.000, 0.069)**	**0.048***	**0.068 (0.027, 0.108)**	**0.001****
	Solar radiation	**0.040 (0.005, 0.075)**	**0.023***	0.031 (-0.009, 0.071)	0.139
	UV-B radiation	-0.031 (-0.068, 0.005)	0.092	**-0.045 (-0.087, -0.004)**	**0.035***
	Body mass	**-0.171 (-0.215, -0.127)**	**<0.001*****	**-0.163 (-0.212, -0.112)**	**<0.001*****
	Sexual dichromatism	**0.235 (0.206, 0.264)**	**<0.001*****	**-0.044 (-0.078, -0.011)**	**0.009****
	Forest dependency	**0.034 (0.008, 0.059)**	**0.010***	**0.079 (0.049, 0.108)**	**<0.001*****
	Frugivore-nectarivore	**0.032 (0.000, 0.063)**	**0.048***	**0.037 (0.000, 0.073)**	**0.045***
	Migratory	-0.010 (-0.037, 0.017)	0.477	**-0.046 (-0.077, -0.014)**	**0.004****
	Ground nesting	-0.002 (-0.028, 0.025)	0.901	0.016 (-0.015, 0.046)	0.318
	Territoriality	0.000 (-0.036, 0.037)	0.985	0.015 (-0.025, 0.056)	0.459
	Community diversity	**0.089 (0.057, 0.122)**	**<0.001*****	**0.106 (0.069, 0.144)**	**<0.001*****
Volume (VS)	(Intercept)	0.236 (-0.514, 0.979)	0.531	0.316 (-0.434, 1.059)	0.406
	Temperature	-0.005 (-0.050, 0.039)	0.818	-0.011 (-0.063, 0.041)	0.684
	Precipitation	**0.062 (0.027, 0.099)**	**0.001*****	**0.109 (0.067, 0.151)**	**<0.001*****
	Net primary productivity	**0.035 (0.001, 0.071)**	**0.047***	**0.067 (0.026, 0.108)**	**0.001****
	Solar radiation	**0.042 (0.006, 0.077)**	**0.021***	0.035 (-0.005, 0.078)	0.097
	UV-B radiation	-0.026 (-0.063, 0.011)	0.167	-0.038 (-0.081, 0.005)	0.085
	Body mass	**-0.175 (-0.219, -0.130)**	**<0.001*****	**-0.170 (-0.219, -0.120)**	**<0.001*****
	Sexual dichromatism	**0.233 (0.203, 0.262)**	**<0.001*****	**-0.050 (-0.084, -0.017)**	**0.003****
	Forest dependency	**0.030 (0.004, 0.056)**	**0.021***	**0.066 (0.035, 0.096)**	**<0.001*****
	Frugivore-nectarivore	0.017 (-0.015, 0.049)	0.310	0.031 (-0.005, 0.069)	0.094
	Migratory	-0.003 (-0.030, 0.024)	0.826	**-0.043 (-0.075, -0.012)**	**0.008****
	Ground nesting	0.003 (-0.025, 0.029)	0.848	0.019 (-0.013, 0.050)	0.240
	Territoriality	-0.001 (-0.037, 0.035)	0.949	0.021 (-0.019, 0.062)	0.303
	Community diversity	**0.090 (0.057, 0.123)**	**<0.001*****	**0.110 (0.072, 0.148)**	**<0.001*****

Supplementary Table 5. Bayesian phylogenetic mixed model results for the effect of predictor variables on male and female colour volume scores across passerine species (*n* = 4,415). All variables were standardised (mean = 0, sd = 1) prior to model fitting. UVS, ultraviolet sensitive; VS, violet sensitive. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. All models were run over 100 posterior phylogenetic trees.

Variable	Term[a]	Estimate (95% CI)	P_{MCMC}
Volume (UVS)	Absolute latitude	-0.059 (-0.082, -0.036)	<0.001***
	Sex	-0.111 (-0.123, -0.100)	<0.001***
	Absolute latitude x Sex	-0.003 (-0.015, 0.009)	0.601
Loci (UVS)	Absolute latitude	-0.066 (-0.090, -0.042)	<0.001***
	Sex	-0.094 (-0.103, -0.084)	<0.001***
	Absolute latitude x Sex	-0.018 (-0.027, -0.008)	<0.001***
Volume (VS)	Absolute latitude	-0.069 (-0.092, -0.045)	<0.001***
	Sex	-0.112 (-0.124, -0.099)	<0.001***
	Absolute latitude x Sex	-0.004 (-0.017, 0.008)	0.488
Loci (VS)	Absolute latitude	-0.081 (-0.104, -0.058)	<0.001***
	Sex	-0.099 (-0.110, -0.089)	<0.001***
	Absolute latitude x Sex	-0.081 (-0.028, -0.008)	<0.001***

Supplementary Table 6. Bayesian phylogenetic mixed model results for the effects of absolute latitude and sex on male and female colourfulness across passerine species (*n* = 4,527 species). All variables were standardised (mean = 0, sd = 1) prior to model fitting. UVS, ultraviolet sensitive; VS, violet sensitive. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. All models were run over 100 posterior phylogenetic trees. [a], For Sex, 'Male' is the reference category.

		Males		Females	
Variable	**Term**	**Estimate (95% CI)**	**P_{MCMC}**	**Estimate (95% CI)**	**P_{MCMC}**
Loci (UVS)	(Intercept)	-0.119 (-0.765, 0.556)	0.715	-0.201 (-0.890, 0.488)	0.554
	Temperature	0.036 (-0.204, 0.279)	0.769	0.141 (-0.126, 0.407)	0.302
	Precipitation	-0.090 (-0.237, 0.056)	0.228	-0.024 (-0.186, 0.139)	0.770
	Net primary productivity	0.133 (-0.009, 0.275)	0.066	0.118 (-0.041, 0.277)	0.146
	Solar radiation	-0.003 (-0.194, 0.186)	0.971	-0.140 (-0.352, 0.069)	0.191
	UV-B radiation	0.088 (-0.145, 0.316)	0.455	0.055 (-0.203, 0.310)	0.671
	Body mass	**-0.226 (-0.329, -0.124)**	**<0.001*** **	**-0.203 (-0.314, -0.092)**	**<0.001*** **
	Sexual dichromatism	**0.327 (0.248, 0.405)**	**<0.001*** **	0.006 (-0.079, 0.091)	0.891
	Social mating system	-0.028 (-0.111, 0.055)	0.504	-0.022 (-0.112, 0.070)	0.640
	Forest dependency	**0.083 (0.012, 0.155)**	**0.023***	**0.121 (0.042, 0.200)**	**0.003****
	Frugivore-nectarivore	0.053 (-0.027, 0.132)	0.193	0.061 (-0.026, 0.148)	0.165
	Migratory	0.021 (-0.067, 0.110)	0.644	-0.055 (-0.153, 0.042)	0.268
	Ground nesting	-0.004 (-0.071, 0.065)	0.920	0.003 (-0.071, 0.078)	0.946
	Territoriality	**-0.096 (-0.177, -0.013)**	**0.022***	-0.036 (-0.125, 0.055)	0.437
	Community diversity	0.080 (-0.022, 0.185)	0.126	**0.116 (0.000, 0.230)**	**0.048***
Volume (UVS)	(Intercept)	-0.164 (-0.787, 0.473)	0.603	-0.247 (-0.874, 0.376)	0.424
	Temperature	0.082 (-0.152, 0.315)	0.492	0.022 (-0.249, 0.297)	0.873
	Precipitation	-0.092 (-0.232, 0.049)	0.201	-0.083 (-0.247, 0.083)	0.323
	Net primary productivity	**0.146 (0.010, 0.283)**	**0.035***	**0.223 (0.064, 0.387)**	**0.006****
	Solar radiation	0.059 (-0.125, 0.242)	0.529	-0.008 (-0.223, 0.209)	0.944
	UV-B radiation	-0.026 (-0.249, 0.196)	0.819	0.024 (-0.240, 0.286)	0.855
	Body mass	**-0.247 (-0.345, -0.148)**	**<0.001*** **	**-0.237 (-0.345, -0.127)**	**<0.001*** **
	Sexual dichromatism	**0.288 (0.211, 0.363)**	**<0.001*** **	-0.059 (-0.146, 0.026)	0.179
	Social mating system	0.041 (-0.039, 0.120)	0.316	0.003 (-0.088, 0.094)	0.955
	Forest dependency	0.053 (-0.017, 0.121)	0.134	0.056 (-0.025, 0.137)	0.175
	Frugivore-nectarivore	0.059 (-0.018, 0.136)	0.132	0.074 (-0.012, 0.163)	0.094
	Migratory	0.004 (-0.081, 0.090)	0.933	-0.080 (-0.181, 0.020)	0.117
	Ground nesting	0.005 (-0.060, 0.071)	0.875	0.018 (-0.058, 0.094)	0.650
	Territoriality	**-0.116 (-0.195, -0.037)**	**0.004****	-0.040 (-0.130, 0.050)	0.382
	Community diversity	0.096 (-0.002, 0.196)	0.057	**0.133 (0.016, 0.250)**	**0.026***

Supplementary Table 7. Bayesian phylogenetic mixed model results for the effect of predictor variables on male and female colour loci scores across passerine species (*n* = 608), including the effect of social mating system. All variables were standardised (mean = 0, sd = 1) prior to model fitting. UVS, ultraviolet sensitive; VS, violet sensitive. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. All models were run over 100 posterior phylogenetic trees.