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RESEARCH ARTICLE

Climate underpins continent-wide patterns of carotenoid-based feather colour consistent with Gloger's observations

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Abstract

1. Animal coloration has long been predicted to vary across geographic and climatic gradients in accordance with a long-standing ecogeographical rule. But further to his widely supported predictions that melanin pigmentation increases towards the Equator, Gloger observed that reds and yellows are more vivid in warm regions and thus more prevalent at lower latitudes, a prediction supported further by Görnitz, who suggested these colours would be more intense in areas with higher rainfall. Yet, studies of the associations between geography or climate and carotenoid-based plumage coloration to test these observations at a continental scale are scarce.
2. Here, we investigated the extent to which yellow and red feather colour varies according to these hypotheses in *Pogoniulus* tinkerbirds with distributions across sub-Saharan Africa. We tested first for associations of feather colour with geography along latitudinal or elevational gradients, and then for associations with the climatic factors of rainfall and temperature that may underpin colour variation on continental scales.
3. We find evidence consistent with Gloger and Görnitz's observations that more saturated colours and warmer hues at lower latitudes were primarily attributed to a relationship of underpart colour with temperature and rainfall. By contrast, forecrown colour, a trait previously associated with sexual selection, had a more complex association with geography and climate, with red forecrown hue associated with rainfall, but red and yellow intensity showing contrasting patterns with latitude.
4. We highlight the complex nature of carotenoid-based plumage coloration, suggesting that although environmental factors affect the abundance of carotenoid availability, plumage coloration is also influenced by other selective pressures.

KEYWORDS
carotenoid pigments, climate variation, ecogeographical rules, geographic patterns, latitudinal gradients, *Pogoniulus* tinkerbirds

1 | INTRODUCTION

Phenotypic plasticity in response to environmental conditions can have pervasive influences on trait differences between populations (Price et al., 2003; Xue & Leibler, 2018), in addition to the underlying spatial heterogeneity in trait values that results from selection and genetic drift (Coyne & Allen Orr, 2004; Endler, 1991; Endler & Houde, 1995). Ecogeographic rules have been proposed to correlate such patterns of phenotypic variation (Allen, 1877; Bergmann, 1847; Delhey, 2018, 2019; Gaston et al., 2008; Gloger, 1833; Mayr, 1956; Rensch, 1929) and are often cited as evidence of adaptation driving phenotypic variation. For example, latitudinal variation in body size in vertebrates, including in birds and mammals, has been relatively well documented, e.g. in accordance with Bergmann's or Allen's rule (Gutiérrez-Pinto et al., 2014; Sebastianelli et al., 2022; Stanchak & Santana, 2019; Symonds & Tattersall, 2010; Youngflesh et al., 2022). There is also abundant evidence for climate-associated adaptation in melanin-based coloration (Cerezer et al., 2024; Hoekstra & Nachman, 2003; Kettlewell, 1973). They follow the premise that broad-scale variation in pelage or plumage traits of animals in warm and humid environments tend to be darker than those in cool and arid habitats for protection against parasites and predators (Gloger, 1833; Delhey, 2019). This ecogeographic pattern—'Gloger's rule' (Delhey, 2018; Galván et al., 2018; Gloger, 1833; Rensch, 1929)—has been studied in the context of melanin-based plumage and coat colours, which are hypothesized to be under strict genetic control (Ducrest et al., 2008) and thus less affected by environmental variation (Mundy, 2005; Nachman et al., 2003). Conversely, variation in carotenoid-based coloration is predominantly influenced by the combination of environmental—carotenoid-based colours are predicted to saturate in areas of high productivity, such as wet and warm areas—and genetic regulators (Hill & McGraw, 2006). Despite this, the effects of geographic and environmental variables on carotenoid-based plumage coloration have seldom been examined at continental scales.

Carotenoids are pigments responsible for the vibrant yellow, pink, orange and red colours observed in birds, and play an important role in visual communication and sexual selection (Hill, 1991). Unlike plants, fungi and bacteria, birds are unable to synthesize carotenoid pigments endogenously and therefore obtain them through their diet (Goodwin, 1984; Saks et al., 2003). Once obtained, some species metabolize these pigments and deposit them into their integuments and plumage, while others deposit them unchanged (Hill & McGraw, 2006). While most yellow plumage primarily arises from the direct deposition of dietary carotenoids with little or no chemical modification, red carotenoid-based coloration typically results from biochemical reactions (Higginson et al., 2016) and cellular processes at the mitochondrial level (Hill et al., 2019), catalysed by specific enzymes (Lopes et al., 2016; Mundy et al., 2016). As such, variation in the quantity and quality of diet affects the type and amount of carotenoids deposited in plumage (Inouye et al., 2001) and can therefore result in diversity in carotenoid-based plumage coloration between and within species (Friedman & Remeš, 2017; Goodwin, 1984; Hill, 2002, 2008).

The variation in the distribution of plumage colour led Gloger to propose that red and yellow colours (since found to have a carotenoid basis) tended to be heavily pigmented towards the tropics where temperatures were higher (Gloger, 1833). Görnitz later added that carotenoid colours would be more intense in areas with higher rainfall (Görnitz, 1923). Gloger suggested that this relationship of greater pigmentation towards the tropics would be more evident in yellows than in reds (Prasetya et al., 2020). The pattern of greater latitudinal effect on yellows than reds is predicted to be more pronounced in humid, high primary productivity regions like the wet tropics, which are expected to harbour species that display more highly saturated yellow carotenoid-based colours (Delhey et al., 2023), as well as in highly productive temperate regions (Zhang et al., 2017). Carotenoid-based colour is also predicted to be influenced by temperature, which also varies along latitudinal and elevational gradients (Prasetya et al., 2020). Studies examining these factors in birds in accordance with Gloger's and Görnitz's observations have yielded conflicting results. For instance, research on Flame-coloured Tanagers *Piranga bidentata* has shown that rainfall negatively affected brightness yet positively affected plumage chroma, in line with Görnitz's observations (Robles-Bello et al., 2022). However, Chui and Doucet (2009) found no correlation between evapotranspiration and crown coloration in Golden-crowned Kinglets *Regulus satrapa*, suggesting that primary productivity is not directly linked with carotenoid abundance. These divergent findings open avenues to investigate how environmental factors drive intraspecific and interspecific variation in carotenoid-based colours.

Here, we test the extent to which continental-scale carotenoid-based plumage colour variation in several species of *Pogoniulus* tinkerbirds can be predicted by geography, and its underlying environmental variation, across sub-Saharan Africa. Tinkerbirds occur across a range of habitats and elevations, and variation in song and morphology has been associated with environmental gradients (Kirschel et al., 2009; Sebastianelli et al., 2022). We focused on the geographic factors of latitude and elevation, and separately on the environmental variables of temperature and rainfall that vary at continental scales, investigating their effects on carotenoid-based plumage coloration in four plumage patches (belly, breast, rump and crown) measured using reflectance spectrometry on museum skins. We hypothesized that carotenoid-based pigments in tinkerbird feathers would exhibit greater intensity (i.e. higher plumage hue and chroma but lower plumage brightness) in regions closer to the equator where there is high precipitation and warmer climates (Figure 1), and as a result, higher primary productivity.

2 | MATERIALS AND METHODS

2.1 | Study species

We focused on tinkerbird species with wide and/or complementary distributions across sub-Saharan Africa: yellow-throated tinkerbird *Pogoniulus subsulphureus*, yellow-rumped tinkerbird

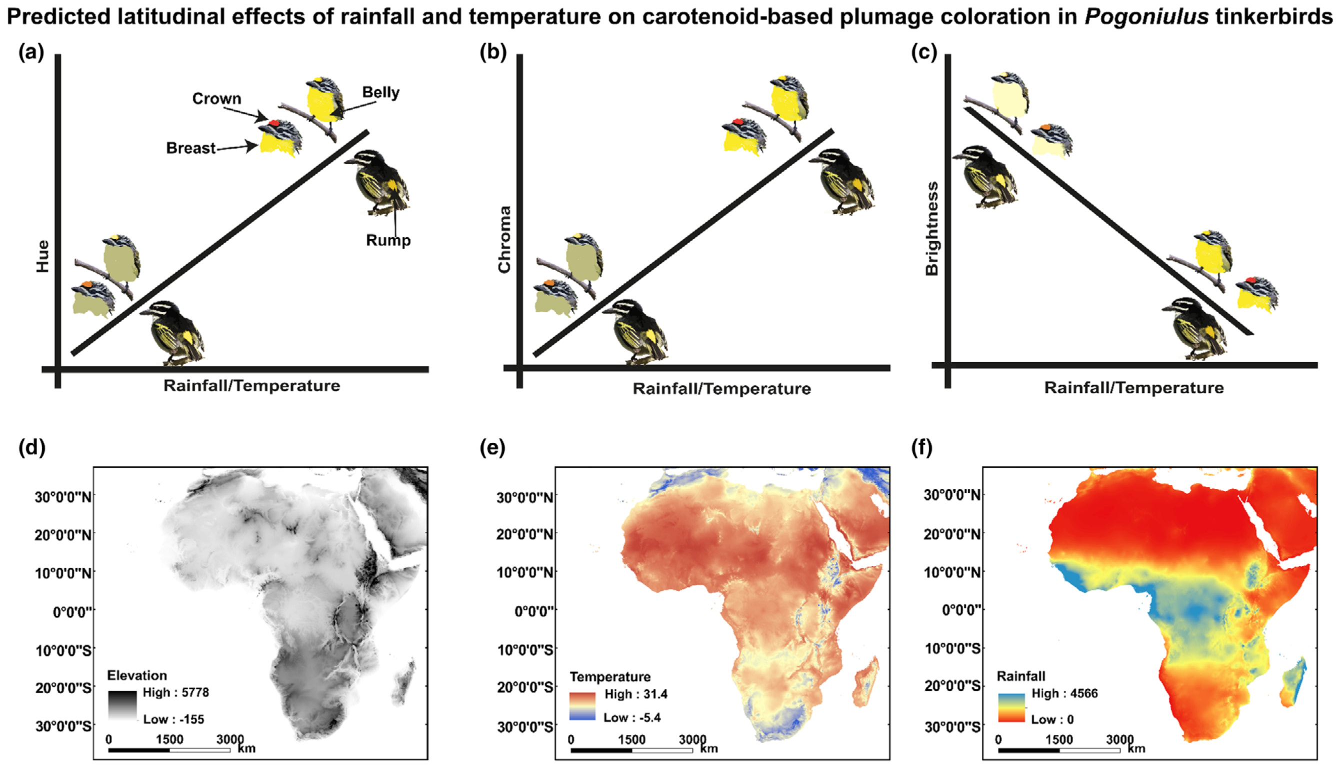

FIGURE 1 A conceptual illustration of the predicted latitudinal effects of temperature and rainfall on carotenoid-based colour of the breast, belly, rump, and crown in *Pogoniulus* tinkerbirds based on measures of (a) hue, (b) chroma and (c) brightness. Birds inhabiting locations, e.g. at (d) lower elevations with (e) higher temperatures and (f) more rainfall are predicted to exhibit high carotenoid saturation (higher-wavelength hue and more intense chroma) compared to areas with lower values, whereas with an increase in temperature and precipitation, brightness is expected to decline. Maps produced in ArcMap 10.7.1 using elevation data from the shuttle radar topography mission and climatic data (temperature and rainfall) from the WorldClim database.

Pogoniulus bilineatus, yellow-fronted tinkerbird *Pogoniulus chrysoconus* and the recently split Northern and Southern red-fronted tinkerbird *Pogoniulus uropygialis* and *Pogoniulus pusillus* (Short & Horne, 2024a, 2024b). The study taxa vary both between and within species in their plumage coloration, with underpart plumage ranging from carotenoid-based yellow to grey-olive and white. *P. pusillus*, *P. uropygialis and P. chrysoconus* differ from the other two taxa in exhibiting a carotenoid-based colour forecrown patch (Kirschel, Nwankwo, Pierce, et al., 2020). Furthermore, the taxa vary in their habitat preferences both between and within species and can be found from pristine tropical rainforest, dry forest and secondary forest and gardens to moist savanna and arid savanna/thorn scrub. While tinkerbirds are chiefly frugivorous, they also forage for insects, such as wood-boring beetle larvae (Lukhele et al., 2022; Short & Horne, 2001).

2.2 | Reflectance spectra measurements and colour analysis

We took reflectance spectra from 575 museum skins (*P. bilineatus*: 226, *P. chrysoconus*: 139, *P. uropygialis: 98*, *P. pusillus*: 28 and *P. subsulphureus*: 83) loaned from or examined at several ornithology collections (see Acknowledgements), of which the *P. subsulphureus* and *P. bilineatus* spectra had previously been used towards studies focusing on regional patterns of variation in those species (Kirschel, Nwankwo, Seal, et al., 2020; Nwankwo et al., 2018). From each study skin, we obtained reflectance spectra of feathers in the 300–700 nm visual range from the breast, belly, rump and crown (the latter where appropriate) of *P. pusillus*, *P. uropygialis*, *P. chrysoconus*, *P. subsulphureus*, and 195 of the *P. bilineatus* study skins using an OceanOptics USB 2000+ diode-array spectrometer, xenon strobe light source (Ocean Optics PX-2), and a fibre-optic reflectance probe (Ocean Optics R-400), with the probe angled 45° to the feather surface. For the remaining 31 of the *P. bilineatus*, from populations in Eastern and Southern Africa, we obtained reflectance spectra from the belly, breast and rump of museum study skins using a JAZ spectrometer (Ocean Optics) with a fibre-optic reflectance probe (Ocean Optics R-200) and PX xenon light source. The reflectance probe was placed in an RPH-1 Reflection Probe Holder (Ocean Optics), at a 90° angle and secured at 2 mm from the aperture of the probe holder. Two measurements were taken per plumage patch, per specimen with the specimen placed flat on a white background perpendicular to the observer and facing to the left, and then rotated 180° for the second measurement, with the probe holder placed horizontally onto the specimen. Each reflectance spectrum was measured relative to the reflectance of a WS-2 white standard (OceanOptics) and a black standard by blocking the light path of the spectrometer by replacing its cap.

We utilized the R package *pavo* (Maia et al., 2013) to average the duplicate measurements from each plumage patch (Figure S1). We smoothed the spectra with a smoothing parameter of 0.25 and used '*addmin*' to correct negative values. We then computed three colour components: brightness—computed as the mean percentage reflectance over the entire reflectance spectrum for the measured feather surface (mean brightness: B2 in pavo), chroma (S9—carotenoid chroma, which quantifies the contribution of carotenoid pigments in the overall colour, therefore is a good proxy for the degree of colour saturation due to carotenoids), and hue (H3—the principal colour reflected by the feathers and measured as the wavelength at which reflectance is halfway between its minimum and maximum). These three values were used as response variables to test for the effects of environmental and geographic factors on plumage coloration in tinkerbirds. There is a distinction, however, in which measures represent carotenoid-based coloration. Hue and chroma are chromatic measures that in tinkerbirds are expected to be associated with uptake of dietary carotenoids and therefore associated with yellow and red colours (Kirschel, Nwankwo, Pierce, et al., 2020). Many carotenoid-based plumage reflectance spectra exhibit a secondary reflectance peak in the UV region (300–400 nm). Hue values computed near 350 nm can therefore be misleading if interpreted as a direct measure of carotenoid deposition. For example, a high UV reflectance peak might result in a low hue value, but this does not necessarily indicate low carotenoid content. Instead, the values could reflect structural coloration and/or lack of carotenoid pigment in those feather patches. Brightness or luminance on the other hand, is achromatic, and variation in this measure may be influenced by the distribution of melanin pigments as well as carotenoid pigments in the feathers (Hegyi et al., 2007).

2.3 | Environmental variables

We assessed the influence of geographic and bioclimatic variables on plumage colour variation by first extracting environmental variables for each specimen locality using the global positioning system (GPS) coordinates of the museum specimens where available. When localities of specimens were not provided, the locations where specimens were collected were established using museum tags, maps, gazetteers and Google Earth (Google.Inc), following methods used in Kirschel, Nwankwo, Seal, et al. (2020). Each locality was assigned an approximate latitude, longitude and elevation. We used the WorldClim database (Fick & Hijmans, 2017) to obtain bioclimatic variables that included annual rainfall (BIO 12, mm), mean annual temperature (BIO 1, °C) and elevation data obtained from the global Shuttle Radar Topography Mission (SRTM, m) (Farr et al., 2007), at a spatial resolution of 2.5 arc-minutes. Furthermore, we used the *extract values to points* function in ArcMap 10.7.1 (ESRI, 2019) to extract environmental data from the bioclimatic variable rasters. Because the localities from some museum specimens returned null values, we interpolated all individual cell values with those from the surrounding eight cells.

2.4 | Statistical analysis

To test the hypothesis that carotenoid-based colours will be more intense (high plumage hue, chroma and low plumage brightness) in low latitudes and elevation in accordance with Gloger's and Görnitz observations on carotenoid-based plumage colours, we fitted generalized least squares (GLS) models implemented in the *nlme* package (Pinheiro et al., 2024) in R (version 4.3.1) with the geographic variables absolute latitude and elevation, taxon and sex (male or female) as fixed effects and hue, chroma, and brightness computed from the reflectance spectra for each body patch as response variables.

We then tested for the specific climatic predictors associated with carotenoid-based colour variation distributed across the landscape, using mean annual temperature and annual rainfall to test the relative effects of the climatic factors implicated by Gloger (1833) and Görnitz (1923) to predict carotenoid-based colour variation in birds. We fitted a model that included mean annual temperature, annual rainfall, taxon and sex as fixed effects, again using hue, chroma and brightness as response variables. Since carotenoid chroma is primarily designed to measure pigment deposition in yellow carotenoid-based colours, we thus fitted models that used carotenoid chroma as a response variable for only *P. chrysoconus*. For the models on crown colour, we assigned taxa according to crown colour to distinguish between possible effects on yellows and reds separately, since red feather colour has a genetic basis (Kirschel, Nwankwo, Pierce, et al., 2020). The crown patch models differed in the inclusion of an interaction between taxa and the geographic or climatic predictors to test for possible differences in their effects on reds and yellows (for hue and brightness), as suggested by Gloger. In both the geographic and climatic models, we accounted for spatial autocorrelation between data points by including a Gaussian spatial correlation structure by fitting a correlation term (~longitude + latitude).

Because shades of colour patches may vary between study species, species-level effects may obscure bioclimatic effects in a multiple-taxon model. Conversely, effects apparent at a multi-taxon level might not reflect selective pressures within each taxon, and instead reflect wider geographic and environmental scale effects that might only be revealed across taxa. Further, respective species distributions could occur at different extremes of ecological gradients; therefore, potentially obscuring bioclimatic effects. We thus also tested for geographic and bioclimatic effects for each taxon separately by fitting GLS models to account for spatial dependence of points. For *P. subsulphureus* and *P. bilineatus*, we tested for geographic variation in carotenoid-based colour by fitting a geographic model including absolute latitude, elevation, sex and taxa as fixed effects. To test for the variation in colour due to climatic effects, we fitted a model that included annual rainfall, mean annual temperature, sex and taxa as predictor variables. Models specifically for *P. pusillus*, *P. uropygialis* and *P. chrysoconus* did not include taxa as a fixed effect. For models testing for effects of geographic and climatic factors on colour traits in these species, we merged *P. chrysoconus*, *P. uropygialis* and *P. pusillus* into a single taxon (hereafter *chrysoconus-pusillus* combined) for two reasons. First, in areas where two of these species meet in sympatry, they frequently interbreed

(Nwankwo et al., 2019). This suggests that numerous individuals possess a mix of ancestral traits in different proportions, making it difficult to assign them to one species or the other. Second, phylogenetic analyses suggest *P. chrysoconus* is not monophyletic with respect to the other two taxa (Kirschel et al., 2021; Nwankwo et al., 2019), thus undermining analysis at the currently recognized species level. *P. bilineatus* and *P. subsulphureus*, on the other hand, are each represented by distinct taxa that are geographically, and in specific traits, phenotypically nonoverlapping (Kirschel, Nwankwo, Seal, et al., 2020). We deemed it necessary to account for these distinct taxa separately in models. We thus separated West African *P. subsulphureus chrysopygus* with their white head markings and paler underpart coloration from Central African *P. subsulphureus flavimentum* with yellow head markings and darker underpart coloration, divided by the Dahomey Gap (Kirschel, Nwankwo, Seal et al., 2020), and Northern and Western populations of *P. bilineatus* formerly known as lemon-rumped tinkerbird (hereafter *leucolaimus*) from Southern and Eastern populations of *P. bilineatus* formerly known as golden-rumped tinkerbird (hereafter bilineatus), divided primarily by the arid corridor of the East African Rift (Ogolowa et al., 2024; Short & Horne, 2001).

We thus fitted taxon-specific models separately for *P. subsulphureus* (with a taxon effect factor to account for differences among subspecies), *P. bilineatus* (with a taxon effect factor to account for differences among subtaxa; see Ogolowa et al., 2024), and *chrysoconus-uropygialis-pusillus* combined into a single taxon (with no taxon effect factor).

We transformed rainfall and elevation data because they were in different orders of magnitude; therefore, ensuring that they are on the same scale. For the rainfall data, we divided each value by 10; thus, effects were calculated on the basis of cL, and for elevation, we divided each value by 100; thus, effects were calculated on a 100 m scale. We checked for multicollinearity between the predictor variables by examining variance inflation factors (VIF) with the *usdm* (Naimi, 2017) R package. Specifically, we used the *vifstep* function, which calculates VIF for all variables, then removes the one with the greatest VIF that exceeds the 2.5 threshold and repeats the operation until no covariate with VIF > 2.5 remains. However, in our models, none of the predictors had a VIF > 2.5 (Table S1). Furthermore, to assess model assumptions and fit, we used performance (Lüdecke et al., 2021) R package. Significant effects were those with $p < 0.05$. All models and statistical tests were performed in R v.4.3.1 (Core Team, 2021).

To further assess whether variation in carotenoid-based coloration covaries consistently with absolute latitude, elevation, mean annual temperature and annual rainfall across different taxa, we also employed a meta-analytical approach. For each taxon and across various body patches, we modelled the effects of (a) geographic variables, including absolute latitude and elevation; and (b) climatic variables, including mean annual temperature and annual rainfall, on hue, chroma and brightness as response variables. We again controlled for spatial autocorrelation through a Gaussian correlation structure using geographic coordinates. We estimated the effect size along with its associated uncertainty (standard error, SE) in each model. These estimates were used as inputs for the meta-analysis, where we weighted each effect size by the inverse of its variance ($1/SE^2$) to account for the precision of each taxon's effect. The weighted estimates were then combined using linear mixed-effects models to derive an overall effect size across taxa for each environmental variable. All meta-analyses were conducted in R using the *metafor* package (Viechtbauer, 2010).

3 | RESULTS

3.1 | Geographic patterns in underpart carotenoid-based coloration are consistent with Gloger's observations

Tinkerbirds exhibited warmer hues and more intense chroma in the belly and breast closer to the Equator, consistent with Gloger's observations (Figures 2 and 3). Specifically, there was a negative association of hue, chroma and brightness with absolute latitude for both underpart colour patches (Tables S2–S4). Effects of elevation on underpart hue and chroma were less evident, with only breast chroma decreasing with elevation (Table S3), but brightness decreased strongly at higher levels in both patches (Table S4).

Meta-analyses revealed the latitudinal patterns in underpart coloration were not consistent across each taxon (Figure 4, Figures S2 and S3, Tables S5 and S6). Although underpart hue, chroma and brightness within the taxon with the widest latitudinal range, *chrysoconus-pusillus* combined, were negatively associated with absolute latitude (nonsignificant for breast hue; Figure S4, Table S8), this pattern was not reflected in the other taxa (Figures S5 and S6, Tables S9 and S10). The negative effect of elevation on breast brightness was consistent across taxa, and although found for *chrysoconus-pusillus* and *bilineatus* belly brightness, it was not evident in *subsulphureus* (Figure S6, Table S10).

3.2 | Rump coloration shows no consistent geographic pattern

There was no association of any geographical variable with rump colour across taxa, and this was confirmed in the meta-analysis (Figure S4, Table S7). Any differences observed were among taxa, something that was evident in most traits examined, reflecting that *bilineatus* rumps had higher-wavelength hue, more intense chroma, and lower brightness than the other taxa in most models (Figure 4, Table S4). Within taxa, rump colour differences were most evident in *bilineatus*, between the two groups *leucolaimus* and *bilineatus* mostly differentiated by observers by rump colour (described as lemon and golden rumps; Kirschel, Nwankwo, Seal, & Grether, 2020), but *bilineatus* rump chroma also increased with latitude and elevation (Figure S6, Table S9). There were also taxon-based differences between *subsulphureus* ssp. with *chrysopygus* exhibiting higher-wavelength hue and more intense chroma than *flavimentum* (Figure S7, Table S10). The only difference in rump

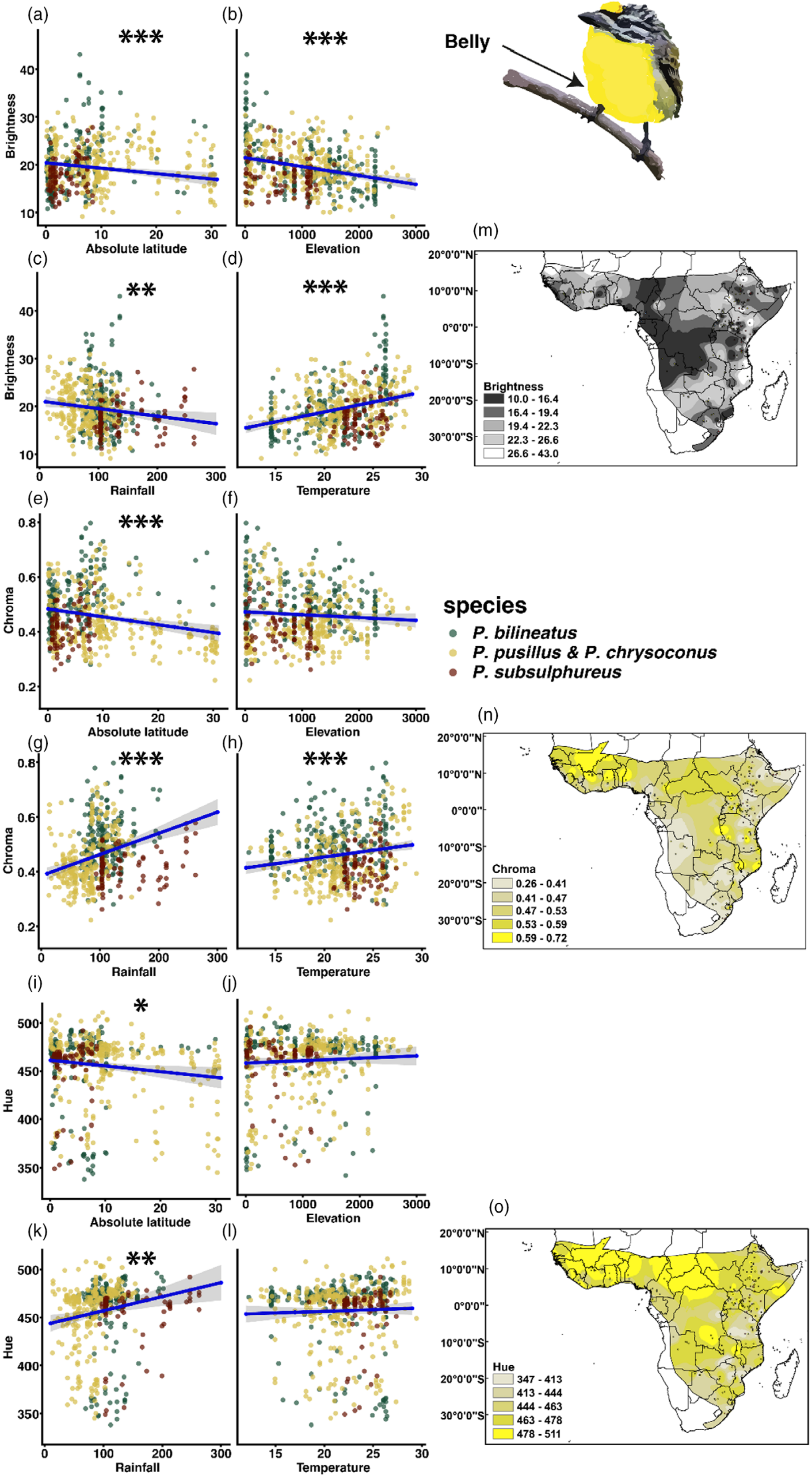

FIGURE 2 Plots showing the model effects of the geographic model (absolute latitude and elevation) and climatic model (annual rainfall and mean annual temperature) on belly patch brightness (a–d); belly patch chroma (e–h); and belly patch hue (i–l). Asterisks signify statistically significant effects (***=<0.001; **=<0.01 and *=<0.05), shading represents 95% CI. Interpolated Inverse Distance Weighting (IDW) maps from ArcMap 10.7.1 illustrate the variation of belly patch colour in tinkerbirds across their distribution range in (m) brightness, (n) chroma and (o) hue.

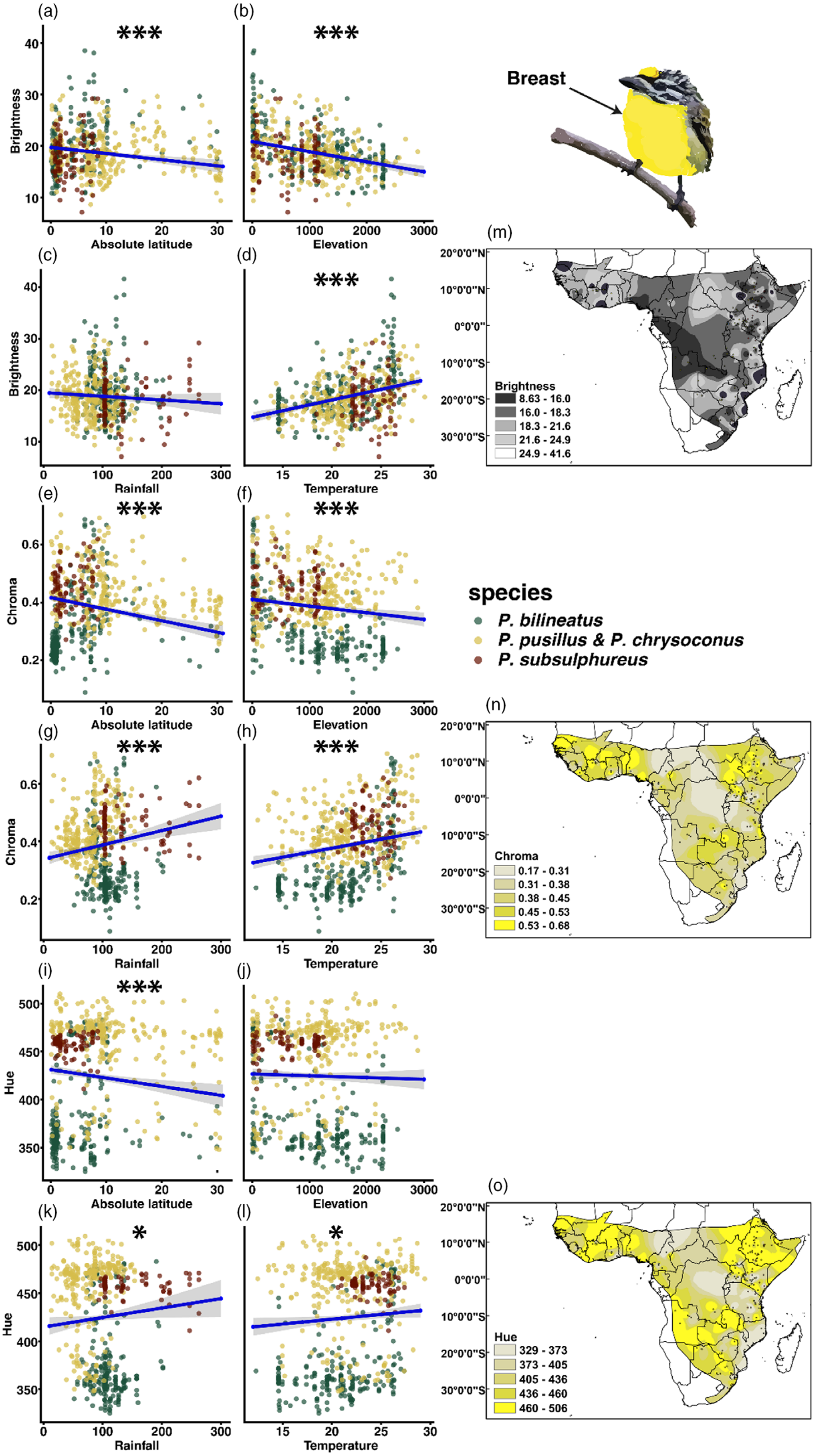

FIGURE 3 Plots showing the model effects of the geographic model (absolute latitude and elevation) and climatic model (annual rainfall and mean annual temperature) on breast patch brightness (a–d); breast patch chroma (e–h); and breast patch hue (i–l). Asterisks signify statistically significant effects (***=<0.001; *=<0.05), shading represents 95% CI. IDW maps from ArcMap 10.7.1 illustrate the variation of breast patch colour in tinkerbirds across their distribution range in (m) brightness, (n) chroma and (o) hue.

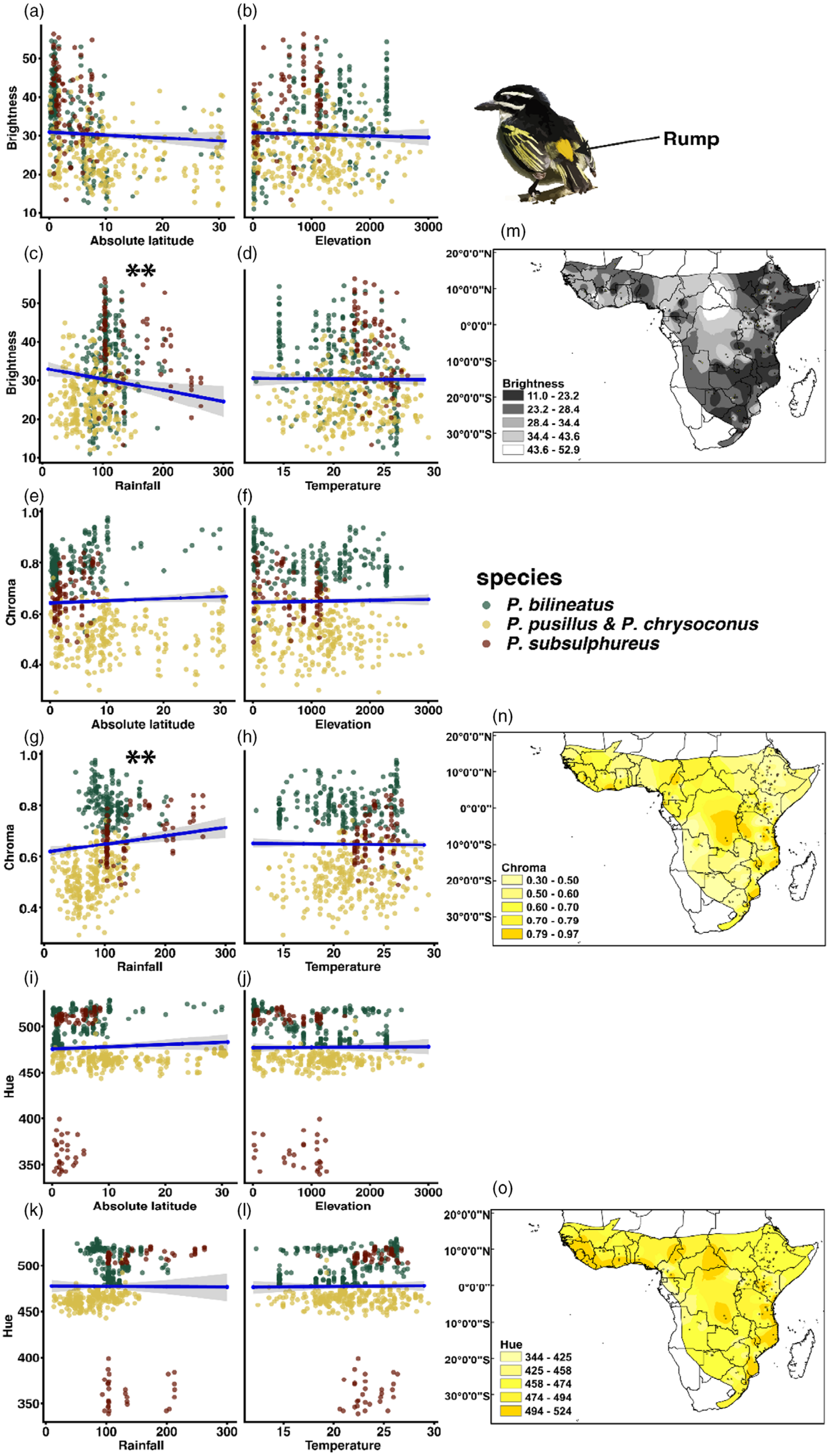

FIGURE 4 Plots showing the model effects of the geographic model (absolute latitude and elevation) and climatic model (annual rainfall and mean annual temperature) on rump patch brightness (a–d); rump patch chroma (e–h); and rump patch hue (i–l). Asterisks signify statistically significant effects (** = <0.01), shading represents 95% CI. IDW maps from ArcMap 10.7.1 illustrate the variation of rump patch colour in tinkerbirds across their distribution range in (m) brightness, (n) chroma and (o) hue.

colour attributable to sex was within species, with *bilineatus* males exhibiting less intense chroma than *bilineatus* females (Figure S6, Table S9).

3.3 | Feather colour is affected by climate

Environmental gradients underpin geographic patterns of variation in carotenoid-based coloration. Rainfall was associated with higher-wavelength hue and more intense chroma in both the belly (Figure 2, Tables S2 and S3) and breast (Figure 3, Tables S2 and S3), while rump chroma was also higher, and belly and rump brightness lower where wetter (Figure 4, Tables S3 and S4). There were, however, no associations of rainfall with breast brightness (Figure 3, Table S4) or rump brightness (Figure 4, Table S4). Temperature was also positively associated with breast and belly chroma and brightness, but not hue, and there were no associations of temperature with rump colour (Figures 2–4, Tables S3 and S4).

Some environmental effects were consistently found among taxa as well. Meta-analyses found consistent positive relationships of both rainfall and temperature with belly chroma and consistent effects of rainfall on belly hue across taxa (Figure S2, Table S5), but no consistent environmental effects on rump colour (Figure S7, Table S7). More specifically, within taxa, rainfall was associated with higher-wavelength belly, breast and rump hue and chroma, and lower brightness in *chrysoconus-pusillus* combined (Figure S4, Table S8), higher belly and breast chroma in *bilineatus* (Figure S5, Table S9), and higher rump chroma in *subsulphureus*, although in the latter we also found the reverse association with rainfall of lower breast hue and chroma (Figure S6, Table S8). Within species, temperature had a positive effect on *bilineatus* breast hue, breast and rump chroma, and belly and breast brightness (Figure S5, Table S9), and on *chrysoconus-pusillus* (combined) belly and breast chroma and brightness (Figure S4, Table S8), but no effect was seen of temperature on colour in *subsulphureus* (Figure S6, Table S10).

3.4 | Geographic and environmental effects on crown plumage

Opposite to Gloger's observation of yellows and reds increasing in intensity at lower latitudes, absolute latitude had a positive effect on crown hue, with reds especially increasing in hue wavelength. There was also a positive effect of latitude on crown chroma, but a negative interaction effect with brightness decreasing with latitude in red crowned birds (Figure 5, Table S11). There was no pattern with elevation nor with temperature on crown hue, chroma, or brightness. Rainfall, however, had a negative effect on crown chroma but a positive interaction effect on crown hue in red-fronted tinkerbird, with higher-wavelength hue with more rainfall compared to *P. chrysoconus*. Not surprisingly, all models found differences between red-fronted and yellow-fronted taxa, with red-fronted exhibiting higher-wavelength hues and lower brightness in the climate model, and within the aforementioned negative interaction with latitude in the geographic model (Figure 5, Table S11).

4 | DISCUSSION

Our study revealed continent-wide patterns of variation in carotenoid-based colour consistent with Gloger's and Görnitz's observations, but the pattern was not evident in all traits examined. The geographic pattern of more intense colours and warmer hues at lower latitudes was underpinned by an especially strong and consistent relationship of feather colour with rainfall, as proposed by Görnitz (1923). Feather patches were also brighter at lower latitudes, and this trait was more closely associated with temperature, but in this case, opposite to Gloger's prediction that colours would be more chromatic towards the Equator. These patterns were most apparent in the continuously distributed colours we examined on the underparts of tinkerbirds (belly and breast colours), less so on the dichotomous rump and crown colours that distinguish taxa.

Indeed, crown hue increased in wavelength with latitude (i.e., birds farther from the equator had deeper red crowns, though this may reflect the fact *P. pusillus* occupies wetter forest in Southern Africa than *P. uropygialis* in dry woodland in East Africa). Yellow feathers crown chroma increased with latitude opposite to Gloger's expectations that yellow feathers will exhibit greater pigmentation towards the tropics. The conversion of yellow carotenoids to red ketocarotenoids in tinkerbird crown feathers is genetically determined (Kirschel, Nwankwo, Pierce, et al., 2020), and thus the relative distributions of yellow and red feathers are not influenced by geographic distributions of dietary resources. A study on Australian birds also found red feathers have a stronger negative relationship with latitude than yellow feathers, with geographic variation in sexual selection intensity considered as one of the possible factors underpinning the pattern (Prasetya et al., 2020; Weaver et al., 2018). Along with song (Sebastianelli et al., 2024), crown colour might be expected to play an important role in sexual selection in tinkerbirds (Kirschel, Nwankwo, Pierce, et al., 2020), as a result potentially obscuring latitudinal gradient patterns. It was the only feather patch with consistent sex-linked differences, with males exhibiting lower reflectance and hues of longer wavelength, and its association with geographic and climatic variables was more nuanced.

Findings at a continental scale were seen within species, but this varied by taxon. The underpart coloration of *chrysoconus-pusillus* combined, the tinkerbird species complex with the widest distributional range (Sebastianelli et al., 2022), followed the overall pattern we found. This species complex has the widest ecological niche as well and provides the context for adaptation to environmental variation. On the other hand, *P. subsulphureus*, with the narrowest ecological niche and distribution entirely in the tropical rainforest belt of the Upper and Lower Guinea forests (Kirschel, Nwankwo, Seal et al., 2020), did not exhibit patterns consistent with Gloger's

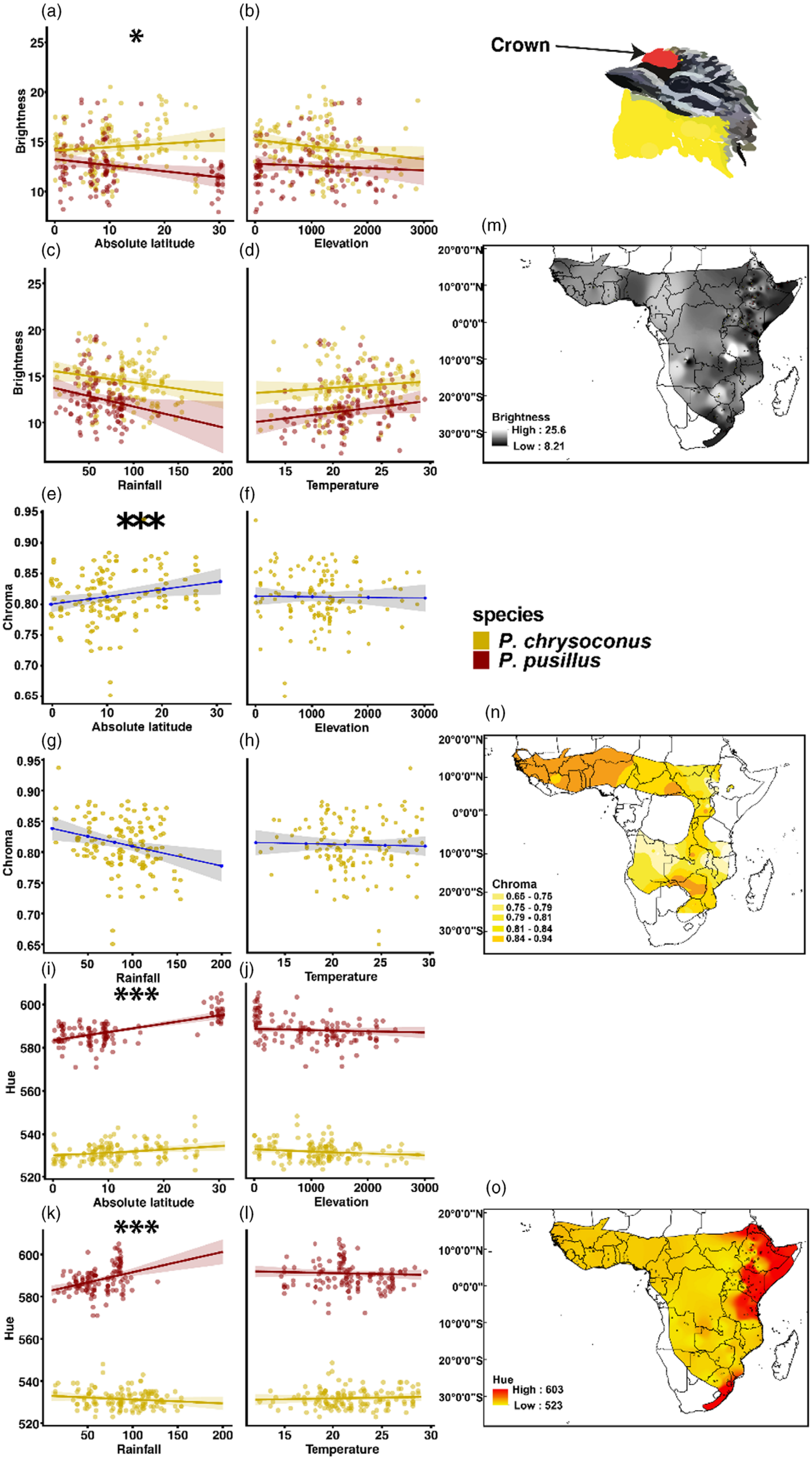

FIGURE 5 Plots showing the model effects of the geographic model (absolute latitude and elevation) and climatic model (annual rainfall and mean temperature) on crown patch brightness (a–d); crown patch chroma (e–h); and crown patch hue (i–l). Red lines represent effects on red crowned birds, yellow lines on yellow crowned birds. Asterisks signify statistically significant effects (*** = <0.001; * = <0.05), shading represents 95% CI. IDW maps from ArcMap 10.7.1 illustrate the variation of crown patch colour in tinkerbirds across their distribution range in (m) brightness, (n) chroma and (o) hue.

observation. This is perhaps not surprising, since climatic effects and geographic patterns are not expected to be evident at such narrow latitudinal ranges in Equatorial regions (Huston & Wolverton, 2011), and this corresponds with the lack of any latitudinal pattern consistent with Bergmann's and Allen's rules in this species (Sebastianelli et al., 2022). The results of taxon-specific GLS models were largely supported by forest plots illustrating results of the meta-analyses, but there were a couple of discrepancies between our combined taxa GLS models and the meta-analyses. These related in particular to breast colour. We attribute this discrepancy to the meta-analysis not accounting for variation among taxa. In particular, the taxon *leucolaimus*, with its greyish breast with limited carotenoid pigment, significantly differed in breast colour from other taxa (Table S3), and this obscured the effect of temperature and rainfall on breast colour in the meta-analysis. As an equatorial taxon, it also obscured the overall pattern in latitude across the continent. Nevertheless, our findings provide insights into the applicability of ecogeographic rules on plumage coloration at continental scales, with predictions of specific effects resulting from individual climatic factors that underpin geographic variation.

In our study, tinkerbirds exhibited significantly greater intensity of carotenoid-based colours in areas of higher rainfall and at lower latitudes and elevations where temperatures are highest, which is manifested in higher chroma of breast, belly and rump patches and/or brighter underpart coloration in these regions. Carotenoids are primarily produced by plants, and birds cannot synthesize them de novo but need to obtain them from their diet (Goodwin, 1984). Regions with high productivity, such as those with high rainfall and temperature, are thought to be associated with greater carotenoid pigment availability, likely due to increased plant biomass and insect diversity (Hill & McGraw, 2006). This increase in plant density in turn leads to increased availability of fruits (Zhang et al., 2017), including berries preferred by tinkerbirds (Lukhele et al., 2022), and insects that are rich in carotenoids (Short & Horne, 2001). Birds that feed on berries and insects can exploit this more abundant source of pigments, potentially leading to higher pigment saturation. Thus, gradients in rainfall and temperature can fundamentally influence the availability of carotenoid-rich food sources, ultimately affecting carotenoid-based plumage coloration (Gaudioso-Levita et al., 2017; Reudink et al., 2015; Salmón et al., 2023).

The greater saturation of carotenoid pigments, and in some cases warmer hues, with higher temperature and precipitation is thus consistent with Gloger's and Görnitz's predictions. One of the challenges of interpreting an ecogeographic pattern that might be manifested at a continental scale is determining the environmental feature that underpins it. We have shown here that in the chromatic measures of carotenoid-based colours, we find support for Gloger's observation at continental scales, and this is underpinned by the geographic extent of rainfall in accordance with Görnitz's prediction. But temperature too was consistently associated with increasing underpart chroma and brightness in the meta-analysis. Temperature has a well-established relationship with body size along latitudinal gradients—Bergmann's rule—a pattern strongly supported in tinkerbirds (Sebastianelli et al., 2022). Previous work on birds has found that relative carotenoid concentrations in blood plasma decrease with body size (Tella et al., 2004), a mechanism that may underpin increased prevalence of carotenoid colours and colour diversity in smaller birds (Delhey et al., 2023; Galván et al., 2013) and in turn, colorfulness along latitudinal gradients (Cooney et al., 2022). This mechanism might also underpin the relationship of temperature with underpart carotenoid colour in tinkerbirds, which are smaller in body size where temperatures are higher (Sebastianelli et al., 2022).

In achromatic measures of plumage patterns, we found that patches were brighter at lower latitudes, underpinned by a positive relationship with temperature and rainfall, and thus opposite to our original predictions. However, Gloger's expectation that feathers would be more intensely pigmented at lower latitudes is challenging to interpret in an achromatic context. More pigmented feathers are darker feathers in a melanin-based context, but in a carotenoid-based context more pigmented feathers could be darker due to more carotenoid pigment deposited, thus lowering reflectance. Indeed, the positive relationship between temperature and both feather chroma and brightness suggests more pigmented feathers are brighter in our study. Beyond the effects of climate on colour variation, studies have shown that carotenoid coloration is heavily influenced by both condition dependence and sexual selection (Hill et al., 2023; Olson & Owens, 1998; Peters et al., 2008). These pigments may be particularly variable among populations due to varying environmental and social pressures. For example, in the context of Endler's sensory drive hypothesis (Endler, 1992)—signals evolve to be effectively transmitted and received in specific environments, some plumage patches in tinkerbirds, such as breast, belly and rump could function as visual communication signals. We found that rump colour was brighter in *P. subsulphureus* and *P. bilineatus*, species which inhabit tropical forests, than in savanna species with a wider latitudinal range. This could reflect stronger selection for bright colour signals in dark environments (Marchetti, 1993).

5 | CONCLUSIONS

We examined if carotenoid colour variation in tinkerbirds relates to environmental variables, and how this varies geographically. We found varying support for patterns consistent with Gloger's and Görnitz's observations that carotenoid-based colours were more intense at lower latitudes where it is warmer and wetter. We found associations of absolute latitude, rainfall and temperature with plumage saturation, but these varied across body plumage patches and between taxa. However, rainfall and temperature overall were positively correlated with colour saturation, mostly in underpart coloration in correspondence with our hypothesis and Gloger's and Görnitz's non-systematic observations. Collectively, our findings demonstrate firstly the complex nature of carotenoid-based plumage coloration at the species level, and secondly that factors in addition to carotenoid availability in the environment (e.g. sexual selection) likely contribute to variation in plumage coloration. Nevertheless, our study sheds light on patterns of carotenoid-based colours at a continental scale across a set of species, suggesting these patterns are manifest in differences among species with different geographic distributions. But we also found that these patterns may apply within and consistently among taxa too, suggesting there are environmental selective pressures, including climatic, that affect colour, even in species with continuous distributions across the continent and thus with ongoing gene flow. Taken together with comparable studies (Chui & Doucet, 2009; Delhey et al., 2023; Prasetya et al., 2020), we suggest that Gloger's and Görnitz's observations on carotenoid-based plumage likely do represent the effects of environmental factors, but that some plumage features are subject to multiple drivers of variation.

AUTHOR CONTRIBUTIONS

Sifiso M. Lukhele and Alexander N. G. Kirschel conceived the ideas and designed the study; Alexander N. G. Kirschel, Nadya E. Seal Faith and Gregory F. Grether collected the data and measured the reflectance spectrum; Sifiso M. Lukhele analysed the data; Sifiso M. Lukhele and Samuel E. I. Jones led the writing of the manuscript. All authors contributed to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.5tb2rbpdt (Lukhele et al., 2025).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Variance inflation factor (VIF) of the predictors that were included in the geographic model and climatic model in the within species and across species models.

Table S2. Generalized least squares models (GLS) results showing the effects of (i) the geographic predictors: absolute latitude and elevation, (ii) climatic predictors: mean annual temperature, and annual rainfall on (a) belly hue, (b) breast hue, and (c) rump hue computed from reflectance spectra taken from study skins of *Pogoniulus* tinkerbirds.

Table S3. GLS results showing the effects of the (i) geographic predictors: absolute latitude and elevation, and (ii) climatic predictors: mean annual temperature and annual rainfall on (a) belly chroma, (b) breast chroma, and (c) rump chroma computed from reflectance spectra taken from study skins of *Pogoniulus* tinkerbirds.

Table S4. GLS results showing the effects of the (i) geographic predictors: absolute latitude, elevation, (ii) climatic predictors: mean annual temperature and annual rainfall on (a) belly brightness, (b) breast brightness, and (c) rump brightness computed from reflectance spectra taken from study skins of *Pogoniulus* tinkerbirds.

Table S5. Results of meta-analysis assessing associations between geographic and climatic predictors and plumage coloration: belly patch brightness, chroma, and hue across species.

Table S6. Results of meta-analysis assessing associations between geographic and climatic predictors and plumage coloration: breast patch brightness, chroma, and hue across species.

Table S7. Results of the meta-analysis assessing associations between geographic and climatic predictors and plumage coloration: rump patch brightness, chroma, and hue across species.

Table S8. GLS results showing the effect of geographic predictors: absolute latitude and elevation and climate predictors: annual rainfall and mean annual temperature on (a) belly hue, (b) belly chroma, and (c) belly brightness, (d) breast hue, (e) breast chroma, (f) breast brightness, (g) rump hue, (h) rump chroma and (i) rump brightness in *chrysoconus-pusillus* combined.

Table S9. GLS results showing the effect of geographic predictors: absolute latitude and elevation and climate predictors: annual rainfall and mean annual temperature on (a) belly hue, (b) belly chroma, and (c) belly brightness, (d) breast hue, (e) breast chroma, (f) breast brightness, (g) rump hue, (h) rump chroma and (i) rump brightness in *P. bilineatus*.

Table S10. GLS results showing the effect of geographic predictors: absolute latitude and elevation and climate predictors: annual rainfall and mean annual temperature on (a) belly hue, (b) belly chroma, and (c) belly brightness, (d) breast hue, (e) breast chroma, (f) breast brightness, (g) rump hue, (h) rump chroma and (i) rump brightness in *P. subsulphureus*.

Table S11. GLS models results showing the effects of the (i) geographic predictors: absolute latitude and elevation, (ii) climatic predictors: mean annual temperature, and annual rainfall on (a) crown hue, (b) crown chroma (for only *P. chrysoconus*), and (c) crown brightness computed from reflectance spectra taken from the study skins of *P. pusillus/uropygialis* and *P. chrysoconus*.

Figure S1. Averaged reflectance spectra obtained from the belly, breast, rump (*chrysoconus-pusillus* combined, and the taxa *P. b. bilineatus*, *P. b. leucalaimus*, *P. s. chrysopygus and P. s. flavimentum*) and crown patch (*P. pusillus/P. uropygialis* and *P. chrysoconus* shown separately).

Figure S2. Forest plots showing the effect size of absolute latitude, elevation, rainfall and temperature on carotenoid-based plumage coloration across *Pogoniulus* tinkerbird belly brightness (a–d), chroma (e–h), and hue (i–l). Also, shown is the overall meta-analytic mean and 95% confidence interval.

Figure S3. Forest plots showing the effect size of absolute latitude, elevation, rainfall and temperature on carotenoid-based plumage coloration across *Pogoniulus* tinkerbird breast brightness (a–d), chroma (e–h), and hue (i–l). Also, shown is the overall meta-analytic mean and 95% confidence interval.

Figure S4. Forest plots showing the effect size of absolute latitude, elevation, rainfall and temperature on carotenoid-based plumage coloration across *Pogoniulus* tinkerbird rump brightness (a–d), chroma (e–h), and hue (i–l). Also, shown is the overall meta-analytic mean and 95% confidence interval.

Figure S5. Forest plots showing model estimates for the fitted geographic (absolute latitude and elevation) and climatic model (annual rainfall and mean annual temperature) on carotenoid-based color variation in *chrysoconus-pusillus* combined.

Figure S6. Forest plots showing model estimates for the fitted geographic (absolute latitude and elevation) and climatic model (annual rainfall and mean annual temperature) on carotenoid-based colour variation in *Pogoniulus bilineatus*.

Figure S7. Forest plots showing model estimates for the fitted geographic (absolute latitude and elevation) and climatic model (annual rainfall and mean annual temperature) on carotenoid-based color variation in *Pogoniulus subsulphureus* combined.

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