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Time spent together and time spent apart affect song, feather colour and range overlap in tinkerbirds

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Most studies on the processes driving evolutionary diversification highlight the importance of genetic drift in geographical isolation and natural selection across ecological gradients. Direct interactions among related species have received much less attention, but they can lead to character displacement, with recent research identifying patterns of displacement attributed to either ecological or reproductive processes. Together, these processes could explain complex, trait-specific patterns of diversification. Few studies, however, have examined the possible effects of these processes together or compared the divergence in multiple traits between interacting species among contact zones. Here, we show how traits of two *Pogoniulus* tinkerbird species vary among regions across sub-Saharan Africa. However, in addition to variation between regions consistent with divergence in refugial isolation, both song and morphology diverge between the species where they coexist. In West Africa, where the species are more similar in plumage, there is possible competitive or reproductive exclusion. In Central and East Africa, patterns of variation are consistent with agonistic character displacement. Molecular analyses support the hypothesis that differences in the age of interaction among regions can explain why species have evolved phenotypic differences and coexist in some regions but not others. Our findings suggest that competitive interactions between species and the time spent interacting, in addition to the time spent in refugial isolation, play important roles in explaining patterns of species diversification.

ADDITIONAL KEYWORDS: bird song – character displacement – ecological gradients – evolutionary diversification – genetic drift – interspecific competition – phylogeography – plumage coloration – refugia.

INTRODUCTION

The processes driving geographical variation among populations of species have long been debated, with competing hypotheses supporting the roles of vicariance, in particular genetic drift in isolation (Haffer, 1969), and natural selection along environmental gradients (Endler, 1977; Smith *et al.*, 1997; Kirschel *et al.*, 2011). In the Congo Basin, the prevailing view has been that refugial isolation is responsible for most diversification, including that in birds (Diamond & Hamilton, 1980; Mayr & O'Hara, 1986). The refugial isolation hypothesis, however, has been challenged

repeatedly by supporters of the theory that ecological gradients drive adaptive differences, including those in characters that function in reproductive isolation (Slabbekoorn & Smith, 2002; Kirschel *et al.*, 2009a, 2011; Smith *et al.*, 2013).

Rather less attention has been paid to the role of interactions among related species in shaping spatial patterns of variation among populations, which could result from character displacement between coexisting species (Brown & Wilson, 1956; Grant, 1972). Such divergence may be driven by interspecific competition for ecological resources (Schluter, 2000), including diet (Losos, 2000; Price, 2008), with shifts manifested in pronounced differences in foraging traits, such as beak shape (Lack, 1947; Grant, 1972; Fjeldså, 1983;

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Schluter *et al.*, 1985; Grant & Grant, 2006; Reifová *et al.*, 2011; Krishnan & Tamma, 2016), or in ecological niche (Schluter & McPhail, 1992; Dufour *et al.*, 2018), both of which might lead to reproductive isolation. Alternatively, selection against hybrids can reinforce incipient premating isolation by driving divergence in traits used in mate choice and species recognition (reviewed by Butlin, 1987; Servedio & Noor, 2003; Coyne & Orr, 2004; Hoskin & Higgie, 2010). Patterns attributed to reinforcement or, more generally, reproductive character displacement include divergence in plumage coloration in *Ficedula* flycatchers (Saetre *et al.*, 1997) and stronger discrimination of songs of those species by females (Wheatcroft & Qvarnström, 2017), in addition to divergence in calls and/or their recognition in several anuran species (Marquez & Bosch, 1997; Hoskin *et al.*, 2005; Pfennig & Pfennig, 2005; Lemmon, 2009; Gerhardt, 2013). Interference competition may also drive displacement of characters in coexisting species, reducing costly interspecific aggression (Grether *et al.*, 2013), which could occur because of intrasexual competition for either mates or resources, such as territories (Lorenz, 1962; Howard, 1993; Noor, 1999; Adams, 2004; Peiman & Robinson, 2007; Grether *et al.*, 2009, 2017; Peiman & Robinson, 2010; Kirschel *et al.*, 2019). Such interspecific social competition has been proposed as a major contributor to speciation, by way of rapid divergence between species in traits that also affect mate recognition (West-Eberhard, 1983).

Given that it is often not known which trait or combination of traits influence ecology, aggressive interactions and reproductive isolation between related species, it is important to examine multiple phenotypic traits before drawing conclusions regarding the extent to which each process might influence character evolution.

Another possibility is that interactions between species at contact zones may be such that they exclude each other from their ranges, either via competitive exclusion or by reproductive exclusion (Hochkirch *et al.*, 2007; Pfennig & Pfennig, 2009; Weir & Price, 2011; Grether *et al.*, 2013, 2017). The factors that allow related species to coexist have received much attention recently (reviewed by Weber & Strauss, 2016), with studies focusing on factors such as phylogenetic distance (Weir & Price, 2011), ecological niche (Pigot & Tobias, 2013; Tobias *et al.*, 2014; Laiolo *et al.*, 2017), habitat segregation (Reif *et al.*, 2018), sexual dimorphism (Cooney *et al.*, 2017) and dispersal ability (Pontarp *et al.*, 2015), whereas parapatric distributions have been attributed to a lack of standing variation in phenotypic traits (Pfennig & Pfennig, 2009) or, conversely, to adaptation to different niches along gradients (Price & Kirkpatrick, 2009). Indeed, the theory on species range limits suggests that gene flow from central population optima may swamp

adaptation at range edges (Kirkpatrick & Barton, 1997; Case *et al.*, 2005; Sexton *et al.*, 2009), and this may also play a role in determining whether species ranges overlap and whether ecological character displacement may then occur (Case & Taper, 2000; Bridle & Vines, 2007). Thus, it could take millions of years for closely related species to diverge sufficiently to enable sympatric coexistence. Mean estimates of divergence times in sympatric sister taxa of birds near the equator are > 3 Myr (Weir & Price, 2011).

Here, we examine patterns of differentiation across multiple traits among three different regions with contact zones between two closely related tinkerbirds (*Pogoniulus* Lafresnaye, 1844). We studied populations of yellow-rumped tinkerbird, *Pogoniulus bilineatus* (Sundevall, 1850), and yellow-throated tinkerbird, *Pogoniulus subsulphureus* (Fraser, 1843), in three regions 900 km apart, which correspond to the three postulated Pleistocene rain forest refugia in sub-Saharan Africa thought to explain most of the diversification in the region (Diamond & Hamilton, 1980; Mayr & O'Hara, 1986). The hypothesis posits that climatic fluctuations in the Pleistocene led to fragmentation of continuous forest, with divided populations diverging by genetic drift in isolation. When more mesic conditions returned, many taxa expanded, and secondary contact occurred, which might have resulted in gene flow if populations had not evolved reproductive isolation. Few studies have tested for the relative effects of different processes in explaining patterns of geographical variation important in species diversification, such as the divergence in traits that may mediate reproductive isolation (e.g. Wilkins *et al.*, 2018). Our ultimate aim here is to determine whether historical isolation in rain forest refugia, adaptation along ecological gradients or the effects of interspecific interactions best explain patterns of geographical variation in phenotypic traits that may mediate reproductive isolation, while also establishing how the age of interaction between the species in each contact zone may influence range overlap and displacement of characters. Kirschel *et al.* (2009b) found evidence for divergent character displacement of song between *P. bilineatus* and *P. subsulphureus* where they coexist in Uganda (East Africa) and Cameroon (Central Africa), and this inference was supported by a divergence in body size and beak shape. Playback experiments suggested that song divergence facilitates species recognition and reduces interspecific aggression among males competing for mates, rather than reinforcement of premating isolation, with no evidence of hybridization between the two species. Plumage coloration also differs between West Africa and Central Africa, at least in *P. subsulphureus*, but whether colour covaries with song and morphology across the range is not known.

Specifically, *P. subsulphureus* in West Africa (Upper Guinea refugium) have whitish throats, superficially resembling those of *P. bilineatus*, instead of the yellow throats further east, from which the vernacular name of the species is derived (Fig. 1; Borrow & Demey, 2001).

We examine variation in song, morphology and plumage coloration in *P. bilineatus* and *P. subsulphureus* by including data from fieldwork on song variation and an examination of morphological and plumage variation from museum specimens extending from West to East Africa. We focus our study here on the 'lemon-rumped' complex of *P. bilineatus* (Kirschel *et al.*, 2018), which is the one whose range coincides with that of *P. subsulphureus*. We contrast ecological gradients across each region and produce a molecular phylogeny and perform population genetics analyses to examine how the duration (age) of interactions affects the extent of range overlap. We also include the presence of red-rumped tinkerbird *Pogoniulus atroflavus* (Sparrman, 1798) as a factor in analyses because it is the closest *Pogoniulus* species to the focal species in plumage coloration and song.

MATERIAL AND METHODS

STUDY SPECIES AND REGIONS

Pogoniulus bilineatus occupies savanna woodland, forest edge, gallery forest and secondary forest and occurs from sea level to > 2000 m in elevation between West and East Africa (Short & Horne, 2002). *Pogoniulus subsulphureus* is primarily a lowland rain forest species and has a disjunct distribution either side of the Mono River in the Dahomey Gap dividing Upper Guinea forest in West Africa and Lower Guinea forest in Central and East Africa (Short & Horne, 2002; Dowsett-Lemaire & Dowsett, 2019). It coexists with *P. bilineatus* primarily in secondary forest and edge, where their respective preferred habitats meet and where they can be found side by side occupying overlapping territories (Kirschel *et al.*, 2009b). They differ phenotypically in the yellow-tinged facial markings of *P. subsulphureus* (compared with white markings in *P. bilineatus*) and in the frequency and pace of their songs (Kirschel *et al.*, 2009b). A more distantly related species in the genus, the larger red-rumped tinkerbird, *P. atroflavus*, has a distinct yellow throat and red rump (Short & Horne, 2002). It occurs primarily in the forested lowlands of West and Central Africa and is absent from most of the lowland rain forest of East Africa. We defined our study populations as follows: East Africa, including data from Uganda; Central Africa, with data from Cameroon; and West Africa, with data from Ghana, Ivory Coast, Sierra

Leone, Liberia and Guinea, thus representing the Upper Guinea refugium.

FIELD RECORDINGS AND SONG ANALYSES

We recorded 43 *P. bilineatus* and 43 *P. subsulphureus* in Ghana in West Africa in October and November 2010 using a Marantz PMD 661 digital recorder with a Sennheiser MKH 8050 directional microphone at a sampling rate of 48 kHz. These recordings were incorporated in analyses with 460 recordings obtained previously by Kirschel *et al.* (2009b) in Cameroon in Central Africa (53 *P. bilineatus* and 85 *P. subsulphureus*) and Uganda in East Africa (175 *P. bilineatus* and 147 *P. subsulphureus*) between July 2004 and September 2007 (see Fig. 1; for recording locations, see Supporting Information, Fig. S1). We approached singing birds up to ~10 m to obtain good-quality recordings of focal birds, aiming to record them for ≥ 120 s when possible. The 546 recordings (271 *P. bilineatus* and 275 *P. subsulphureus*) were imported into RAVEN v.1.4 (Cornell Laboratory of Ornithology), in which spectrograms and power spectra were generated using a fast Fourier transformation size of 4096, a window size of 1269, 3 dB filter bandwidth of 50 Hz and a frequency resolution of 11.7 Hz. We measured the first ten clean songs from recordings of each individual and calculated the mean peak frequency from the power spectrum for each song typically, 1–3 s long, including inter-note intervals. We calculated song pace in accordance with methods previously described (Kirschel *et al.*, 2009b; Clark *et al.*, 2016).

DENSITY ESTIMATION FOR SONG ANALYSES

In accordance with Kirschel *et al.* (2009b) we consider that one species is unlikely to have an impact on the song of the other if it is absent or rare at a site, meaning that encounter rates are low (see also Lemmon, 2009). Specifically for the song analyses, we defined a site as low density for a species if its congener was found (heard) in no more than one 1 km² pixel for sites encompassing at least four 1 km² pixels. The justification for this method is that some field sites in the preferred habitat of one species, such as a closed forest for *P. subsulphureus*, might have an edge where the occasional *P. bilineatus* could appear. Most of those *P. subsulphureus* in the closed forest do not encounter *P. bilineatus*, and this absence of syntopy means that the majority are unlikely to be affected by the nearby presence of their congener. In contrast, for *P. bilineatus*, which regularly encounters the common *P. subsulphureus* at the forest edge, its congener is considered to be at high density. This method was used as a proxy for differences in encounter rates with heterospecifics when the two species coexist,

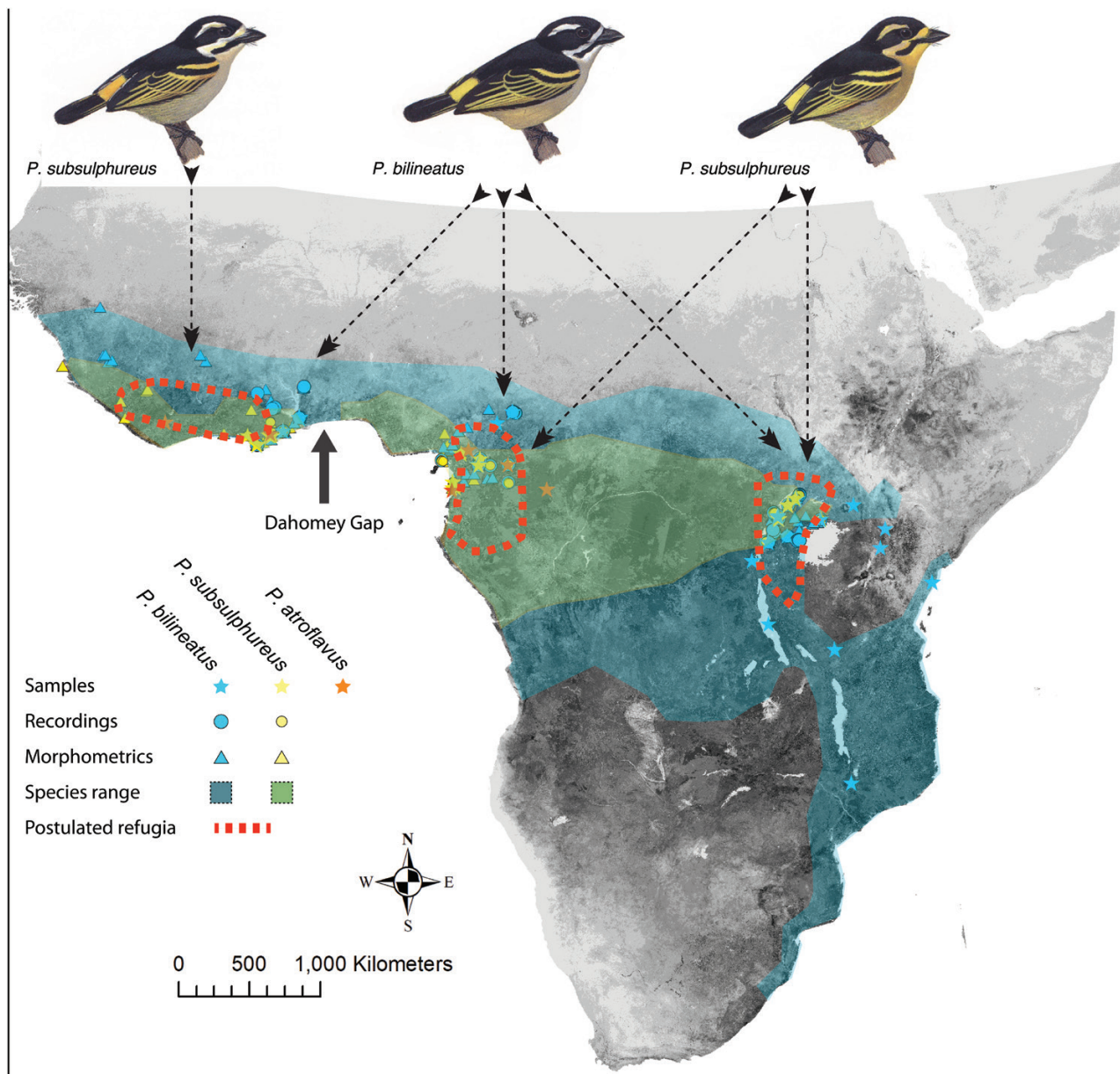


Figure 1. Enhanced vegetation index (EVI) map, illustrating localities from which samples, recordings and morphometric data were obtained within the three contact zones of *Pogoniulus subsulphureus* and *Pogoniulus bilineatus* in West, Central and East Africa. Approximate ranges of *P. subsulphureus* and *P. bilineatus* are shaded in green and blue, respectively. Red dotted lines surround areas postulated as rain forest refugia. In Central and East Africa there is substantial overlap in the species ranges, but in West Africa *P. bilineatus* is distributed in ecotone habitat around the forest zone where *P. subsulphureus* is found (the forest–savanna ecotone reaches the coast in Eastern Ghana in the Dahomey Gap, resulting in parapatry between the species along the coast). Species illustrations indicate the colour phenotype of *P. subsulphureus* and *P. bilineatus* in West, Central and East Africa, highlighting differences in throat colour in *P. subsulphureus* between West Africa and further east (from Borrow & Demey, 2001).

without having data on the encounter rates (see further justification and examples in the [Supporting Information](#), Expanded methods). Using this method of assessing high and low density of heterospecific presence, from a total of 38 sites, *P. bilineatus* was

recorded at 30 sites, 13 with *P. subsulphureus* at low density (five West, two Central and six East Africa) and 17 with *P. subsulphureus* at high density (three West, seven Central and seven East Africa), and *P. subsulphureus* was recorded at 26 sites, 11 with

P. bilineatus at low density (three West, seven Central and one East Africa) and 15 with *P. bilineatus* at high density (three West, five Central and seven East Africa).

MUSEUM SPECIMENS

In total, we took morphometric measurements from 241 *P. bilineatus* and 125 *P. subsulphureus* museum skins that were collected between 1872 and 2007 in the three study regions of West, Central and East Africa, where refugia are purported to have existed (see Fig. 1; Supporting Information, Table S1). Using digital callipers, we measured wing chord, tarsus and tail length, bill length and width and lower mandible length. We identified the localities where specimens were collected from museum tags and used maps, gazetteers and Google Earth (Google, Inc.) for those specimens where coordinates were not given, and we assigned the approximate latitude and longitude coordinates and elevation for each locality.

Given that specimens were collected historically and that information on relative densities of the focal species at the collection locality was not available, we could not use the same approach as with song to determine congener density. Instead, we estimated whether the species present was allopatric or sympatric from its congener based on our field experience in the region, which species were represented by study specimens from each collecting locality, and examination of the habitat and elevation there. We assumed that all specimens from West Africa were from allopatric populations because we found no specimens of the two species from the same locality, and the species have largely allopatric/parapatric distributions there, with *P. subsulphureus* found in the forest zone and *P. bilineatus* in the transition zone to savanna (Fig. 1; Supporting Information, Fig. S1A; see the Supporting Information for a list of references supporting their parapatric distribution). Even where *P. bilineatus* has been found within the forest zone, it is in deforested areas, such as farmbush adjacent to forest (Dowsett-Lemaire & Dowsett, 2014) and thus not in syntopy with *P. subsulphureus*. In total, we took morphometric measurements from 27 allopatric and 15 sympatric sites for *P. bilineatus* and from 12 allopatric and 20 sympatric sites for *P. subsulphureus*.

COLOUR MEASUREMENTS

We took reflectance measurements on 222 of the study skins, representing 23 allopatric and nine sympatric sites for *P. bilineatus* and 18 allopatric and eight sympatric sites for *P. subsulphureus*. We measured reflectance spectra (300–700 nm) of feathers on the throat, breast, belly, rump, shoulder and the crown

of the head using a diode-array spectrometer (Ocean Optics USB2000) with a fibre-optic reflectance probe (Ocean Optics R-400) and xenon strobe light source (Ocean Optics PX-2), in reference to dark (blocked light path) and white standards (Ocean Optics WS-1). A total of 25 measurements were taken per individual while holding specimens such that the dorsal surface of feathers was placed up to a 1.3-mm-diameter aperture in a razor-thin steel plate. The reflectance probe was oriented at 45° relative to the upper surface of the plate, positioned such that the edges of the aperture matched the acceptance angle of the detector light path and held such that the light source pointed in the direction of the feather rachis (in perpendicular orientation) or the feather base (in parallel orientation; see the Supporting Information, Expanded methods, for more detail on plumage reflectance measurements).

Replicate reflectance spectra were averaged and processed further to yield quantum cone catch (Q_j) and cone excitation estimates (E_j) using typical cone λ_{\max} values for Piciformes (Endler & Mielke, 2005). We used the irradiance spectrum that prevails under open canopy and cloudy conditions (Endler, 1993; J.A. Endler, personal communication, 2006) because tinkerbirds spend much of their time singing from exposed perches in the forest canopy or foraging in the canopy for fruit (the results were qualitatively the same with a forest shade irradiance spectrum). Values for E_j were used to calculate cone contrasts and coordinates in tetrahedral colour space. Four colour variables were used in the data analysis: (1) the sum of the four cone excitations (E_{sum}) to represent luminance (brightness); (2) the distance from the achromatic centre of tetrahedral colour space to represent colour saturation (chroma); (3) the medium- to short-wave cone contrast E_{ms} (hue); and (4) the short- to ultraviolet-wave cone contrast E_{su} (ultraviolet contrast). To determine whether the differences in colour between populations detected by the spectrometer are likely to be perceptible by birds, we used Vorobyev & Osorio's (1998) receptor noise model to estimate colour differences (ΔS) in units of just noticeable difference (JND). To calculate ΔS from the quantum catch (Q_j) estimates, we used the R package pavo, with noise set to 'neural', cone densities set to the ratio 1:2:2:4 (from shortest to longest wavelength), and the Weber fraction set to the default value (0.1).

GENETIC SAMPLING AND ANALYSIS

Samples were obtained during fieldwork performed in Uganda (45 samples) and Cameroon (32 samples) in 2007 (Kirschel *et al.*, 2009b), in Uganda in 2009 (six samples), in Ghana in 2010 and 2015 (23 samples), and in Kenya and Tanzania in 2011–2014 (six samples; Kirschel *et al.*, 2018). Tinkerbirds were captured using targeted mist netting with conspecific playback,

ringed, and blood samples were obtained from the ulnar superficial vein (wing) and transferred into 2 mL cryovials containing 1 mL Queens Lysis Buffer (Hobson *et al.*, 1997). Further tissue and extracted DNA samples were obtained from other sources (see Fig. 1; for DNA sample locations, see Supporting Information, Fig. S1; Table S1).

DNA was extracted using a Qiagen DNeasy blood and tissue kit, following the manufacturer's protocols (Qiagen, Valencia, CA, USA). Polymerase chain reaction (PCR) was performed to amplify DNA of the mitochondrial genes ATPase subunit 6/8 using the primers C02GQL and C03HMH (Eberhard & Bermingham, 2004), and PCR products were sent to Macrogen Europe (Amsterdam, The Netherlands) for sequencing.

PHYLOGENETIC ANALYSIS

Sequences obtained were aligned with 20 sequences from GenBank resulting from previous work (Kirschel *et al.*, 2018) using MUSCLE (Edgar, 2004) in MEGA v.7 (Kumar *et al.*, 2016). Different models of evolution were compared using the R package phangorn (Schliep, 2011) based on our aligned sequences. We performed a Bayesian analysis within RevBayes v.1.0.3 (Höhna *et al.*, 2016) based on a Hasegawa–Kishino–Yano (HKY) substitution model (Hasegawa *et al.*, 1985) with Gamma distribution (Hasegawa *et al.*, 1985), which was the highest-scoring model based on both the Akaike information criterion corrected for small sample sizes (AICc) and Bayesian information criterion scores. We estimated divergence dates in RevBayes v.1.0.3 (Höhna *et al.*, 2016) based on a modification of the method described by Nwankwo *et al.*, (2018). The sampling probability was set to the ratio of the tips and the estimated total number of described bird species within the Lybiidae family (52), and we ran the burn-in phase of the Monte Carlo sampler for 100 000 iterations under two independent replicates using 13 different moves in a random move schedule, with 44 moves per iteration and a tuning interval of 250. The main phase of the Markov chain Monte Carlo analysis was run for five million generations, sampling every 500 generations, with two independent runs and the same random moves as in the burn-in phase.

We screened sites from the ATPase gene of 109 individuals that were obtained from 30 different localities to determine populations with fixed alleles and removed the uninformative sites from downstream analyses. The overall quality of our genotype loci data was examined, including a search for missing data and rare alleles. We showed the contribution of the major haplotypes to the genotypes of the two species by

producing a haplotype network (minimum spanning network) using PopART (Leigh *et al.*, 2015).

ECOLOGICAL DATA

We examined variation in ecological preferences between species and among regions by using remote sensing to extract the ecological variables of elevation obtained from the shuttle radar topography mission (SRTM), and the enhanced vegetation index (EVI) and vegetation continuous field (VCF) products from the MODIS satellite. For VCF (percentage tree cover), because cloud contamination can affect the values from specific areas with extensive cloud cover, we obtained data from the years 2000, 2010 and 2016 and used the maximum value among the 3 years in our analyses. The VCF data were obtained for the year 2000 at a resolution of 1 km², and SRTM data were also aggregated to a resolution of 1 km², consistent with what was used for a subset of the data previously and what was used to define the pixel size (Kirschel *et al.*, 2009b), whereas VCF data for 2010 and 2016 were at a spatial resolution of 250 m. For EVI, we used values for the 16 day period 6–21 March 2010 at a spatial resolution of 250 m.

DATA ANALYSIS

We used generalized linear mixed models (GLMMs) in lme4 (Bates *et al.*, 2015) and car (Fox & Weisberg, 2011) statistical packages implemented within R v.3.5.1 (R Core Team, 2017) to test for the relative effects of species identity, geographical isolation, ecological gradients and interactions with congeners on song peak frequency and pace (both log₁₀-transformed). Specifically, we included the geographical and ecological variables of longitude, latitude, region, elevation (square-root transformed), EVI and VCF (log₁₀-transformed) as predictors in the model, in addition to the interactions between region and sympatry, species and sympatry, and species and region as fixed effects, with site included as a random effect. Best models were selected based on the lowest AICc score. Residuals were tested for normality by examining the correlations between predicted and fitted values, and fitted values and residuals and their associated plots in R, and Moran's *I* tests were used to test for spatial autocorrelation. We also performed a generalized linear model on peak frequency (log₁₀-transformed) in order to visualize the effects of congener density in each region after controlling for the environmental factors EVI, VCF (log₁₀-transformed) and elevation (square-root transformed).

For the analyses of data from museum specimens, we focused on broad regional patterns of variation and

compared those with the extent of range overlap of the two species. To determine whether there were any differences in the colour of feather patches between males and females, we ran regressions on each feather measurement used in the analyses with sex as the predictor variable, controlling for region. We used analysis of distance (AOD) in STATA v.14.2 (StataCorp, 2015) to quantify the extent of differentiation in colour between *P. bilineatus* and *P. subsulphureus* in each of West, Central and East Africa. Analysis of distance is conceptually similar to MANOVA but free of distributional assumptions (Gower & Krzanowski, 1999; Fenty, 2004). We calculated the Minkowski distance in tetrahedral colour space and used a permutation test with 10 000 iterations to calculate *P*-values. We then used a canonical discriminant analysis (CDA) with leave-one-out cross-validation to determine the extent to which it could classify individual specimens to East, Central and West Africa based on the tetrahedral *x*, *y* and *z* colour measurements of each feather patch. We also used AOD to test for character displacement in throat colour in Central and East Africa, but not in West Africa because we had no museum specimens from sympatric populations from there. Moreover, because of the possibility that the feather colour of museum specimens might change over time (Armenta *et al.*, 2008; Doucet & Hill, 2009), we tested for any effect of collection year by running a GLMM in R on throat colour hue, chroma, brightness and ultraviolet contrast, with species identity, sex and region included in the model.

We used principal components analysis (PCA) to reduce the set of morphometric measurements to two principal components (PCs). We ran GLMMs in R to test for the relative effects of species identity, sex, sympatry with one another, longitude, latitude, region and elevation, in addition to the interactions between region and sympatry, species and sympatry, and species and region on PCs, with diagnostics and spatial autocorrelation tests performed as described above for song.

In order to test for possible differences in habitat preferences between the species and among regions, we ran GLMM in R on EVI, VCF and elevation with species and region as predictors.

RESULTS

SONG

Songs varied significantly between species and among regions. In peak frequency, most variation was explained by differences between the two species, followed by intraspecific differences between high and low heterospecific density sites, indicating significant

song divergence among sites as a result of the presence of the congener in both species (Table 1), consistent with previous findings for the two species (Kirschel *et al.*, 2009b). Heterospecific density had the strongest effect on song frequency in Central Africa, with greater differences between populations with high congener density than low density there and also in East Africa, but no difference in West Africa (Fig. 2). Frequencies were also higher at lower elevations, further west (Fig. 2) and in the presence of the red-rumped tinkerbird (*P. atroflavus*), although this last effect was not included in the final model (Table 1). Pace also differed mostly between the species and by region, with slower songs in both species in West Africa and faster songs in East Africa (Fig. 2), but there were no significant effects of interactions with other congeners or the environment on song pace.

SEX DIFFERENCES

We found no significant differences between the sexes in throat, rump, belly or breast colour ($0.109 < P < 0.976$), but there was evidence of sexual dimorphism in tarsus length and some bill characteristics of *P. subsulphureus*. We thus incorporated all data, including unsexed individuals, in subsequent colour analyses, but we controlled for sex in morphological analyses, removing data of unsexed specimens.

COLORATION

Examination of mean reflectance spectra revealed that *P. subsulphureus* and *P. bilineatus* were more similar in West Africa, especially in throat colour (Fig. 3), but also in rump and breast colour (Supporting Information, Figs S2, S3). *Pogoniulus subsulphureus* in West Africa was more similar in throat colour to *P. bilineatus* and less similar to that of *P. subsulphureus* in Central and East Africa (Fig. 3E). *Pogoniulus subsulphureus* was also more similar in rump and breast colour to *P. bilineatus* in the west, mostly because of greater similarity in the ultraviolet range. These patterns were particularly evident in chroma (Fig. 2A; Supporting Information, Figs S2A, S3A), ultraviolet contrast (Fig. 3C; Supporting Information, Figs S2C, S3C) and, for throat colour, in hue (Fig. 3B). No difference was found between the species in brightness (Fig. 3D; Supporting Information, Figs S2D, S3D), and there was substantial overlap between the species across the range in all measurements of belly colour (Supporting Information, Fig. S4).

We found significant differences in plumage between the species in each region using AOD (all $P < 0.05$). However, the extent of differences in colour between patches varied geographically, with evidence for greater differences further east (Table 2). Application

Table 1. Results of generalized linear mixed models examining song variation in terms of peak frequency (A) and pace (B) (both \log_{10} -transformed)

A. Peak frequency (log): $N = 546$, populations = 38	Estimate	SE	t -value	P -value
Intercept	3.0712913	0.0057573	553.46	$< 2 \times 10^{-16}$
EVI	-9.13×10^{-3}	9.31×10^{-3}	-0.98	0.33
VCF (\log_{10} -transformed)	-5.09×10^{-3}	6.07×10^{-3}	-0.838	0.40
Red-rumped tinkerbird presence	1.54×10^{-2}	7.20×10^{-3}	2.142	0.04*
Elevation (square-root transformed)	-0.0007	0.0003	-2.90	0.005
Heterospecific density (high)	-0.01	0.004	-3.23	0.002
Species (YTT)	0.09	0.005	19.67	$< 2 \times 10^{-16}$
Region (East Africa)	-4.35×10^{-2}	2.74×10^{-2}	-1.585	0.12
Region (West Africa)	3.08×10^{-2}	2.01×10^{-2}	1.530	0.13
Longitude†	-0.0005	0.0002	-2.52	0.015
Heterospecific density (high): YTT	0.02	0.005	3.39	0.001
Heterospecific density (high): East Africa	1.48×10^{-2}	7.51×10^{-3}	1.97	0.059
Heterospecific density (high): West Africa	1.00×10^{-2}	7.85×10^{-3}	1.28	0.21
Species (YTT): region (East Africa)	-2.79×10^{-3}	4.91×10^{-3}	-0.57	0.57
Species (YTT): region (West Africa)	1.10×10^{-2}	6.29×10^{-3}	1.75	0.08
B. Pace (log): $N = 546$, pops = 38				
Intercept	0.369495	0.005	80.20	$< 2 \times 10^{-16}$
EVI	6.02×10^{-3}	1.46×10^{-2}	0.41	0.68
VCF (\log_{10} -transformed)	-1.05×10^{-2}	8.98×10^{-3}	-1.17	0.24
Red-rumped tinkerbird presence	9.40×10^{-3}	7.80×10^{-3}	1.20	0.24
Elevation (square-root transformed)	1.43×10^{-4}	3.62×10^{-4}	0.39	0.69
Heterospecific density (high)	2.42×10^{-3}	8.59×10^{-3}	0.28	0.78
Species (YTT)	0.32	0.006	57.03	$< 2 \times 10^{-16}$
Region (East Africa)	0.05	0.005	9.43	4.56×10^{-14}
Region (West Africa)	-0.05	0.007	-7.26	5.27×10^{-11}
Longitude‡	-6.43×10^{-4}	1.82×10^{-3}	-0.35	0.73
Heterospecific density (high): YTT	9.41×10^{-3}	7.42×10^{-3}	1.27	0.22
Heterospecific density (high): East Africa	2.86×10^{-3}	8.39×10^{-3}	0.34	0.74
Heterospecific density (high): West Africa	-1.81×10^{-2}	9.51×10^{-3}	-1.91	0.06
Species (YTT): region (East Africa)	0.03	0.007	4.40	1.70×10^{-5}
Species (YTT): region (West Africa)	-0.04	0.009	-4.01	8.20×10^{-5}

The models test for patterns attributable to species differences (YTT = *Pogoniulus subsulphureus*), patterns consistent with character displacement based on an effect of high heterospecific density, patterns of variation between regions, and the effects of elevation, longitude, latitude and two remotely sensed measures of habitat, namely the enhanced vegetation index (EVI) and percentage canopy cover (VCF). The models also examine the effect of the presence of the red-rumped tinkerbird, and various interactions between regions, species and presence of the congener at a high heterospecific density. Bold text indicates that the variable was included in the best-supported model according to its Akaike information criterion corrected for small sample sizes (AICc) score. In A, the AICc score for the best-supported model was -2736.92; for the full model it was -2664.11. In B, the AICc score for the best-supported model was -2193.29; for the full model it was -2108.08.

*Red-rumped tinkerbird shows a significant effect on peak frequency, but this effect is diminished in subsequent models.

†The full model would not converge with latitude included, but replacing longitude with latitude in the above model showed that the latter had no effect ($P = 1.0$).

‡The full model would not converge with latitude included, but replacing longitude with latitude in the above model showed that the latter had no effect ($P = 0.9999$).

of the receptor noise model (Vorobyev & Osorio, 1998) suggested that differences in throat colour in West Africa were at the threshold of perceptibility ($\Delta S = 0.998$), but were considerably greater in Central and East Africa ($\Delta S > 3$), with rump colour distinct between the species, and breast and belly colour mostly above the 1.0 threshold (Table 2).

The CDA confirmed the pattern of convergence in West Africa. Canonical discriminant analysis

revealed that 83.3% of West African *P. subsulphureus* were misclassified as *P. bilineatus* based on throat colour, but only 4.5% of Central and East African *P. subsulphureus* were misclassified. In *P. bilineatus*, the single individual misclassified was also from West Africa. No individuals were misclassified based on rump colour (Supporting Information, Table S2). Based on breast colour, the CDA misclassified 21 and 4% of *P. subsulphureus* in West Africa and

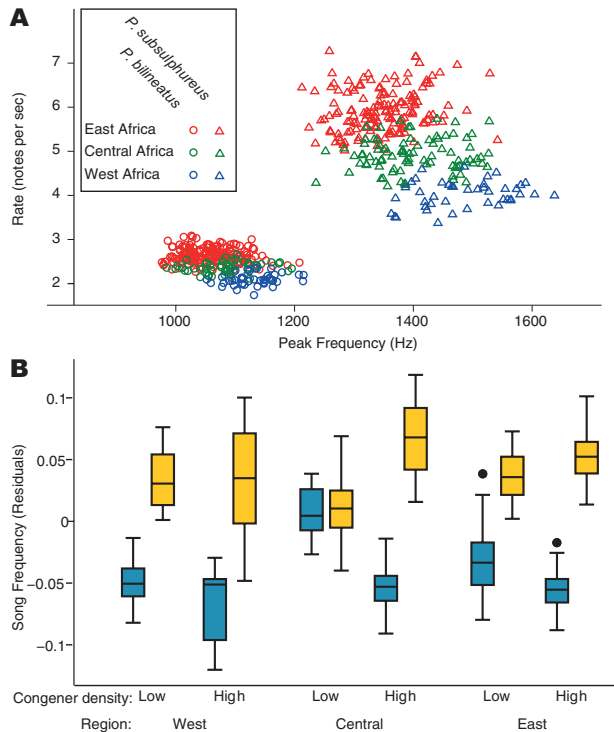


Figure 2. A, scatter plot of individual tinkerbird songs in peak frequency and rate, illustrating differences between species within and between regions. Note that where songs of *Pogoniulus subsulphureus* (YT) are closer in rate (further west in Ghana) to *Pogoniulus bilineatus* (YR), they are more different in frequency. In addition, there is a distinct pattern eastwards of lower frequencies and faster rates in both species. B, box plots of peak frequency (\log_{10} -transformed) residuals of *P. bilineatus* (blue) and *P. subsulphureus* (yellow) in each region in populations with high and low heterospecific density. Where the congener coexists at high density, song pitch is more different in East and, especially, in Central Africa than where the congener occurs at low density (or is absent). There is no difference in peak frequency between sites with low and high congener density in West Africa.

Central + East Africa, respectively, and one individual of *P. bilineatus* from Central + East Africa; belly colour could not be used to discriminate among the species within any region (Supporting Information, Table S2). We did not find evidence of divergence in throat colour between sympatry and allopatry within regions in *P. subsulphureus* [Central Africa, (Minkowski) distance = 0.1034, $P = 0.87$; East Africa, distance = 0.3445, $P = 0.34$], whereas in *P. bilineatus* there was marginal evidence of displacement in Central Africa (distance = 0.9485, $P = 0.0096$) and in East Africa (distance = 0.0684, $P = 0.054$), although the direction of divergence differed (converging

towards *P. subsulphureus* in Central Africa, but away from it in East Africa). The GLMM testing the role of specimen age showed no effect on throat colour (chroma, $t = -0.05$, $P = 0.96$; hue, $t = 0.70$, $P = 0.48$; brightness, $t = -1.38$; $P = 0.17$; ultraviolet contrast, $t = 0.08$, $P = 0.94$; Supporting Information, Table S3).

MORPHOMETRICS

Using PCA with varimax rotation, we extracted the first two PCs explaining 73% of the variation in morphology, with PC1 being positively correlated with larger body size and, specifically, wing, tail and tarsus length, and PC2 positively correlated with bill size measurements. The GLMM revealed body size (PC1) differences between regions (*P. bilineatus* largest in the east, and *P. subsulphureus* smallest in the west), species (*P. subsulphureus* smaller), sexes (females smaller) and elevations (larger individuals at higher elevations) (Table 3; Fig. 4). Evidence for character displacement was also found in body size, with *P. subsulphureus* being significantly smaller and *P. bilineatus* larger (though not significantly) where the two species occurred together (Supporting Information, Fig. S5). Much less variation was explained in bill size (PC2) in the GLMM, with the only significant difference attributed to the smaller size of *P. subsulphureus* in West Africa (Table 3). There was no evidence of spatial autocorrelation in any of the models.

PHYLOGENETIC RECONSTRUCTION

We successfully sequenced 94 fragments of 832 bp of ATPase subunit 6/8 (for GenBank accession numbers, see Supporting Information, Table S1). Bayesian analyses revealed that *P. bilineatus* and *P. subsulphureus* were reciprocally monophyletic, and they were estimated in RevBayes to have been diverging for 4.37 Myr, with *P. atroflavus* a more distant relative and with a common ancestor to the *P. bilineatus*/*P. subsulphureus* clade 6.31 Mya (Fig. 5A). Populations in West and Central Africa have been diverging for 0.99 Myr in *P. subsulphureus* and 0.64 Myr in *P. atroflavus*, but for considerably less time in *P. bilineatus*, with the population from West Africa sharing a common ancestor with most populations from Central and East Africa 0.335 Mya. In contrast, there was deep divergence among different subspecies of *P. bilineatus* in East Africa, with some populations in Kenya alone diverging for 2.31 Myr. In *P. subsulphureus*, there were similar levels of structure east and west of the Dahomey Gap (Fig. 5B), whereas in *P. bilineatus* greater structure was more evident in the east than in the west (Fig. 5C).

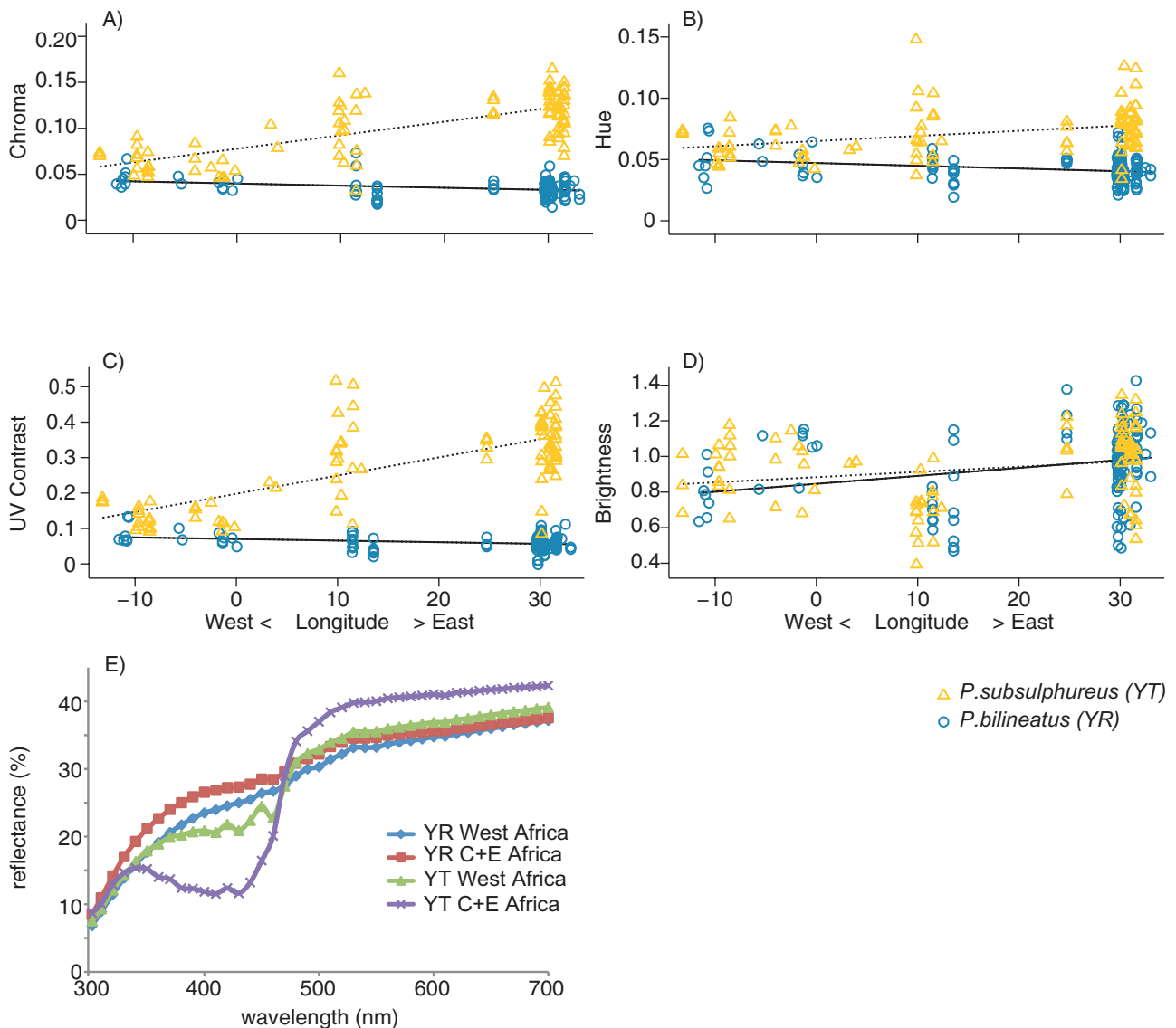


Figure 3. Throat colour is more similar between species in the west. This can be seen in three measurements representing chroma (A), hue (B) ultraviolet (UV) contrast (C), but there is no difference in brightness (D). E, raw reflectance spectra also show greater similarity in the west.

ECOLOGICAL GRADIENTS

A GLMM revealed no differences overall in VCF ($N = 546$, $F = 2.59$, $P = 0.11$) or EVI ($N = 539$, $F = 1.71$, $P = 0.19$) between the species, but there were differences in elevation ($N = 545$, $F = 5.86$, $P = 0.016$). Nonetheless, there were regional differences in all three environmental variables (VCF, $F = 7.68$, $P = 0.0017$; elevation, $F = 38.15$, $P < 0.0001$; EVI, $F = 8.13$, $P = 0.0012$), with the highest VCF, EVI and elevation in East Africa, followed by Central Africa, and the lowest in West Africa, although West Africa was not significantly lower than the other two regions in VCF and EVI. Differences were found between the species in VCF ($N = 85$, $F = 9.08$, $P = 0.003$;

Supporting Information, Fig. S6A) in West Africa, with *P. subsulphureus* in more densely forested areas than *P. bilineatus*, and in elevation in Central Africa ($N = 138$, $F = 10.67$, $P = 0.001$; Supporting Information, Fig. S6C), with *P. bilineatus* found at higher elevations, but no differences were found in EVI (Supporting Information, Fig. S6B). There were no significant differences in habitat among the species in East Africa.

DISCUSSION

We found evidence for phenotypic divergence in several traits among the allopatric populations

Table 2. Minkowski distances in tetrahedral colour space (d) and receptor noise based colour distances (ΔS) between *Pogoniulus bilineatus* and *Pogoniulus subsulphureus* in each contact region

Region	Rump		Throat		Breast		Belly	
	d	ΔS	d	ΔS	d	ΔS	d	ΔS
West Africa	15.996	2.71	1.946	0.998	4.048	1.25	3.691	1.31
Central Africa	18.713	2.95	33.726	3.044	5.967	1.12	4.049	1.81
East Africa	22.926	3.32	42.133	3.818	13.597	1.87	0.884	0.47

Measurements of the left side of the rump, right side of the throat, middle of the breast and left side of the belly (both in parallel orientation) were used because they scored higher than other sides of those patches in consistency tests. A value of $\Delta S \geq 1$ indicates that the mean colour differences between populations are likely to be perceptible by birds (Vorobyev & Osorio, 1998; Vorobyev *et al.*, 2001). The Minkowski distances are squared and multiplied by 10^4 for ease of presentation.

in different regions. This divergence reflected independent evolution in geographical isolation, consistent with patterns of either genetic drift or sexual selection. Allopatric divergence in phenotypic traits such as song has been reported before, including in barn swallow (*Hirundo rustica*; Wilkins *et al.*, 2018) and yellow-rumped tinkerbird in East Africa (Nwankwo *et al.*, 2018). But in contrast to barn swallow song, which is learned, tinkerbird song is understood to develop innately (Kirschel *et al.*, 2009b), and thus population differences in song are likely to have a genetic basis. In addition to differences in song among regions, we found evidence of character displacement in song between the two tinkerbird species within regions.

MECHANISMS

Drift and adaptation

Traits differed among regions, consistent with divergence in refugial isolation over long periods of time that could be associated with genetic drift. The distributions of both species are continuous across Central and East Africa in the present day, as in many species purported to have been restricted to refugia during the Pleistocene, and a pattern of clinal variation could result from gene flow after secondary contact. Similar patterns could be explained instead by adaptation to specific allopatric habitats. We found no evidence, however, of ecological gradients being associated with variation in phenotypic traits, based on the factors we tested, although elevation was found to exhibit a significant negative correlation with song frequency and positive correlation with body size. A negative correlation between song frequency and body size is expected (Ryan & Brenowitz, 1985; Seddon, 2005; Kirschel *et al.*, 2009b), and a positive relationship between body size and elevation is in accordance with predictions of Bergmann's rule of larger body size at higher latitudes and elevations

(Ashton, 2002; Nwankwo *et al.*, 2019). We did not find an effect of latitude, but that is likely to be attributable to the small latitudinal range of the study.

Competitive and reproductive interactions

In accordance with previous work on these species (Kirschel *et al.*, 2009b), we found evidence that interactions between tinkerbird species in sympatry have led to character displacement. This is particularly evident in Central and East Africa, where the two species commonly occur in sympatry at high relative abundances and where character displacement occurs in song frequency and body size (although unlike previous work based on fieldwork, not in bill shape, cf. Kirschel *et al.*, 2009b) and where greater differences in throat and rump colour are found. The pattern of similarity in rump and breast colour and, more strikingly, in throat colour, where there is very little range overlap in West Africa, suggests a possible role for competitive or reproductive exclusion (see Gröning & Hochkirch, 2008; Price & Kirkpatrick, 2009). The lack of evidence for divergence between sympatric and allopatric populations of *P. subsulphureus* in throat colour suggests that throat colour diverged sufficiently throughout the time when *P. subsulphureus* has been interacting with *P. bilineatus* in Central and East Africa. Divergence in throat colour might have evolved to aid species recognition, possibly by way of historical reproductive character displacement after secondary contact, with gene flow then spreading the yellow throat colour into allopatric populations, but not across the Dahomey Gap (Fig. 6).

Indeed, phylogenetic evidence supports the possibility that the two species have been present and thus likely to be interacting for considerably longer in East Africa than in West Africa. This hypothesis is supported by the deeper population genetic structure east as opposed to west of the Dahomey Gap (see also Nwankwo *et al.*, 2018; Kirschel *et al.*, 2018). Specifically,

Table 3. Results of generalized linear mixed models examining variation in morphology in terms of principal component (PC) 1 (A), which is positively associated with wing chord, tarsus and tail length, and PC2 (B), which is positively associated with greater bill size (bill length, bill width, upper bill depth and lower mandible length)

A. PC1: $N = 299$, populations = 65	Estimate	SE	t -value	P -value
Intercept	-3.535×10^{-1}	3.206×10^{-1}	-1.102	0.28
Red-rumped tinkerbird presence	5.26×10^{-3}	3.27×10^{-1}	0.02	0.99
Elevation	5.67×10^{-4}	2.00×10^{-4}	2.832	0.007
Sympatry	1.26×10^{-1}	2.11×10^{-1}	0.60	0.56
Species (YTT)	-1.33	3.34×10^{-1}	-3.97	0.0003
Sex (female)	-3.05×10^{-1}	9.66×10^{-2}	-3.16	0.002
Region (East Africa)	9.25×10^{-1}	1.91×10^{-1}	4.85	1.9×10^{-5}
Region (West Africa)	5.57×10^{-1}	3.27×10^{-1}	1.71	0.09
Longitude	2.36×10^{-2}	3.39×10^{-2}	0.70	0.49
Latitude	7.76×10^{-2}	7.20×10^{-2}	1.08	0.29
Sympatry: YTT	-7.76×10^{-1}	3.30×10^{-1}	-2.35	0.023
Sympatry: East Africa	-5.71×10^{-1}	3.78×10^{-1}	-1.51	0.14
Sympatry: West Africa	-3.89×10^{-1}	3.12×10^{-1}	-1.25	0.21
Species (YTT): region (East Africa)	-2.57×10^{-1}	2.48×10^{-1}	-1.04	0.30
Species (YTT): region (West Africa)	-1.82	4.30×10^{-1}	-4.23	6.2×10^{-5}
B. PC2: $N = 299$, pops = 65	Estimate	SE	t -value	P -value
Intercept	0.6151	0.2420	2.542	0.014
Red-rumped tinkerbird presence	1.44×10^{-2}	6.12×10^{-1}	0.02	0.98
Elevation	-5.74×10^{-5}	4.35×10^{-4}	-0.13	0.90
Sympatry	7.34×10^{-1}	7.00×10^{-1}	1.05	0.30
Species (YTT)	-0.6263	0.33	-1.93	0.056
Sex (female)	-1.82×10^{-1}	1.92×10^{-1}	-0.95	0.34
Region (East Africa)	-0.53	0.30	-1.76	0.09
Region (West Africa)	0.33	0.50	0.67	0.50
Longitude	2.00×10^{-3}	6.34×10^{-2}	0.03	0.97
Latitude	1.27×10^{-1}	1.27×10^{-1}	1.00	0.32
Sympatry: YTT	-8.02×10^{-1}	7.07×10^{-1}	-1.14	0.27
Sympatry: East Africa	-4.44×10^{-1}	6.65×10^{-1}	-0.67	0.51
Sympatry: West Africa	9.32×10^{-4}	5.93×10^{-1}	0.002	1.00
Species (YTT): region (East Africa)	0.21	0.45	0.46	0.64
Species (YTT): region (West Africa)	-1.54	0.65	-2.35	0.02

The models test for patterns attributable to species (YTT = *Pogoniulus subsulphureus*) and sex differences, patterns consistent with character displacement based on sympatry with its close relative, patterns of variation between regions, and the effects of elevation, longitude and latitude, presence of the red-rumped tinkerbird, and various interactions between regions, species and sympatry with the congener. Bold text indicates that the variable was included in the best-supported model according to its Akaike information criterion corrected for small sample sizes (AICc) score. In A, the AICc score for the best-supported model was 751.04; for the full model it was 766.01. In B, the AICc score for the best-supported model was 1121.16; for the full model it was 1153.91.

while populations of *P. bilineatus* have been diverging in East Africa from > 2 Mya, divergence between West Africa and East Africa was estimated to be no earlier than 0.335 Mya (Fig. 5), suggesting more recent expansion westwards. *Pogoniulus subsulphureus* diverged either side of the Dahomey Gap ~1 Mya, suggesting that the two species have been interacting an order of magnitude less time in West Africa than in East Africa.

Song functions more in intrasexual competition (Kirschel *et al.*, 2009b); therefore, might plumage play a more important role in intersexual interactions? Reproductive exclusion resulting from similarity

in plumage coloration (see Hochkirch *et al.*, 2007; Vallin *et al.*, 2012) could potentially maintain reduced range overlap in West Africa. This outcome might be more likely for plumage than for song if interspecific intersexual interactions are costlier than intraspecific intrasexual interactions, owing to the costs of hybridization (Kishi *et al.*, 2009; Ord & Stamps, 2009; Seddon & Tobias, 2010). Nwankwo *et al.* (2019) found that distinct crown colour differences in two other tinkerbird taxa (*Pogoniulus chrysoconus* and *Pogoniulus pusillus*) did not maintain reproductive isolation despite a divergence time of ~4 Mya. The songs of those two species overlap in pace and

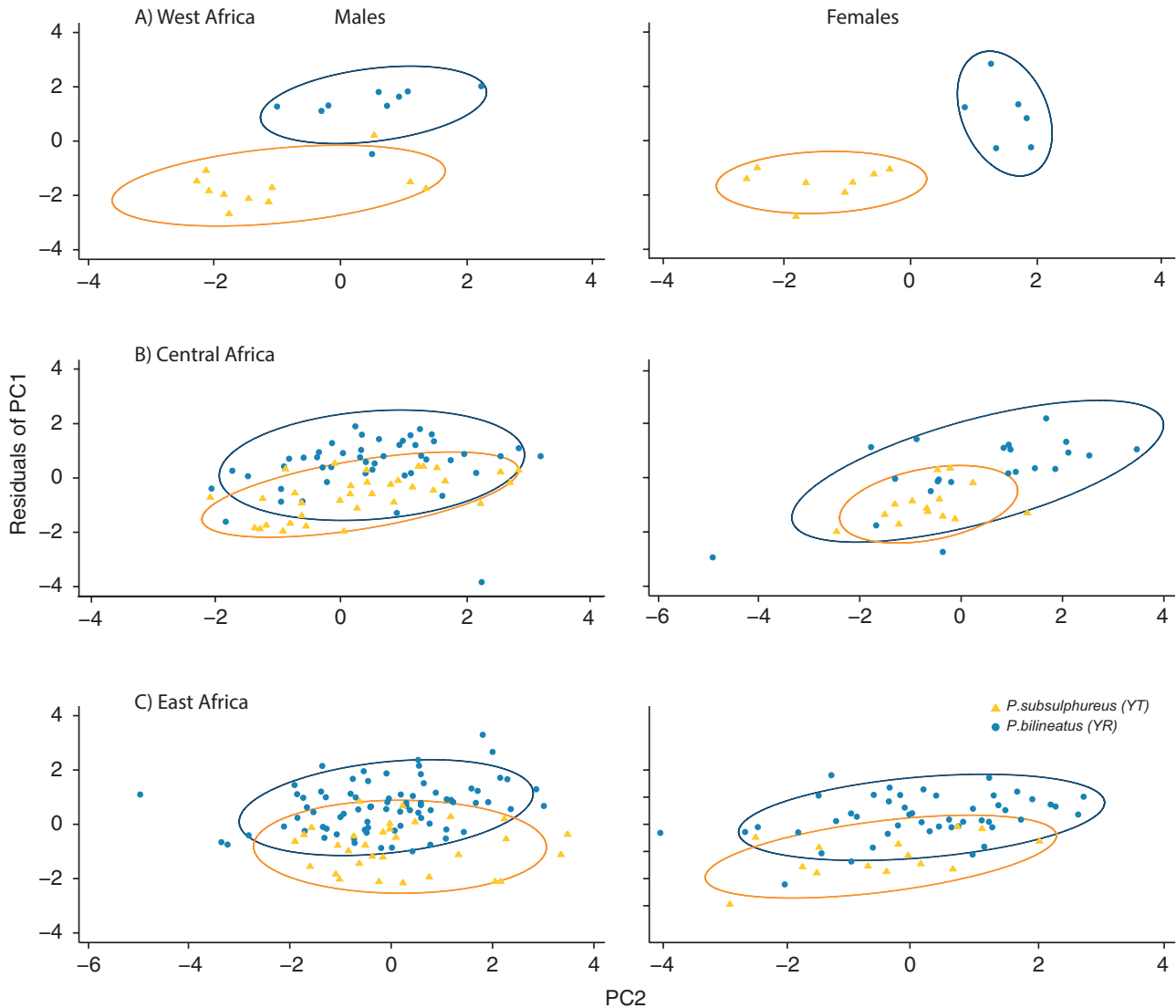


Figure 4. Scatter plots and 95% confidence ellipses (means centred) of body size and bill size. There is minimal overlap in morphology between *Pogoniulus bilineatus* and *Pogoniulus subsulphureus* in West Africa (A), but substantial overlap in both Central (B) and East Africa (C). We took residuals of principal component (PC) 1, positively correlated with body size, after controlling for elevation (which explained much variation in body size, as reported by Kirschel *et al.*, 2009b). Principal component 2 is positively correlated with bill size.

frequency, with playback experiments supporting the hypothesis that they do not recognize differences in song (A. Kirschel, unpublished data). In contrast, the two species examined here do not overlap in song characters and differentiate one another's song in sympatry (Kirschel *et al.*, 2009b). Furthermore, there is no present evidence of hybridization, although the finding of convergence in *P. bilineatus* throat colour in sympatry with *P. subsulphureus* in Central Africa might be suggestive of some introgressive hybridization. Although interspecific intrasexual interactions owing to song similarity might also lead to competitive or reproductive exclusion (Irwin & Price, 1999; Seddon,

2005; but see Laiolo, 2017), in the present study the songs might be sufficiently diverged to facilitate coexistence. An alternative explanation is that because of plumage similarity, interspecific aggression is so strong that range boundaries are maintained in West Africa despite differences in body size and song, but divergent plumage and character displacement in song and body size facilitates coexistence further east.

Divergence in morphology in Central and East Africa could be explained by competition for resources and thus ecological character displacement (Brown & Wilson, 1956; Schluter, 2000; Price, 2008). This raises the possibility of song (a trait important in non-random

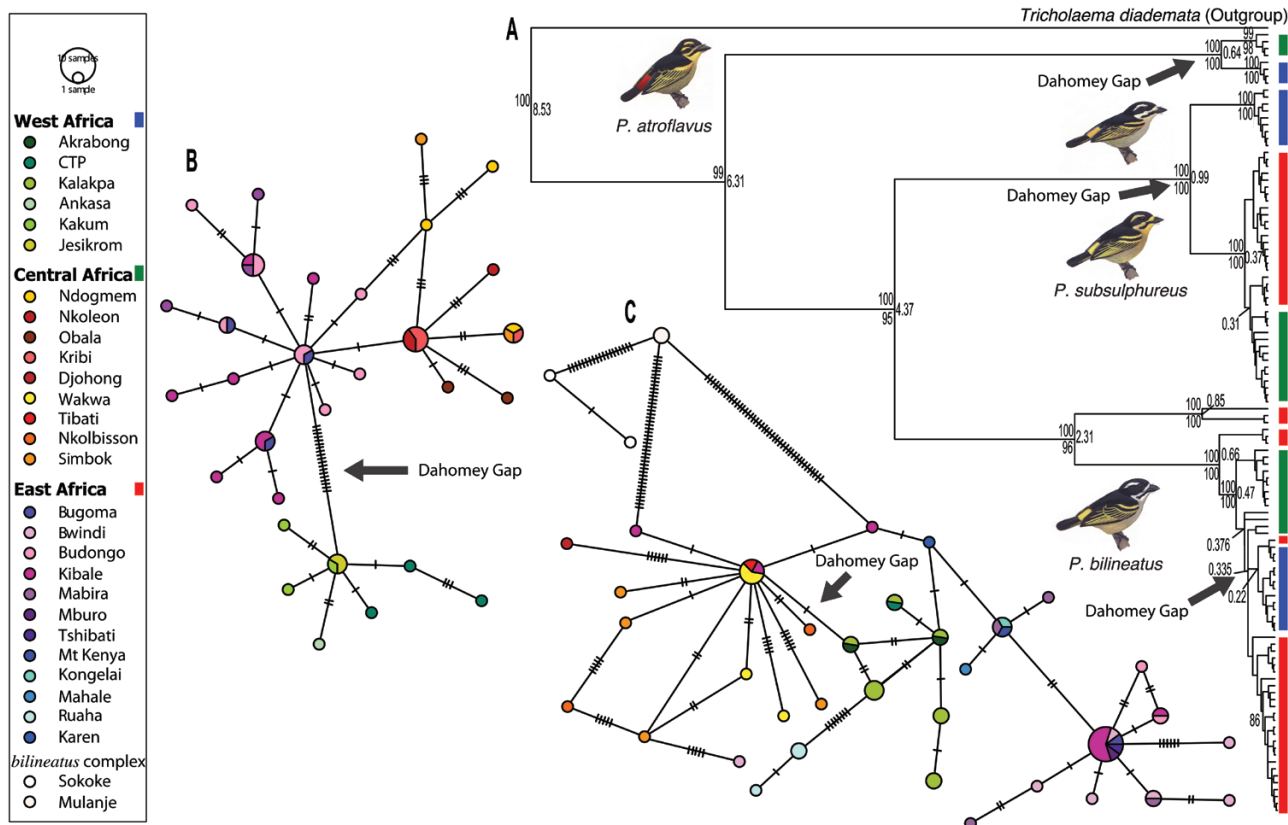


Figure 5. A, molecular phylogeny including samples of *Pogoniulus bilineatus*, *Pogoniulus subsulphureus* and *Pogoniulus atroflavus* based on the Bayesian inference consensus tree of an 832 bp fragment of ATPase subunit 6/8, with *Tricholaema diademata* as the outgroup. On the left side of nodes are the posterior probabilities and bootstrap values above and below the branch, respectively, with node ages on the right side of the node, calculated using RevBayes v.1.0.5. Populations of *P. subsulphureus* and *P. atroflavus* have diverged either side of the Dahomey Gap in West Africa (blue line) and Central Africa (green line) for longer than populations of *P. bilineatus*, which has longer divergence times in East Africa (red line) than to the west, suggesting more recent westward expansion. B, C, minimum spanning networks show a clear east–west division in *P. subsulphureus* (B), whereas in *P. bilineatus* (C) there is greater structure in East Africa (incorporating the *P. bilineatus* complex from further east and further south in Africa), while West Africa haplotypes appear nested within a cluster including Central and East Africa haplotypes. Species illustrations are from [Borrow & Demey \(2001\)](#).

mating) diverging as a by-product of divergence in morphology ([Servedio et al., 2011](#)) because of the physical constraints of body size on sound frequency ([Ryan & Brenowitz, 1985](#); [Seddon, 2005](#); [Amezquita et al., 2006](#); [Gilbert & Bell, 2018](#)). Nonetheless, results of playback experiments suggest that song divergence helps to reduce interspecific aggression in sympatry ([Kirschel et al., 2009b](#)), with weaker responses to heterospecific song in sympatry than in allopatry. Thus, even if song originally diverged as a by-product of divergence in morphology, selection for reduced interspecific aggression probably reinforced the species differences in song.

When ecological competition is strong, theory suggests that range limits should be sharper along steeper environmental gradients ([Case & Taper, 2000](#); [Case et al., 2005](#); [Goldberg & Lande, 2006](#); [Price &](#)

[Kirkpatrick, 2009](#)). We found that differences in tree cover were greater among the species where they are largely allopatric in West Africa, suggesting that tree cover might play a role in determining the species range limits. Indeed, such differences in habitat suggest that *P. bilineatus* might have become adapted to a different habitat in West Africa, where it is found much more in gallery forest in savanna rather than in secondary forest, where it is often found further east. However, *P. bilineatus* also occurs in gallery forest in Central Africa and in much more densely vegetated habitat elsewhere, suggesting that it is not because of habitat preferences that it is absent from dense forest in West Africa, although directional or stabilizing selection there specifically cannot be ruled out. Elevational distributions were also significantly different among the species, with steeper gradients (i.e.

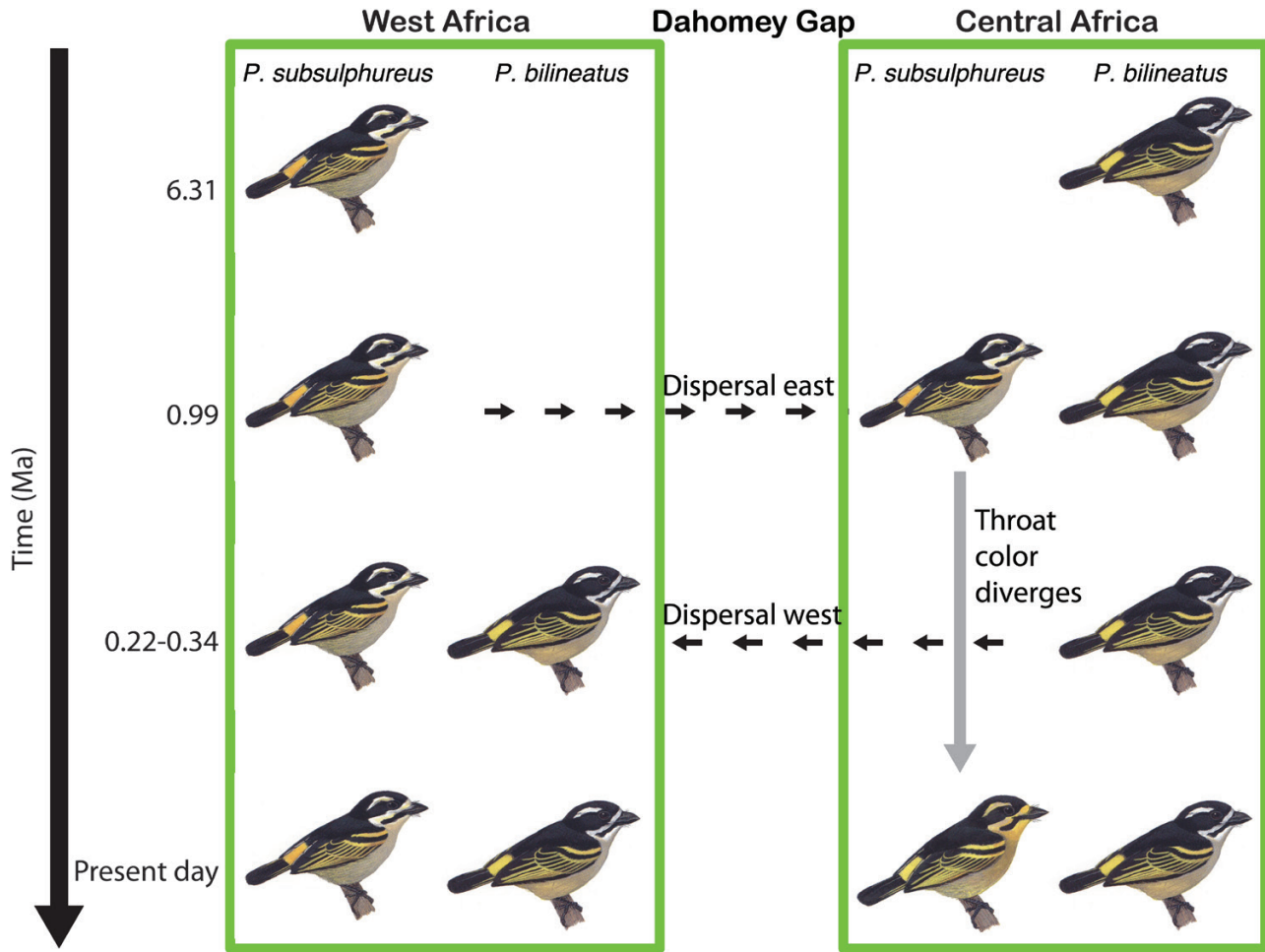


Figure 6. Schematic diagram of a possible scenario resulting in divergence in throat colour in *Pogoniulus subsulphureus*. The two species diverge from a white-throated common ancestor 6.31 Mya, and here we present a plausible example of divergence across the Dahomey Gap, with *P. subsulphureus* west and *Pogoniulus bilineatus* east. *Pogoniulus subsulphureus* disperses east of the Dahomey Gap to Central Africa 0.99 Mya. The eastern populations interact with *P. bilineatus*, driving character displacement in throat colour and thus divergence from white-throated *P. subsulphureus* west of the Dahomey Gap. Yellow throat colour spreads throughout the eastern populations in allopatry and sympatry with *P. bilineatus* as a result of gene flow. *Pogoniulus bilineatus* disperses west of the Dahomey Gap much more recently and with little or no range overlap with *P. subsulphureus*. Species illustrations are from [Borrow & Demey \(2001\)](#).

greater differences in elevation) between the species in Central Africa than in West Africa, suggesting that less overlap would be expected in Central than in West Africa, but the reverse is found, thus the steepness of environmental gradients is unlikely to explain the extent of range overlap between the two species, with competitive or reproductive interference from related species potentially playing a more important role.

EFFECTS OF SPECIES ASSEMBLAGES

Another factor that could contribute to different traits diverging in different contact zones is geographical variation in the species assemblage ([Hoskin & Higgie,](#)

[2010](#)). In particular, the strength of interaction between two focal species might be affected by other key species ([Case *et al.*, 2005](#)). One possibility here is that another forest tinkerbird, the larger *P. atroflavus*, also interacts with one or both focal species where its range overlaps with theirs in primary and secondary forests in West and Central Africa. Despite larger body size in East African *P. bilineatus* and *P. subsulphureus* populations where *P. atroflavus* is mostly absent, the presence or absence of *P. atroflavus* did not appear to explain variation in body size. Song frequency was higher in the presence of *P. atroflavus*, suggesting a possible shift in frequencies in both species when coexisting with the lower-pitched red-rumped tinkerbird.

However, the effect of the presence of *P. atroflavus* was not included in the best-supported model, with significant effects in the full model diminishing after other non-significant factors were removed. There was no effect of the presence of *P. atroflavus* on song pace. Nevertheless, species interactions might otherwise explain *P. subsulphureus* throat colour. In West Africa, *P. subsulphureus* is largely allopatric from *P. bilineatus*, which also have white throats, but commonly sympatric with *P. atroflavus* with yellow throats. Divergence in throat colour might thus have occurred for species and mate recognition in *P. subsulphureus* in either West Africa, in the presence of *P. atroflavus*, or in Central and East Africa, where it commonly coexists with *P. bilineatus*. It is not known which throat colour is ancestral in *P. subsulphureus*, although white would be more parsimonious considering that its sister taxon *P. bilineatus* has a white throat (Fig. 6). In any case, a number of species in the genus have either yellow or white throats, or both (Short & Horne, 2002), suggesting that there is ancestral genetic variation in colour genes that, via selection and recombination, explains the appearance of yellow or white throats in different lineages (e.g. Stryjewski & Sorenson, 2017). However, because yellow is likely to be carotenoid based (Shawkey et al., 2006), we cannot rule out a possible role of diet in explaining differences among populations and species.

How long species have been in contact is expected to play a crucial role (see Price, 2011), with *P. subsulphureus* in West Africa likely to have coexisted for longer with *P. atroflavus* (0.64 Myr) than with *P. bilineatus* (< 0.335 Myr). *Pogoniulus bilineatus* is largely absent from the forest zone in West Africa (Dowsett-Lemaire & Dowsett, 2014), suggesting that *P. subsulphureus* and *P. atroflavus* might occupy suitable ecological niches and preclude syntopy with *P. bilineatus* (see Price, 2011), although we cannot rule out the possibility that *P. bilineatus* in West Africa has not been able to colonize and adapt to closed forest there after expansion westwards. Further east, however, *P. bilineatus* coexists in forested habitat with *P. subsulphureus*, whereas in coastal eastern and southern Africa it is primarily a forest bird (Nwankwo et al., 2018).

CONCLUSIONS

Several processes may influence the phenotypes of both species and their distributions. Different processes may play a more significant role in each contact zone. In this study, in West Africa, patterns of variation are consistent with drift in refugial isolation, a shorter interaction time and competitive and/or reproductive exclusion. In contrast, in Central and East Africa, agonistic character displacement after a longer period

of time interacting in syntopy provides a better explanation of divergence in phenotypic characters. These different processes acting on different traits and their consequent evolutionary trajectories might even drive allopatric divergence among regions (Hoskin et al., 2005; Lemmon, 2009; Hoskin & Higgie, 2010). Displacement in characters can result between species where they meet (Grant, 1972), within species between sympatric and allopatric populations (Kirschel et al., 2009b) and among contact zones based on different trajectories of displacement (Hoskin et al., 2005). These processes can be affected by time spent in isolation and time interacting after secondary contact, the relative abundances of the interacting species, and the extent of their phenotypic similarity at each contact zone (see Pfennig & Pfennig, 2010). We suggest that such species interactions can play a profound role in species diversification, and these interactions can be shaped by historical biogeography and geological history, which can be explored using phylogeographical analyses. We recommend that future studies focusing on divergence between allopatric populations using phylogeographical methods should also examine the possible role of interactions within the communities in which the populations are found, in addition to interactions among populations that might diverge in allopatry as a result of their interactions with related species in sympatry.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Supplementary File. Expanded methods: estimation of congener density; tinkerbird distributions in West Africa; plumage reflectance details; References; Figures S1–S7; Tables S1–S3.

Figure S1. Separate enlarged sections of Figure 1 representing West (A), Central (B) and East Africa (C), allowing for greater scrutiny of the distribution of data collection coordinates (triangles, museum specimens; circles, recordings; stars, DNA samples) for *Pogoniulus subsulphureus* (yellow shapes), *Pogoniulus bilineatus* (blue shapes) and *Pogoniulus atroflovus* (orange stars for DNA samples only). Each map is an enhanced vegetation index image (from the MODIS satellite for the 16 day period from 6 to 21 March 2010), with darker shades representing densely vegetated areas and pale shades more open habitat.

Figure S2. Box plot of principal component 1 (PC1) residuals representing body size per species per region in populations in sympatry and allopatry after controlling for elevation. There is a pattern of character displacement in body size in East and Central Africa, with overlap in sympatry, but with *Pogoniulus bilineatus* (blue) larger than *Pogoniulus subsulphureus* (yellow) where they coexist in sympatry. In West Africa, *P. bilineatus* is considerably larger than *P. subsulphureus* in allopatry, and no specimens were obtained from any sympatric populations where the two species have non-overlapping distributions.

Figure S3. Rump colour varies between east and west, but the species are most similar in chroma in the west. This can be seen in the measurement representing chroma (A) and, to some extent, ultraviolet contrast (C) and raw reflectance spectra (E), but hue (B) is distinct throughout, and there is no difference in brightness (D).

Figure S4. Breast colour is more similar between species in the west in chroma (A) and ultraviolet contrast (C), and there is substantial overlap throughout in hue (B) and brightness (D). E, reflectance spectra show greater similarity among species within regions rather than within species across regions in short wavelengths, suggesting convergence, but the reverse is found at longer wavelengths.

Figure S5. There is overlap in belly colour in chroma (A), hue (B), ultraviolet contrast (C) and brightness (D) between species in the west and east. E, reflectance spectra show greater similarity among species within regions rather than within species across regions in longer wavelengths.

Figure S6. Environmental variables per species (YR, *Pogoniulus bilineatus*; YT, *Pogoniulus subsulphureus*) per region of Africa. Differences in habitat among the species within each region are found only in the percentage of tree cover (VCF) in West Africa (A) and in elevation in Central Africa (C), with no significant differences in enhanced vegetation index (EVI) across the study area (B).

Figure S7. Examples of low-density and high-density sites for a species based on the presence of its congener. Low-density sites for yellow-rumped tinkerbird (YR) include where yellow-throated tinkerbird (YT) is present in one pixel (1 km²) out of six (A) and where YT is absent from the two pixels (C). High-density sites for YR include where YT is present in four of six pixels (B) and where YT is present in one of two pixels (D).

Table S1. Classification of individual specimens of *Pogoniulus bilineatus* (YR) and *Pogoniulus subsulphureus* (YT) based on rump, throat, breast and belly colour. The numbers indicate correct/total classification.

Table S2. Results of generalized linear mixed models (GLMMs) testing for the effects of specimen age on throat colour, in addition to species identity, sex and region, in terms of: (i) tetrahedral chroma; (ii) *Ems* (hue); (iii) *Esum* (brightness); and (iv) ESU (ultraviolet contrast). There is no effect of specimen age on any measure, with variation explained mostly by species identity and region.

Table S3. Catalogue numbers for samples, species, their sampling locality and country, source of sample and GenBank accession numbers. Museum catalogue/field number abbreviations: CTR, Center for Tropical Research at University of California Los Angeles Institute of the Environment and Sustainability; FMNH, Field Museum of Natural History; LSUMZ, Louisiana State University Museum of Natural Science; UCY, University of Cyprus; ZMUC, Zoological Museum of the University of Copenhagen.