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Plio-Pleistocene Climatic Fluctuations and Divergence With Gene Flow Drive Continent-Wide Diversification in an African Bird

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ABSTRACT

Diversification mechanisms in Sub-Saharan Africa have long attracted research interest, with varying support for either allopatric or parapatric models of speciation. However, studies have seldom been performed across the entire continent, a scale which could elucidate the relative importance of allopatric and parapatric models of divergence. To shed light on continental-scale patterns of African biogeography and diversification, we investigated the historical demography of a bird with a continent-wide distribution in Sub-Saharan Africa, the Yellow-Rumped Tinkerbird, *Pogoniulus bilineatus*. We sampled populations from across the continent and, using genomic data, assessed genetic diversity, structure, and differentiation, reconstructed the phylogeny, and performed alternative demographic model selection between neighbouring clade pairs. We uncovered substantial genetic structure and differentiation patterns which corroborated the phylogenetic topology. Structure was chiefly influenced by the arid corridor, a postulated biogeographical barrier in Sub-Saharan Africa. Moreover, peak genetic diversities coincided with postulated refugial areas while demographic reconstructions between genetic lineages supported allopatric models consistent with the Pleistocene Forest Refuge hypothesis. However, within lineages, divergence with gene flow was supported. Continent-wide patterns of diversification involve an integration of both allopatric and parapatric mechanisms, with a role for both periods of divergence in isolation and across ecological gradients. Furthermore, our study emphasises the importance of the arid corridor as a primary biogeographical feature across which diversification occurs, yet one that has hitherto received scant attention regarding its importance in avian diversification in Sub-Saharan Africa.

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1 | Introduction

Sub-Saharan Africa has long been studied by evolutionary biologists to understand the primary mechanisms of diversification, with many African vertebrate taxa widely distributed across habitat types. However, there is little consensus on their genetic differentiation and divergence mechanisms, with two broad categories of competing models proposed to explain species distributions and divergence in Sub-Saharan Africa: allopatric and parapatric models. Allopatric models highlight the historical effect of physical isolation via geographic and/or climatic processes, and these include vanishing-refuge and disturbancevicariance hypotheses. The most prominent allopatric models are the Pleistocene Forest Refuge hypothesis (Diamond and Hamilton 1980; Mayr and O'Hara 1986), the Riverine Barrier hypothesis (Wallace 1854), and the Elevation Barrier hypothesis (Hardy et al. 2013). The parapatric mode of divergence, on the other hand, evokes disruptive selection across ecological gradients (Endler 1982; Rice and Hostert 1993; Moritz et al. 2000; Smith et al. 2001).

The Pleistocene Forest Refuge hypothesis posits that forested areas contracted and expanded repeatedly due to Pleistocene climatic fluctuations, and these in turn led to the isolation of populations. The climatic fluctuations should be reflected in concordant patterns in effective population sizes (N_{a}) (Haffer 1969, 2008); nonetheless, such vicariant effects are not exclusively limited to the Pleistocene (Lovett 1993). The Riverine Barrier hypothesis might be important because sub-Saharan Africa is composed of a landscape traversed by several major rivers, including the Volta, Niger, Congo and Zambezi rivers. It postulates that populations on opposite banks of rivers diverged, with the river forming a barrier to gene flow, except at head-water regions. This hypothesis has been supported in Africa in fish (Bartáková et al. 2015), amphibians (Penner et al. 2011; Gehring et al. 2012), reptiles (Allen et al. 2021), birds (Fjeldsa 1994; Voelker et al. 2013), and especially mammals (Telfer et al. 2003; Anthony et al. 2007; Haffer 2008; Nicolas et al. 2008; Jacquet et al. 2013; Mitchell et al. 2015; Dommain et al. 2022). The prediction of the riverine hypothesis is similar to that of the elevational barrier hypothesis, with no expected change in N_e, but with gene flow expected at narrower headwater regions and differentiation at lower parts of rivers. The elevational barrier hypothesis proposes regions of high elevation as barriers to gene flow, supported by studies showing that the continental mountain ranges of the Cameroon Volcanic Line (CVL) acted as a genetic break between populations (Hardy et al. 2013; Shirley et al. 2014). However, the CVL has also been proposed both as a refugium and a geographic barrier because it has been reported to have high species diversity and endemic taxa (Maley 1996; Budde et al. 2013; Hardy et al. 2013). Allopatric models focus on the absence of gene flow as the main factor in speciation, but over the years, there has been increasing focus on divergence with gene flow (parapatric divergence).

Parapatric divergence occurs without a physical barrier between populations and instead is driven by adaptation through natural selection to specific ecological niches along environmental gradients in climate, vegetation, and topography across geography (Huntley 2023). The hypothesis was proposed for Africa due to morphological divergence observed in Little Greenbuls along forest-savanna ecotones (Moritz et al. 2000; Smith et al. 2001; Plana 2004) and supported by studies in plants (Duminil et al. 2010; Heuertz et al. 2014) and vertebrate taxa, including amphibians (Vences et al. 2012; Hughes et al. 2018), fish (Burress 2015), reptiles (Nunes et al. 2022), and birds and mammals (Fjeldså and Lovett 1997; Smith et al. 1997; Orr and Smith 1998; McCormack and Smith 2008; Kirschel et al. 2011; Lewin et al. 2016). Parapatric speciation involves continuous/ historical gene flow followed by isolation where population boundaries are expected to coincide with ecological gradients (Moritz et al. 2000; Couvreur et al. 2021).

Support for both allopatric and parapatric modes of speciation suggests either could drive genetic divergence, as emphasised by Endler (1982). It is therefore best to consider alternative hypotheses for divergence simultaneously, preferably using a widely distributed species. This is because various factors may have influenced their divergence across wide geographic ranges, yet such studies are scarce (e.g., Bowie et al. 2004; Fuchs et al. 2017, 2018, 2021; Dongmo et al. 2019), even in birds with wide distributions across sub-Saharan Africa.

The Yellow-Rumped Tinkerbird (Family: Lybiidae) has a wide range that extends from the Guinean-Congolian forest (West and Central Africa), through East Africa to Southern Africa (Fry et al. 1988; Short and Horne 2001); thus, it is distributed across refugial areas, forest and savanna ecotones as well as the CVL. Previous phylogenetic reconstructions based primarily on mitochondrial DNA revealed deep genetic structure among its subspecies (Kirschel et al. 2018, 2020; Nwankwo et al. 2018); however, the mechanism of diversification is not known. The species was previously divided into two distinct species based on rump colour but then lumped into one after two forms were found interbreeding in Burundi (Prigogine 1980; Short and Horne 2002). Following previous phylogenetic work on two mitochondrial genes and one nuclear intron (Kirschel et al. 2018), we refer to the bilineatus complex, a form with a golden yellow rump (including nominate P. b. bilineatus, P. b. fischeri and its synonymized taxon conciliator, which previous work found was morphologically and genetically distinct (Friedmann 1929; Nwankwo et al. 2018)), and the leucolaimus complex, which includes all forms with a lemon yellow rump (P. b. mfumbiri, P. b. poensis and P. b. leucolaimus), a synonymized taxon sharpei, which again earlier work suggested is distinct (Kirschel et al. 2020), as well as P. b. jacksoni with a golden rump, in line with those phylogenetic reconstructions.

In this study, we used genome sequencing to identify genetically distinct populations and assess the extent to which genetic boundaries coincide with forest refugia, elevation barriers, rivers or ecological gradients, assessing concordance of spatial patterns of effective migration and genetic diversity with divergence scenarios. We also reconstructed the evolutionary relationship between populations, using the inferred phylogeny to identify populations with which to compare alternative divergence scenarios of allopatric and parapatric models using a demographic model-testing framework. Our goal was to assess the divergence mechanisms in Yellow-Rumped Tinkerbird and determine the predominant modes of divergence. Examining the phylogeographic structure and demography of Yellow-Rumped Tinkerbird will not only improve our understanding of the mechanism of its divergence but also provide insights into continental-scale diversification processes in the region.

2 | Methods

2.1 | Genomic DNA Extraction and ddRADseq Library Preparation

We collected 50ul of whole blood from 142 tinkerbirds using mist nets and conspecific playbacks (Kirschel et al. 2009, 2020; Nwankwo et al. 2018) from across their range in Sub-Saharan Africa representing all six currently recognised subspecies and the two synonymised taxa (Figure S1, Table S1). Blood was collected from the brachial vein and preserved in 1mL Queen's lysis buffer. We further obtained muscle tissue from 41 museum specimens (Table S1). We extracted genomic DNA of all 183 samples using a DNeasy Blood and Tissue kit as per the manufacturer's protocol (Qiagen, USA) and we quantified them on a Qubit 2.0 fluorometer (Thermo Fisher Scientific Inc.) to standardise input DNA for double digest restriction-site associated DNA sequencing (ddRADseq) library preparation following Brelsford et al. (2016). Briefly, we digested DNA with SbfI and MseI restriction enzymes to which we then ligated SbfIbarcoded unique adapters (4-8 bp in size) to each sample that allowed downstream equimolar pooling of all 183 samples. We purified each barcoded sample using Agencourt AMPure XP DNA beads followed by amplification using the Illumina PCR primers with the cycling program (98°C for 30s; 20 cycles of: 98°C for 20s, 60°C for 30s, 72°C for 40s; final extension at 72°C for 2 min). We pooled and purified the final library and sent it for 2×150 nt sequencing in an Illumina Hiseq X Ten platform by Novogene Inc. (Sacramento, CA, USA).

2.2 | SNP Calling and Bioinformatic Processing of ddRADseq Data

We processed sequenced data in Stacks v2.3 (Rochette et al. 2019) using the process-radtags module to demultiplex raw reads and the '-rescue' flag (permitting a single mismatch in the observed-to-expected barcode sequence). We next aligned the processed reads to the chromosomal-level assembly of the Pogoniulus pusillus reference genome (Accession no. GCA_015220805.1, Sebastianelli et al. 2024) using bwa-mem v0.7.15 (Li 2013). Aligned files were converted to BAM files via samtools v1.14 (Li et al. 2009) during which we removed alignments with a minimum mapping quality of MQ < 20 and generated a variant catalogue and genotyping in the Stacks gstacks and populations modules. We used VCFtools v0.1.17 (Danecek et al. 2011) to exclude individuals with very low sequence quality (-missing-indv), retaining individuals with \leq 50% missingness. We then re-ran Stacks using only the sequences of the individuals retained, then filtered SNPs in VCFtools (-max-missing 0.8, -min-meanDP 3, -max-meanDP 20 and -mac 2). We also used Stacks to process a dataset to reconstruct the phylogeny with ddRADseq, using the dataset for population structure analyses plus four outgroup samples. For demographic analyses, we processed VCF files per pairs of clades to include only samples

within pairs of clades following Warmuth and Ellegren (2019). We refer to a clade here as a monophyletic lineage that is distinct from other lineages as reported from our phylogenetic and population structure analyses (see Section 3). Further filtering included a clone filter to remove duplicates, and to ensure that SNPs are unlinked, we applied linkage disequilibrium (LD) pruning via *PLINK* v1.9 (Chang et al. 2015) (--indep-pairwise, window size of 50 SNPs, sliding window of 5 SNPs, genotypic correlation threshold of 0.5) to population structure, differentiation and genetic diversity data. While for demographic analyses, we selected one random SNP per locus via the *Stacks populations* module but retained only individuals with a missingness of \leq 20% for both datasets.

We obtained whole genome sequences from 19 individuals that represented six currently recognised subspecies and the two synonymised taxa, of which 15 samples were also included in the ddRADseq. We used a single individual each for P. subsulphureus and Tricholaema leucomelas (Accession No. GCA_013400475.1) as outgroups (Short and Horne 2002; Kirschel et al. 2020) (Table S1). We generated genomic libraries with the NEBNext DNA Prep kit and the manufacturer's protocol (Ipswich, MA, USA) and sequenced the libraries in the 2×150 nt configuration on an Illumina Novaseq 6000 platform with a mean insert size of 350 bp by Novogene Inc. (San Diego, CA, USA). We processed the whole genome sequences following Shultz (2018) except for the BQSR because we had less than 30 samples. First, we trimmed the remnant adapters using cutadapt v3.5 (Martin 2011) followed by mapping to the Pogoniulus pusillus reference genome (Accession no. GCA_015220805.1, Sebastianelli et al. 2024). We followed the same post-processing conversion as for ddRADseq and then marked duplicate reads using MarkDuplicate in Picardtools v2.26.10 (Broad Institute 2019). We then generated intermediate GVCF files per sample using the HaplotypeCaller module of GATK v4.2.6.1 and merged them across samples using the GenomicsDBImport module before joint genotyping all samples in the GenotypeGVCFs module with the inclusion of nonvariant sites. We used hard filtering via the VariantFiltration module to filter out poor quality SNPs using recommended values by GATK (Shultz 2018; Broad Institute 2022): quality by depth (QD < 2), Mapping Quality (MQ = 60), FisherStrand test (FS>60), StrandOddRatio test (SOR>3), ReadPosRankSum test (< 80) and MappingQualityRankSum test (-12.5). The hardfiltered variants were then selected by applying the SelectVariant module and used for downstream analyses.

2.4 | Genetic Structure Analyses of ddRADseq Data

To identify genetically distinct populations, we conducted a principal component analysis (PCA) using *flashpca* v1.2.6 (Abraham and Inouye 2016) and visualised the results in R v4.3.0's *ggplot2* package (Wickham 2016). We combined the PCA method with a maximum-likelihood clustering approach in *ADMIXTURE* v1.3.0 (Alexander et al. 2009) and used a cross-validation method with a fold value of 10 to assess the

best number of genetic clusters (*K*). We analysed K = 1-10 with three replicates each. We selected the genetic partition with the lowest cross-validation estimate as the best fit, aligned the replicate cluster proportions (Q) in CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007) and visualised the results with the program distruct 1.1 (Rosenberg 2004) and based on the results, we assessed further substructuring within each estimated cluster. To assess genetic structuring with geography, we displayed the spatial representation of ancestry coefficients (Q-matrix) across geography via the LEA R package v3.10.1 (Frichot and François 2015) by modifying the script at https://github.com/Tom-Jenkins/admix ture_pie_chart_map_tutorial. Lastly, we tested the reliability of the ddRADseq dataset number of SNPs by randomly downsampling the number of SNPs into dataset sizes of 1000 (1 K), 2, 5, 10, 50 and 100 K, ran PCAs with each down-sampled dataset, and compared PC1-PC3 with those from the full dataset using a regression model (see Data S1).

2.5 | Phylogenetic Analyses From Whole Genome Sequences

To assess evolutionary relationships between subspecies and inspect if their boundaries were concordant with geography, we reconstructed phylogenetic relationships via the maximum likelihood method as implemented in IQ-TREE v2.1.2 (Nguyen et al. 2015) and the multispecies coalescent approach as implemented in ASTRAL v5.7.8 (Rabiee et al. 2019). While the former considers all loci to follow the same topology, the latter considers incomplete lineage sorting (ILS) as a source of phylogenetic heterogeneity across genomic loci. In IQ-TREE, models of evolution were evaluated using ModelFinder (Kalvaanamoorthy et al. 2017), while branch support was assessed using 1000 bootstrap trees with ultrafast bootstrap approximation (Hoang et al. 2017), SH-aLRT and aBayes tests (Anisimova et al. 2011). To confirm consistency between whole genome and ddRADseq data, we reconstructed the phylogeny with the ddRADseq. We used autosomal chromosomes to assess phylogeny in both ASTRAL and IQ-TREE and retained high-quality SNPs with VCFtools (Danecek et al. 2011) for the whole genomes (-minQ 30, -min-alleles 2, -max-alleles 2, -mac 2, -minDP 3, -min-meanDP 4, -max-meanDP 60 and -max-missing 0.5 for ASTRAL but -max-missing 0.8 for IQ-TREE). For ASTRAL analyses, we used R's ape package to produce a set of unrooted Neighbour-Joining gene trees from the autosomal chromosomes at genomic intervals of 500 SNPs (Rancilhac 2024) and for the whole genomes, we filtered the resulting trees to retain trees with a maximum of 20% missingness and a sliding window size of 50,000 and 10,000 between windows. We then combined each filtered tree into a single file for input in ASTRAL.

To estimate the time of split between the resulting neighbouring phylogenetic clades, we scaled the time demographic parameters of the best-fit models reported from our demographic analyses to real values (See Section 2.7 below) and corroborated the estimated times using the Multiple Sequentially Markovian Coalescent (MSMC) method from the whole genome sequences following the workflow of Rick (2022). For the MSMC analyses, we generated the mask files per chromosome for samples and reference genome using SNPable Regions (Li 2009) and msmctools scripts (Wang et al. 2020) on samples' BAM files via bcftools mpileup (Danecek et al. 2021). To generate input data for MSMC, we used the merged GVCF file produced via GATK. First, we removed indels and filtered both variant and invariant sites with hard-filtering via GATK (QD < 2, MQ=60, FS>60, SOR>3, ReadPosRankSum test<80 and MappingQualityRankSum test = -12.5), then used VCFtools to generate separate VCFs of invariant sites (-min-alleles 1, -maxalleles 1) per sample per chromosome using the aforementioned filters as well as a multi-sample VCF with all chromosomes for variant sites only (-min-alleles 2, -max-alleles 2). Next, we phased the variant sites via physical phasing in WhatsHap v2.2 (Patterson et al. 2015), then statistical phasing in beagle v5.3 (Browning et al. 2021). After which, we separated phased variant sites into single-sample VCFs per chromosome, merged phased variant and unphased invariant sites per sample per chromosome via bcftools and used the merged files to generate the MSMC input data per clade based on phylogenetic results. Lastly, we used MSMC2 (Schiffels and Wang 2020) to estimate the relative cross-coalescent rate (rCCR) between clades, where the mid-point of the rCCR at 0.5 indicates the time of separation between clades and scaled times to years using a mutation rate of 2.42×10^{-9} for Downy woodpecker Dryobates pubescens (Nadachowska-Brzyska et al. 2015), which is the closest relative with an available mutation rate within the order Piciformes, and used a generation time of 2.28×10^{-7} for *P. bi*lineatus (Bird et al. 2020). We used block-bootstrapping to obtain confidence intervals of split time on 20 bootstrap replicates via the *msmctools* which generates artificial bootstrap datasets from the MSMC input dataset by chopping up the input dataset into blocks of 5 Mb long and randomly sampling the blocks with replacement to create an artificial 3Gb-long genome. In our determination of the time of split between the bilineatus and leucolaimus complexes, we selected representative samples from the geographically closest populations, specifically conciliator for the bilineatus complex and one sample each from jacksoni and mfumbiri for the leucolaimus complex. We used two samples each for the bilineatus, conciliator and fischeri clades. For the mfumbiri/jacksoni clade, we used one sample of mfumbiri and one sample of jacksoni, while for the leucolaimus/sharpei/poenis clade, we used one sample of leucolaimus, sharpei and poenis respectively (see Section 3).

2.6 | Genetic Diversity, Differentiation and Spatial Connectivity Using ddRADseq Data

To assess the genetic diversity of subspecies across geography, we used the entropy analysis (Sherwin et al. 2017) implemented in the *dart*R v2.7.1 package (Gruber et al. 2018; Mijangos et al. 2022) using R v4.3.0 (R Core Team 2023). We estimated the diversity of allelic richness (q=0), Shannon Information (q=1) and observed heterozygosity (q=2) with the expectation of higher genetic diversity within refugia areas and lower genetic diversity between refugia areas. To assess the degree of differentiation between subspecies, we analysed pairwise $F_{\rm ST}$ via the *hierfstat* package (Goudet and Jombart 2022) in R v4.3.0. We also accounted for the effect of geographic distance on differentiation through isolation by distance (IBD) analyses in the *dart*R v2.7.1 package. The package uses the Mantel test to perform correlation

analyses between geographic distance and population-based $F_{\rm ST}/1 - F_{\rm ST}$ analyses. We estimated effective migration surface (EEMS) to visualise how spatial patterns of genetic diversity and gene flow deviate from the expectation of IBD (Petkova et al. 2016). EEMS uses Markov Chain Monte Carlo (MCMC) to estimate posterior mean diversity (q) and migration rates (m) in geo-referenced data and illustrates spatial patterns of genetic variation. We did an initial run of different deme sizes of 200, 400 and 700 but chose a deme size of 700 because it had a denser grid as recommended by the manual (Petkova et al. 2016). With the 700-deme size, we conducted three independent analyses as replicates using the RUNEEMS_SNPS of EEMS with a burn-in of 200,000 and 10,000,000 MCMC iterations. We used the default values of hyperparameters and set the parameter values such that proposal variances were accepted 20%-30% of the time as recommended by the manual (Petkova et al. 2016). We combined the results of the three independent runs, assessed convergence, and generated the effective diversity (q) and migration rate (m) surfaces using the R package rEEMSplots (Petkova et al. 2016). For analyses of genetic diversity, F_{ST} and IBD, we excluded *poensis* because the subspecies was only represented by a single sample.

2.7 | Demographic History Reconstruction

To assess demographic inference between pairs of clades, we tested alternative demographic models using the joint site frequency spectrum (JSFS) approach that is based on the diffusion approximation method implemented in *δaδi* v2.0.3 (Gutenkunst et al. 2010). To check for consistency in our results, we compared results from $\delta a \delta i$ with results from the ordinary differential equation method as implemented in moments v1.1.0 (Jouganous et al. 2017). The ordinary differential equation method is like the diffusion approximation method except that it computes the frequency spectrum directly without solving the diffusion system. We generated two-dimensional joint allele frequency spectra (2D-JSFS) between pairs of clades and fitted model parameters to the 2D-JSFS across all pairs of clades that were monophyletic and neighbours in the reconstructed phylogeny. To generate the JSFS without missingness, we used the easySFS.py script: https://github.com/isaacovercast/easySFS to project down the sample sizes of the 2D-JSFS per pair of clades. The script computes the number of segregating sites per possible number of alleles in the dataset. The number of alleles with the highest number of segregating sites was then used as the sample size to generate the final 2D-JSFS data. Initial exploration of the 2D-JSFS showed excess shared heterozygosity between clades, thus suggesting the presence of collapsed paralogs. Consequently, we applied the McKinney et al. (2017) method through a paralog-finder tool (https://github.com/edgardomor tiz/paralog-finder) to explore and filter out paralog from each clade after which we analysed the final 2D-JSFS in moments and *δaδi*.

We then examined 20 divergence models (Table S2) per pair of neighbouring clades, with models corresponding to allopatric and parapatric divergence scenarios using a $\delta a \delta i$ pipeline (Portik et al. 2017), and the *moments* pipeline of Leaché et al. (2019). Allopatric models involve simple models with no gene flow followed by isolation to complex models with instantaneous size change, gene flow after instantaneous size change (secondary contact models), as well as gene flow and size change across two or three discrete time intervals (epoch models). The allopatric model is consistent with the riverine model if the lineage pair boundaries coincide along rivers where migration is expected at headwater regions, and consistent with the refuge hypothesis if it has secondary contact and size expansion with the lineage boundaries coinciding with refugia areas. Also, it is consistent with the elevational barrier model if the lineage pair boundaries coincide with high elevational areas. Parapatric models are models with historical (ancient migration models) or continuous gene flow followed by population split with or without size change. To examine divergence between fischeri population in Kenya and Zanzibar Island, we included 14 island models from the $\delta a \delta i$ pipeline (Table S2).

To estimate demographic parameters per model, we performed three rounds of parameter optimisations using the Nelder–Mead method (*optimize_log_fmin*) with 40, 50 and 100 replicates for the first, second and third rounds, respectively, thus making a total of 190 replicates. The first round used a threefold randomly perturbed starting parameter set with a maximum of 30 iterations per step, while the second and third rounds used the best scoring replicate from the previous round as the starting parameter value with a two-fold perturbed parameter set and a maximum of 40 iterations, and one-fold perturbed parameter set with a maximum of 50 iterations respectively (Portik et al. 2017). We used a multinomial approach to estimate the log-likelihood of the data JSFS given the model and the 'Summarize_Outputs.py' (Portik et al. 2017) to summarise our results across replicates and models per pair of clades.

To select the model that best fits the data, we used the Akaike Information criterion (AIC) values generated from the computation to estimate AIC differences (Δ AIC) and Akaike weights (ω_l) per model (Burnham and Anderson 2002). To estimate the divergence time between clades, we converted split times (*T*) recovered from the best-fit model to absolute values from both $\delta a \delta i$ and moments analyses per pair of clades. The split time was returned by *T*=2Nref×generation time. 'Nref' was derived from the population mutation parameter (θ)=4Nref μ L, where ' μ ' is the per-base mutation rate of the population, and *L* is the sequence length. We used the mutation rate of 2.42×10⁻⁹ for Downy woodpecker (Nadachowska-Brzyska et al. 2015), a generation time of 2.28×10⁻⁷ (Bird et al. 2020) and estimated *L* from the data following the relationship described by Warmuth and Ellegren (2019) where:

 $L = L(unfiltered) \times \frac{\text{segregating sites in filtered dataset}}{\text{segregating sites in unfiltered dataset}}$

3 | Results

3.1 | SNP Calling and Bioinformatic Processing of ddRADseq and Whole-Genome Sequencing Data

We sequenced 183 ddRADseq samples and, after removing individuals with low sequencing quality, we retained 157 individuals, which generated 139,865,740 sites, of which 1,296,882 were variants. *VCFtools* filtering retained 250,608 quality SNPs with mean depth of 16 (Figure S2a,b), and, upon LD pruning, 164,208 SNPs remained with 148 individuals having missingness $\leq 20\%$. For demographic analyses, the number of individuals and SNPs retained per clade is summarised in Table S3. For the ddRADseq phylogeny dataset, we had 161 individuals (157 ingroup samples plus four outgroup samples) with 180,124,546 sites, of which 1,752,372 were variants. Filtering for *ASTRAL* retained 522,543 SNPs with 151 individuals with $\leq 20\%$ missingness, and for *IQ-TREE* retained 503,837 sites with 152 individuals with $\leq 20\%$ missingness.

From the whole genome sequencing, we obtained 1,229,478,670 sites of which 148,032,461 were variants and retained 96,922,833 SNPs after hard filtering for 19 individuals of the focal subspecies and two outgroup individuals. Filtering the SNPs for analyses in *ASTRAL* via *VCFtools* retained 58,295,278 SNPs used to generate 125,343 neighbourjoining gene trees from a sliding window of 500 SNPs, with 46,506 gene trees retained after filtering. The filtering applied for *IQ-TREE* analyses retained 50,796,863 SNPs. The ddRAD dataset generated 1066 gene trees.

3.2 | Genetic Structure Reveals a Divide Between *leucolaimus* and *bilineatus* Complexes Coinciding at the Arid Corridor

An assessment of clustering from genome-wide SNPs revealed two major groups with further clustering within each group. The two major groups were separated at PC1, and consistent with the leucolaimus complex and the bilineatus complex (Figure 1a,b). Within the bilineatus complex, there were three distinct genetic clusters separated at PC3 consistent with the subspecies bilineatus, fischeri and conciliator (Figure 1b). Similarly, within the *leucolaimus* complex, there were three genetic clusters separated at PC1, but the clusters had some degree of admixture between them. The clusters represented a combined group for jacksoni/mfumbiri, one for leucolaimus and one for sharpei/poensis combined. Results from the PCA analyses were congruous with those obtained from ADMIXTURE analyses but detected further subdivision with *fischeri* and *mfumbiri*, with K=8 best supported in the cross-validation report. As in the PCA, genetic distinction was evident within the bilineatus complex, while extensive admixture was revealed between clusters within the leucolaimus complex (Figure 1c). Further admixture analyses within each of the clusters revealed K = 2 as best supported within *concili*ator and bilineatus (Figure 1c) while K = 1 was best supported for all other clusters. Within fischeri, populations in Kenya (hereafter fischeri Kenya) were genetically distinct from populations in Zanzibar (hereafter fischeri Zanzibar), while variation within bilineatus was clinal between the south (hereafter bilineatus south) and east (hereafter bilineatus east). Spatial representation of the ancestry coefficients (Q-matrix) revealed a strong northwest-southeast phylogeographical break between the leucolaimus complex and the bilineatus complex (Figure 1d) with the break coinciding with the arid corridor (Figure 1e). Clusters within the bilineatus complex showed no evidence of admixture, while considerable admixture was evident between clusters within the *leucolaimus* complex at transitions between *mfumbiri/jacksoni* and *leucolaimus* in East Africa and *sharpei/poensis* and *leucolaimus* in West and central Africa. Lastly, testing reliability of the ddRADseq dataset number of SNPs showed a strong correlation between the reduced and full dataset with consistency in plots at PC1–PC3 (Figure S3).

3.3 | Phylogenetic Reconstruction Reveals Reciprocal Monophyly Between *leucolaimus* and *bilineatus* Complexes

Our reconstructed phylogenies corroborated the genetic structure results with a concordance in topology from the coalescent method in ASTRAL (Figure 1f) and concatenation-based maximum-likelihood in IQ-TREE (Figure S2c). Both topologies recovered two major clades consistent with the leucolaimus and the *bilineatus* complexes with a split time of approx. 3.003-4.473 million years ago (Pliocene epoch) estimated from MSMC analyses (Figure 1f, Table S4). Each major clade was further subdivided with strong branch support. Within the bilineatus complex, nominate bilineatus and fischeri were recovered as sister clades with a split time of approximately 146-162 kya while conciliator was sister to the clade containing bilineatus and fischeri with a split time from bilineatus of approximately 572 kya-1.1 million years ago (Table S4). Within the leucolaimus complex, the clade containing jacksoni and mfumbiri was sister to the clade containing leucolaimus, sharpei and poensis (split time approx. 125-181 kya) but *mfumbiri* was paraphyletic with respect to *jacksoni*. Similarly, in the leucolaimus/sharpei/poensis clade, leucolaimus was paraphyletic with respect to sharpei and sharpei was paraphyletic with respect to poensis (Figures 1f and S2c). The topologies obtained using ddRADseq from ASTRAL and IQ-TREE (Figure S2d,e) were also consistent with those obtained from whole genomes and closely matched the results obtained from ADMIXTURE. Although the topologies revealed an mfumbiri sample nested within leucolaimus, ADMIXTURE suggested it is an individual with shared leucolaimus and mfumbiri ancestry (Figures 1c and S2d,e).

3.4 | Similar Genetic Diversity With Higher Differentiation Within *bilineatus* Complex Than in *leucolaimus* Complex

Statistical analyses revealed that genetic diversity overall within the *leucolaimus* complex was not significantly different from that within the *bilineatus* complex (Figure S2d), although plots of genetic diversity showed relatively higher levels within subspecies of the former (Figure 2a). This was especially evident in subspecies with relatively larger sample sizes, *mfumbiri* and *leucolaimus*. As expected, Pairwise $F_{\rm ST}$ estimates were lower between pairs of subspecies within each complex compared to between complexes (Figure 2b, Table S5), with the highest levels recorded between *jacksoni* and subspecies in the *bilineatus* complex. Also, IBD (Figure 2c) was not significant (R^2 =0.0881, p=0.114). Between *bilineatus* and *leucolaimus* complexes, we calculated $F_{\rm ST}$ =0.345



FIGURE 1 | Legend on next page.

(0.342–0.349 CI). EEMS further illustrated the deviation from IBD expectations (Figure 2d,e) and showed that peak genetic diversity coincided with postulated forest refugia (Figure 2d) in Cameroon/Gabon, the Albertine Rift Forest and the Eastern Arc mountains. Meanwhile, the effective migration surfaces

between individuals revealed that genetic similarity decayed more quickly at refugia (Figure 2e). A high decay of genetic similarity was also evident at the arid corridor, thus suggesting it to be a strong migration barrier between the *leucolaimus* and *bilineatus* complexes. **FIGURE 1** | Population and phylogenomic analyses of 164,208 SNPs from 148 individuals reveal distinct separation between *bilineatus* and *leucolaimus* complexes in a PCA visualisation of (a) components 1 and 2, and (b) components 1 and 3. (c) Maximum-Likelihood genetic clustering in *ADMIXTURE* according to the best supported values of *K* with arrows pointing to admixed individuals in Figure S2d. (d) Geographic map of admixture proportions at K = 8 (colour scheme match those in panel c) with arrows pointing to *poensis* and *jacksoni* populations included within the *leucolaimus* complex. Tinkerbird illustrations highlight primary phenotypic differences in rump colour between *jacksoni* (golden) and the rest of the *leucolaimus* complex (lemon). The *bilineatus* complex also exhibits golden rumps. (e) Satellite image illustrates the boundary between *bilineatus* and *leucolaimus* complexes coinciding at the arid corridor (colour scheme matches those in panel b). (f) A multispecies whole genome coalescent tree reconstructed in ASTRAL (46,506 gene trees from 20 individuals) with node branch support indicated with the split time between clades from MSMC projected unto the tree (colour scheme matches those in panels a and b).

3.5 | Demographic History Supports Both Allopatric (Consistent With Pleistocene Refuge Hypothesis) and Parapatric Models

We reconstructed the demographic history between pairs of neighbouring clades in the phylogeny. We focused first on the two major clades, the *leucolaimus* and *bilineatus* complexes, and then between pairs of subclades. Within the *leucolaimus* complex, we compared between *mfumbiri/jacksoni* and *leucolaimus/sharpei/poensis*, and within the *bilineatus* complex, we compared between *bilineatus* and *fischeri* and *bilineatus* and *conciliator*. Because of the structure identified within *fischeri* and *bilineatus*, we also reconstructed the demographic history within *fischeri* (between Kenya and Zanzibar) and within *bilineatus* (between southern and East Africa) (Table S6).

Demographic reconstruction revealed a complex demographic history across pairs of clades in $\delta a \delta i$ (Table S7) and moments (Table S8). First, the divergence between the *bilineatus* and *leucolaimus* complexes was consistent with a refugial model of a split with no gene flow, followed by size (N_e) expansion with continuous symmetrical migration, a result corroborated in moments (Table S6), and consistent with changes in N_e based on MSMC (Figure 3a).

Within the bilineatus complex, the two methods did not correspond in their best-fit model reconstructions whereby between the bilineatus and conciliator pair, a parapatric model was selected in $\delta a \delta i$ (Table S6) but the model overestimated shared alleles (Figures 3b and S4). However, the estimated asymmetric gene flow rate was low at a proportion of 9.4×10^{-6} from *bilin*eatus to conciliator and vice versa at 9.08×10^{-8} . In moments, on the other hand, the best-supported model was a refugial model involving a split with no gene flow (Table S6, Figure S5). The refugial model is more likely for this pair considering the low gene flow rate reported from $\delta a \delta i$. The best support model for bilineatus and fischeri in δaδi (Figure S4) was of refugial isolation followed by size expansion and continuous asymmetric migration (Table S6, Figure 3c), which was corroborated by results from moments (Figure S5). Within subspecies, for the bilineatus south and bilineatus east pair, we selected a parapatric model (Figure 3d) which was the best fit model in $\delta a \delta i$ (Figure S4) but second best in moments (Table S6, Figure S5). The best-fit model in *moments* was a refugial epoch model, but the second-best-fit model was within the range of equivalence of the best model (Table S6) at $\Delta AIC = 1.8$ and $\omega_i = 0.29$ (Burnham and Anderson 2002) and corresponded with the best model from *δaδi*. Between *fischeri* Kenya and *fischeri* Zanzibar, the two methods did not correspond, in *babi* a parapatric model was

selected (Table S6, Figures 3e and S4), but in *moments* it was a refugial model (Tables S6, Figure S5). Still, both involved continuous migration following the split (Tables S7 and S8).

Within the leucolaimus complex, the two methods again did not correspond in their best models between the mfumbiri/ jacksoni clade and the leucolaimus/sharpei/poensis clade. The best-fit model in $\delta a \delta i$ was a parapatric model and from moments, a refugial epoch model. However, the second-best fit models corresponded between $\delta a \delta i$ and moments analyses of a refugial model with no gene flow (Table S6, Figures 3f, S4 and S5), and these captured the range of allele frequency data better than the top models (Figure S6). Further, the scaled asymmetric gene flow rate of the best-supported model from $\delta a \delta i$ had a low proportion of migrants per generation at a rate of 1.38×10^{-2} to 2.15×10^{-4} , suggesting a parapatric model of divergence is unlikely. Moreover, the best-fit models overestimated the split time when compared to MSMC estimates, with estimates from $\delta a \delta i$ suggesting a split time earlier than that between the major clades.

4 | Discussion

In this study, we investigated diversification mechanisms across the entire range of a widespread sub-Saharan African bird, the Yellow-Rumped Tinkerbird. We found strong support for monophyly between two major clades consistent with the *leucolaimus* and *bilineatus* complexes in line with previous reconstructions based on two mitochondrial genes and a nuclear intron (Kirschel et al. 2018). The break between the lineages suggested a primary role of the arid corridor in their divergence. Demographic history reconstruction further highlighted the role of Pleistocene Forest Refugia as a major driver of divergence across biogeographical barriers between lineages while parapatric divergence played a major role within subspecies.

4.1 | Phylogeography and Taxonomic Importance

This is the first study to use whole genome sequences to reconstruct a comprehensive and strongly supported phylogeny of Yellow-Rumped Tinkerbird. The phylogeny revealed a major division in the species (i.e., between *bilineatus* and *leucolaimus* complexes) and had a break that coincided with the arid corridor. The break was corroborated by both the population clustering analyses and evident from EEMS which showed a strong migration barrier at the arid corridor. The arid corridor,



FIGURE 2 | Genetic diversity indices and spatial pattern of genetic variation showed similar genetic diversities with higher differentiation within subspecies consistent with the *bilineatus* complex compared to those of the *leucolaimus* complex than expected under IBD using 164,208 SNP ddRADseq loci. (a) Comparison of genetic diversity indices between subspecies (populations) for allelic richness (q=0), Shannon diversity index (q=1), and observed heterozygosity (q=2). (b) Mean pairwise genetic differentiation (F_{ST}) across all loci between subspecies. (c) IBD plot of genetic distance with geographic distance using a mantel test using population $F_{ST}/1 - F_{ST}$ analyses. Pairwise comparisons represented by inner and outer circle colours. (d) Effective diversity surface (q): blue colours indicate areas of higher genetic dissimilarity than expected under IBD, while orange colours indicate areas of lower genetic dissimilarity. (e) Effective migration surface: blue colours indicate areas of higher effective migration rate, while orange colours indicate areas of lower effective migration rate. White shading indicates genetic dissimilarity expected under IBD. (circled areas indicate postulated refugia).



FIGURE 3 | Legend on next page.

FIGURE 3 | Demographic history reconstructions. Left column: MSMC plots of separation between clades and time of separation from relative CCR. Centre: the model schematic view. Right: 2D-JSFS for the data from $\delta a \delta i$ with sample sizes in number of alleles for (a) *bilineatus* complex (n=120) and *leucolaimus* complex (n=114), (b) *bilineatus* (n=46) and *conciliator* (n=22), (c) *bilineatus* (n=44) and *fischeri* (n=52), (d) *bilineatus* south (n=20) and *bilineatus* east (n=20), (e) *fischeri* Kenya (n=29) and *fischeri* Zanzibar (n=17), (f) *mfumbiri/jacksoni* (n=44) and *leucolaimus/sharpei/poensis* (n=74). MSMC sample sizes of 8 haplotypes were used for (a), (b) and (c), 4 haplotypes for (d) and (e) and 6 haplotypes for (f).

a dispersal route for arid-adapted species, extends from the horn of Africa through Kenya, Tanzania, Zambia, Zimbabwe and Botswana to the Namib desert in southwestern Africa, formed in the Miocene by the combination of the northward movement of Africa towards Eurasia and the Miocene uplift of the Central African Plateau. It divides the coastal forest of East and southern Africa from the Guinean-Congolian and Albertine Rift forests and forms a major geographical barrier to east-west migration for forest organisms (Lovett 1993; Bobe 2006).

The major clades we recovered showed further subdivisions that are of taxonomic importance. Within the bilineatus complex, conciliator was distinct from fischeri and corroborated by distinct clustering and high differentiation (Figure 2), and thus supported the findings of Nwankwo et al. (2018) that recovered conciliator as distinct. We therefore recommend the resurrection of conciliator as a distinct taxon from fischeri. Within conciliator, there was subdivision coinciding with isolated mountain ranges (Udzungwa and Nguru), supporting studies postulating the Eastern Arc mountains as centres of endemism (Diamond and Hamilton 1980; Mayr and O'Hara 1986; Staurt et al. 1993; Burgess et al. 1998, 2007; Fjeldså and Bowie 2008; Barratt et al. 2018). The bilineatus and fischeri clades, on the other hand, have no known biogeographic barrier between them, yet are not known to occur together, and experiments demonstrated that distinct vocal differences might maintain reproductive isolation in times of contact (Nwankwo et al. 2018). In contrast, we observed weaker genetic divergence between subspecies within the leucolaimus complex along with substantial admixture between subspecies, with jacksoni clustering with mfumbiri and poensis nested within sharpei, currently synonymised with leucolaimus, within which it was also nested. Thus, leucolaimus is paraphyletic with respect to both sharpei and poensis, questioning present taxonomy.

Although *leucolaimus* was genetically distinct from *mfumbiri*, there is admixture between them along the Albertine Rift. A subdivision within *mfumbiri* coincides with a vegetational gradient between the Albertine Rift, a forested Afromontane centre of endemism, and West Tanzania and Zambia representing the Zambezian regional centre of endemism characterised by open woodland vegetation (White 1983; del Hoyo et al. 2020; Gill et al. 2024). The admixture between these populations suggests gene flow across the ecotone.

Another well-established barrier to gene flow in the region is the Kenyan Rift, including in plants (Chen et al. 2015; Kebede et al. 2007; Mairal et al. 2017; Ruiz Guajardo et al. 2010), but also fish (van der Merwe et al. 2021) and mammals (Huhndorf et al. 2007). However, in our study, one taxon—*jacksoni*—occurs on both sides of the Kenyan Rift, and although our *jacksoni* samples included in population genetics analyses were all from its eastern side (Table S1), their genetic ancestry clustered within *mfumbiri* from further west, thus suggesting the Kenyan Rift may be a weak barrier to gene flow in tinkerbirds. Our study also sheds light on the mystery of hybridization between the golden-rumped *jacksoni* and lemon-rumped *mfumbiri* forms that led to two previously recognised species, golden-rumped (*P. bilineatus*) and lemon-rumped tinkerbird (*P. leucolaimus*) being lumped into a single species (Prigogine 1980). Our study confirms that *jacksoni*, despite its golden rump, is nested within *mfumbiri* and is thus part of the *leucolaimus* complex. The mystery that remains is whether its golden rump resulted from ancestral introgression from *bilineatus* or *conciliator* across the arid corridor, or convergent evolution.

Taxonomic revisions are clearly needed in Yellow-Rumped Tinkerbird, with *leucolaimus* and *bilineatus* complexes longdiverged lineages meriting species status, potentially with further species-level recognition of the three distinct lineages within the *bilineatus* complex.

4.2 | The Role of Pleistocene Forest Refugia and Parapatric Divergence

The most prominent geographic feature dividing lineages of Yellow-Rumped Tinkerbird was the arid corridor, which differentiates entire assemblages of species in Africa, though it has received the most attention as a potentially important biogeographical barrier in mammals (e.g., Smith et al. 2013, Lorenzen et al. 2012; Bertola et al. 2016). It is also present in insects (Wilfert et al. 2006) and birds (Cohen 2011). Although the arid corridor may have formed in the Miocene, we recovered a Pliocene split time. Its effect as a barrier later in the Pliocene was due to its expansion and contraction in response to the climatic cycles of the Plio-Pleistocene (Williamson 1985; Cerling et al. 1997; DeMenocal 1995; Bobe 2006). This is supported by a Pliocene divergence recorded in plants across the arid corridor (Couvreur et al. 2008; Bobe 2006). The effects of climatic cycles on species divergence have been reported in both plants and animals (Taylor et al. 2012; Migliore et al. 2019; Jacquet et al. 2013; Couvreur et al. 2021) as well as in birds (Voelker et al. 2010; Fjeldså and Bowie 2021). Moreover, the boundaries of the two complexes coincided with proposed refugia (Diamond and Hamilton 1980) with the bilineatus complex in the coastal forest of eastern and southern Africa, and the leucolaimus complex restricted to the Guinea-Congolian forest. The Pleistocene times of split between clades within each complex suggest that glacial cycles in the Pleistocene drove divergence among populations whereby changes in the extent of forest cover in the region played a major role in diversification.

Within the *bilineatus* complex, a parapatric model was selected between *bilineatus* and *conciliator* in $\delta a \delta i$ while a refugial model in *moments* was used; however, the model allele frequency spectra from *moments* (Figure S5b) better matched the data (with lower residuals) than the model from $\delta a \delta i$ (Figure S4b) with

the boundaries of both lineages coinciding at postulated refugias of the montane habitats of the Eastern Arc Mountains for conciliator and the coastal forest of East and southern Africa for bilineatus. Nevertheless, the pattern may also correspond with a dispersal from the highlands of the Eastern Arc mountains into lowland forests during unstable climatic periods of the Pleistocene, as reported for several species that extended their ranges northwards along coastal forests (Fjeldså and Bowie 2021). However, the northernmost range of bilineatus is limited to North-East Tanzania along the coast, with a possible southward expansion suggesting it persisted there. The southward expansion of bilineatus is further supported by the parapatric divergence recovered from both *δaδi* and *moments* between its southern and eastern populations. This is supported by a continuous distribution on the coast from northern Tanzania to southern Africa, along an environmental gradient from the equator to higher latitudes (Sebastianelli et al. 2022). The environmental gradient is due to uneven climatic conditions, including rainfall along the coast, where there is relative climatic stability in Tanzania but higher variability from Mozambique southwards (Burgess et al. 1998). The gradient effect is further supported by the extensive admixture and the negative F_{ST} recovered between them, thus suggesting higher genetic differentiation within than between populations, which could result from extensive gene flow. A similar scenario was recovered between fischeri Kenya and fischeri Zanzibar in δaδi. Although moments reported a refugial model, we believe parapatric divergence is more plausible. Although the populations are allopatric, they occurred along an ecological gradient during divergence. This is possible because, first, Zanzibar was separated from mainland Africa around 6000-9000 years ago (Prendergast et al. 2016), long after the split times reported by *δaδi* (61,000 years), moments (104,000 years) and MSMC analyses (62,998 years), suggesting no physical barrier between them at the time of the split. And second, they occupy the Zanzibar-Inhambane vegetational zones, which consist of a vegetational gradient and ecotones of a moist Northern forested areas in Kenya (fischeri Kenya) that transition southwards to a drier woodland (fischeri Zanzibar). A drier habitat in Zanzibar is further supported by archaeological studies that reported a dominance of large mainland mammals in the Pleistocene, thus suggesting a dominance of drier, open habitat prior to the separation of Zanzibar (Prendergast et al. 2016). Moreover, the island models were poorly supported in $\delta a \delta i$ (Table S5), thereby making an island speciation model unlikely and coupled with low $F_{\rm ST}$ between them, a parapatric divergence is likely. Our results are supported by those recovered by Nwankwo et al. (2018) where fischeri Zanzibar was nested within fischeri Kenya, in effect suggesting the dispersal of continental populations to Zanzibar when permitted by a land bridge. However, between fischeri and bilineatus, a refugial model was supported in both $\delta a \delta i$ and *moments*, with a split time in the late Pleistocene climatic fluctuation period. A refugial model is plausible because the lineages are allopatric, with their boundaries coinciding with postulated refugia areas of the coastal regions of East and southern Africa (Diamond and Hamilton 1980; Crowe and Crowe 1982; Mayr and O'Hara 1986; Lovett 1993; Levinsky et al. 2013) and separated by low mosaic vegetation of savanna and thicket (Burgess et al. 1998). Whereby, fischeri is distributed along the coastal forest of Kenva and on the island of Unguja, Zanzibar, while bilineatus ranges from the coast of northern Tanzania, extending southwards as far as Eastern Cape, South

Africa. Thus, the divide may have expanded during the dry interpluvial periods of the Pleistocene, thereby isolating and driving divergence between the two lineages.

Within the *leucolaimus* complex, we considered the refugial model of divergence most likely between the *mfumbiri/jacksoni* and *leucolaimus/sharpei/poensis* clades. The Pleistocene divergence times between them suggest that climatic fluctuations within this period may have influenced divergence, given that their distribution coincides with postulated Pleistocene refugia (Diamond and Hamilton 1980; Mayr and O'Hara 1986), in the Albertine Rift Forest (*mfumbiri/jacksoni*) and the Cameroon/Gabon refugium (*leucolaimus/sharpei/poensis*). The refugial model is further supported by diversity surface analyses from EEMS, which highlighted these areas as the most diverse.

4.3 | Allopatric and Parapatric Divergence Implications in Sub-Saharan Africa

We found support for both allopatric and parapatric models in different populations of Yellow-Rumped Tinkerbird, confirming its complex demographic history across the continent. Pleistocene Forest Refugia have long been associated with diversification in African birds and mammals (Bowie et al. 2006: Nicolas et al. 2008; Lorenzen et al. 2012; Fuchs and Bowie 2015; Bertola et al. 2016; Fuchs et al. 2021) in which diversification was shown to have been shaped by biogeographical barriers formed during the Pleistocene pluvial cycles. Despite the substantial support for the refuge hypothesis in driving divergence in sub-Saharan Africa, some studies have argued that some species in proposed refugial areas diverged before the Pleistocene (Fjeldsa 1994), and that refugial areas act more like museums rather than speciation centres (Fjeldså and Lovett 1997), thus predicting genetic diversity with no geographic structure in widespread taxa. Although we recovered an earlier split in the Pliocene, we recovered substantial geographic structure, as similarly reported by Marks (2010) for Hylia prasina with subsequent splits in the Pleistocene. Moreso, the refugial model is supported by evidence from paleopalynological studies which showed increased aridity in Africa in the Pleistocene glacial climate, suggesting forest contraction during the period (Lovett 1993; DeMenocal 1995; Maley 1996). The parapatric divergence recovered within subspecies has similarly been found to drive divergence in African birds (Smith et al. 1997; Slabbekoorn and Smith 2002; Kirschel et al. 2011). In Yellow-Rumped Tinkerbird, morphology, plumage and song vary continent-wide along the climatic gradient (Sebastianelli et al. 2022; Lukhele et al. 2025). Although gene flow could impede divergence, with strong disruptive selection presumed for speciation to occur, theoretical models found rapid parapatric speciation plausible despite high migration rates because it depends on complex interactions between population range sizes, local densities, mutation rates and the degree of genetic changes required for speciation (Gavrilets et al. 2000). Moreover, there is increasing support for parapatric divergence in natural populations both in plants and animals (Rice and Hostert 1993; Pinho and Hey 2010; Zhao et al. 2018). Our findings suggest that Yellow-Rumped Tinkerbird has a complex biogeographical history with divergence influenced by climatic effects of the Plio-Pleistocene as well as across ecological gradients.

5 | Conclusions

Yellow-Rumped Tinkerbird is a widely distributed taxon across sub-Saharan Africa, and by studying its phylogeography and demographic history, we identified the prominent mechanisms involved in its diversification. The arid corridor was the major geographical feature that separated lineages on either side, while climatic fluctuations in the Pliocene and Pleistocene were important in the divergence of populations. There was no support for other vicariant mechanisms such as the riverine and elevational barrier hypotheses as divergence did not coincide with rivers and the CVL, respectively. An island model was also not supported within fischeri. By contrast, within two subspecies, parapatric divergence was supported. We caveat that on occasions, the methods we used for demographic history reconstruction did not always produce consistent results. This may be due to insufficient sample sizes of whole genome sequences for MSMC and the limitations in using ddRADseq data in JSFS analyses. Nevertheless, we tested an extensive set of demographic models, and overall, estimated split times were largely similar across the three methods: MSMC, *δaδi* and *moments*. Our findings here highlight the key mechanisms that may drive diversification in many assemblages of animals in Africa and anticipate further continent-wide studies to determine their applicability across birds and other organisms.

Author Contributions

A.N.G.K. conceived the study and designed it with B.O.O., A.B., A.F. and B.M.v.H., with contributions from R.G.M., J.F. and T.B.S., A.B., A.F. and B.M.v.H. provided computational resources and analytical tools, and J.F., R.G.M. and T.B.S. provided samples. B.O.O., L.H., E.C.N. and A.N.G.K. performed fieldwork; B.O.O., A.B., E.C.H., M.M. and E.C.N. performed labwork; and B.O.O., A.B. and L.R. analysed data. B.O.O. wrote the paper, with contributions from all authors.

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Disclosure

In accordance with the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilisation to the Convention on Biological Diversity, this study was conducted in accordance with all ethical guidelines in accordance with permits from the following authorities: SAFRING permit 15966; The Ghana Wildlife Service, Cameroon Biodiversity Conservation Society, Uganda Wildlife Authority, Uganda National Council for Science and Technology, Kenya Wildlife Service, Kenya's National Council for Science and Technology, National Museums of Kenya, Tanzania Wildlife Research Institute (TAWIRI), Tanzania Commission for Science and Technology (COSTECH), Tanzania National Parks (TANAPA), Eswatini Big Game Parks, Ezemvelo KZN Wildlife, Mpumalanga Tourism and Parks Agency. Research was conducted according to approved protocols of the Cyprus Ministry of Agriculture, Rural Development and Environment Veterinary Services, University of Jos, Nigeria and UCLA's Animal Research Committee.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Whole genome and ddRAD sequences with accompanying metadata have been deposited on the NCBI SRA database with BioProject PRJNA1240703, available at http://www.ncbi.nlm.nih.gov/bioproject/ 1240703. The raw fastq reads of the ddRADseq data along with metadata and scripts used for analyses have been deposited in the Dryad Digital Repository, available at http://datadryad.org/share/IEWdd s9N7Pk0dvmLr0g5Eco-P6fnham4ruBQLe8pAvM.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.