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Multiple mountain-hopping colonization of sky-islands on the two sides of Tropical Africa during the Pleistocene: The afroalpine *Festuca* grasses

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Abstract

Aim: The afroalpine sky-islands present one of the most interesting models to study discrete biogeographic patterns in a terrestrial island system. Here, we performed range-wide sampling of the afroalpine clade of fine-leaved *Festuca* grasses and address a set of hypotheses on its origin and dispersal. We focus on the widespread species *F. abyssinica* and explore the role of the eastern and western African sky-islands.

Location: Tropical Africa.

Taxon: Afroalpine *Festuca*.

Methods: We combine data from field surveys, phylogeography, coalescence-based dispersal modelling, and environmental niche and dispersal costs analyses to infer patterns of genetic diversity, genealogical relationships, colonization routes and range shifts under two Quaternary climates (current – to represent warm periods; and Last Glacial Maximum (LGM) – to represent cold periods).

Results: The westernmost populations in East Africa show higher genetic diversity and higher similarities with the West African populations and the Ethiopian Simen Mountains than with the more closely situated East African populations. Dispersal models and ecological niche predictions of *F. abyssinica* supported multiple long-distance dispersals (LDD) among the eastern African sky-islands, and at least two dispersal events between the two sides of Africa (0.86 Ma and 0.52 Ma), probably facilitated by bridging suitable habitats during the coldest periods of the Pleistocene.

Main conclusions: We reconstruct an afroalpine mountain-hopping dispersal model, with migrations occurring between adjacent sky-islands in eastern Africa, and through a Central Africa–Sudan pathway connecting afroalpine patches on the two sides of the continent.

KEYWORDS

afroalpine *Festuca*, dispersal cost analysis, ecological niche modelling, long-distance dispersal, mountain-hopping colonization, phylogeography, Tropical Africa

1 | INTRODUCTION

Island systems, surrounded by sea or situated within a continent, have in common their role as generators of diversity and constitute ideal scenarios to study the evolutionary processes that produced it (McCormack et al., 2009; Warren et al., 2015). On oceanic islands, the organisms must necessarily have arrived via cross-oceanic dispersal, whereas on continental islands the organisms may have arrived from adjacent areas, for example, through habitat suitability pockets formed under past climates and/or through range distribution shifts (Kebede et al., 2007; McCormack et al., 2009).

The mountains of Tropical Africa form a mosaic of scattered high-altitude peaks and plateaus subjected to a high degree of isolation, where the communities of plants and animals stand out from those of the intervening lowlands for their endemism (Gehrke & Linder, 2014; Mairal, Sanmartín, Herrero, et al., 2017). In eastern Africa, the most remarkable geological structure is the Great Rift Valley (Figure 1). In its northern part, the Rift Valley is surrounded by the Ethiopian Massif, a region formed by high basaltic plateaus and volcanic peaks and divided into two large units, the Abyssinian Massif to the northwest and the Harar Massif to the southeast (Figure 1). In the southern part, the Rift Valley is divided into two large volcanic arches: the Gregory Rift (Eastern Rift) and the Albertine Rift (Western Rift), which are re-united further down in southern Tanzania. These arches are splashed by numerous geographical features such as old massifs, high plateaus and several

volcanoes of more recent origin. In western Africa, the Cameroonian Volcanic Line (CVL) includes volcanoes of various ages such as Mt. Cameroon on the mainland and Basilé Peak on the island of Bioko (Figure 1). The eastern and western African mountains represent one of the most isolated mountain systems in the world (Sklenář et al., 2014), further subjected to long-term isolation among them due to geographical features such as the Congo Basin and the Sahara desert. This, together with the rugged topography, has strongly shaped their biota (Hedberg, 1969, 1970; Mairal, Sanmartín, Herrero, et al., 2017; Sklenář et al., 2014). Nonetheless, the biotic communities appear to be fairly uniform and these mountains share a large number of species (Galley et al., 2007; Kingdon, 1990; Popp et al., 2008; Sklenář et al., 2014). In fact, most of the Tropical African sky-islands have in common three well-defined altitudinal vegetation belts, the lowermost afroalpine forest (1,300–3,000 m), the transitional ericaceous belt (subalpine; 3,000–4,100 m) and the uppermost afroalpine belt (>3,550 m; Hedberg, 1951).

During the last decades, the colonization of the different Tropical mountains of Africa has been alternatively explained by long-distance dispersal (LDD hypothesis; Ayele et al., 2009; Hedberg, 1970) or via forest reconnections (Montane forest bridge (MFB) hypothesis; Kebede et al., 2007; Kadu et al., 2011; Mairal, Sanmartín, Herrero, et al., 2017; Mairal, Sanmartín, Pellissier, 2017), when the afroalpine vegetation belt descended 1,000–1,500 m and connected isolated patches through vegetation bridges during the glacial phases (Coetzee, 1964; Kebede et al., 2007). However,

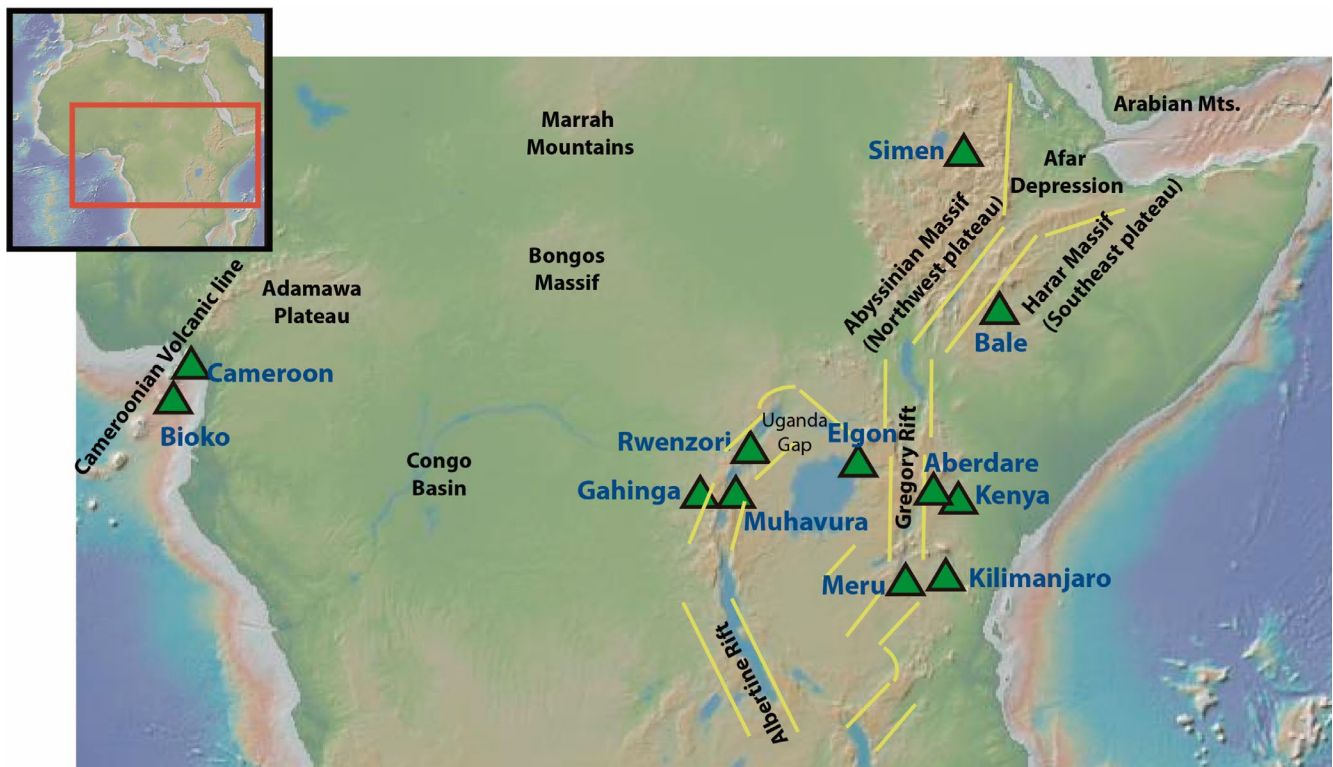


FIGURE 1 Map showing sampling sites for the studied afroalpine *Festuca* species (green triangles). The red square encompasses the Tropical African Mountains (small inset). Yellow lines represent the Great Rift Valley (major tectonic faults). The map was generated using the software GeoMapApp (v. 2.3 (<http://www.geomapapp.org>))



recent phylogeographic and ecological niche modelling studies have shown that these two hypotheses are not mutually exclusive (Chala et al., 2017; Gizaw et al., 2013; Mairal et al., 2015; Mairal, Sanmartín, Herrero, et al., 2017). It has been argued that the ranges of afro-montane plant species can be explained by both LDD and MFB, aided by short-range or stepping-stone dispersal between adjacent mountains (Coetzee, 1964; Kadu et al., 2011; Kebede et al., 2007). The most remarkable examples of afro-montane disjunctions are provided by plant species that are shared between sky-islands located on different sides of Tropical Africa (Kingdon, 1990). Despite their extreme isolation under the current climate, it has been proposed that past vegetation corridors could have connected the ranges of some afro-montane lineages throughout equatorial Africa (Hall, 1973; Kadu et al., 2011; Mairal et al., 2015; Mairal, Sanmartín, & Pellissier, 2017; Pokorný et al., 2015), while for others a LDD process has been proposed (Gizaw et al., 2016; Popp et al., 2008).

By contrast, it has been reasoned that MFB contact for species restricted to the afroalpine belt would have been impossible between most mountains in East Africa (Chala et al., 2017), and definitely between mountains situated on opposite sides of the continent, leaving intermountain LDD as the only plausible explanation for the afroalpine colonizations (Assefa et al., 2007; Chala et al., 2017; Ehrich et al., 2007; Gizaw et al., 2016; Hedberg, 1970; Wondimu et al., 2014). However, there are so far no studies addressing the connections between the two sides of Tropical Africa for plant species that are typical of the uppermost, afroalpine vegetation belt.

Here, we examine the patterns of genetic diversity and phylogeographic history of the afroalpine fine-leaved *Festuca* species found on both the eastern (from Ethiopia to Uganda) and the western Tropical African sky-islands. These fescues form a single afroalpine clade of polyploid (mostly tetraploid) species (Namaganda et al., 2006) originated at the Messinian–Pliocene transition, remarkably from northern South American ancestors (Minaya et al., 2017). Most of these fescues are restricted narrow endemics; by contrast, *F. abyssinica* sensu lato is widespread in mountains on both sides of Tropical Africa (Figure 1b; Clayton, 1970). In this sense, the afroalpine *Festuca* species may therefore be good candidates for studying migration patterns both within eastern Africa and between the eastern and western Tropical African sky-islands.

We address a set of hypotheses on the origin and spread of the afroalpine *Festuca* species. Our first hypothesis assumes that the afroalpine *Festuca* clade originated by dispersal from South America (Minaya et al., 2017), and therefore we predict that the lineage first arrived in West Africa and then spread to East Africa. For migration between West and East Africa, we hypothesize three possible scenarios: (a) direct LDD, (b) stepping-stone LDD and (c) MFB, that is, gradual migration. Based on previous evidence, we expect a west–east colonization through LDD or through suitable habitat pockets that might have facilitated migration between the two sides of the African continent. Here, different pathways might be expected: a northern path through the mountain massifs of the Sahara (Désamoré et al., 2011; Mairal, Sanmartín, Herrero, et al., 2017),

a Central African pathway (Kadu et al., 2011; Wickens, 1976) or a Southern Migratory Tract (SMT; White, 1981). Through hypothesis testing scenarios, we expect to find additional cues to discern between the LDD and MFB hypotheses, and to unveil the connection path presumably followed by this and other afroalpine plants between the two sides of the continent.

In the present study, we performed range-wide field sampling of the afroalpine *Festuca* clade in Tropical Africa, to explore dispersal patterns and speciation in the eastern and western African sky-islands. We present a dated phylogeny of the afroalpine *Festuca* species using non-coding plastid (pDNA) markers and the nuclear ribosomal (nrDNA) ITS region. We use a large sample of intraspecific DNA sequences and Amplified Fragment Length Polymorphism (AFLP) markers covering the entire range of *F. abyssinica* and other afroalpine fescues. These markers have been shown to be suitable for detecting ancient phylogeographical signals of genetic diversity, especially when covering wide geographic scales (Bonin et al., 2007; Mairal, Sanmartín, Herrero, et al., 2017). We also performed coalescent-based dispersal modelling analysis to unravel the colonization scenario of the Tropical African sky-islands by the widespread afroalpine *F. abyssinica*. We modelled the distribution of *F. abyssinica* under the current and the Last Glacial Maximum (LGM) climates, and hypothesized that suitable habitat pockets might have facilitated stepwise migration between western and eastern Africa and within eastern Africa. We further computed least cost path-based resistance estimates of migration between the populations from the two sides of Africa. Our main aims were to (a) determine the geographical distribution of genetic variation in afroalpine *Festuca*, with a focus on *F. abyssinica*, (b) examine the role of the afroalpine sky-islands as refugia of genetic diversity, (c) reconstruct the phylogeographic history including ancestral ranges and migration paths between *F. abyssinica* populations and (d) detect suitable habitat pockets and identify potential migration routes between western and eastern Tropical Africa during past colder periods.

2 | MATERIALS AND METHODS

2.1 | Population sampling and DNA extraction

We performed several field campaigns and range-wide sampling of the afroalpine *Festuca* in the Tropical eastern African mountains, and also obtained samples from West Africa from herbaria and as silica-dried leaf collections (Tables S1 and S2). We covered the total distribution ranges of the species and sampled at least two populations of *F. abyssinica* ($2n = 4x = 28$; Namaganda et al., 2006) on each of nine high mountains in Eastern Africa (Figure 1): Bale and Simen Mts in Ethiopia; Aberdare Mts, Mt Elgon and Mt Kenya in Kenya; Mt Kilimanjaro and Mt Meru in Tanzania and Rwenzori Mts and Virunga Mts (including Mt Gahinga and Mt. Muhavura) in Uganda, and on two West African mountains, Mt Cameroon in Cameroon and Basilé Peak in Equatorial Guinea (Bioko Island). In addition, other species of the *F. abyssinica* clade (*F. borbonica*, *F. camusiana*, *F. gilbertiana*, *F.*

macrophylla, *F. perrieri*; Minaya et al., 2017) and other, less closely related afroalpine species (*F. chodatiana* ($2n = 4x = 28$; Namaganda et al., 2006), *F. obturbans* and *F. pilgeri* ($2n = 4x = 28$; Namaganda et al., 2006) were sampled in the field for this study (see Table 1; Tables S1 and S2). Samples were collected from individuals scattered across the whole occupancy area of each population, and preserved in silica gel. Total genomic DNA was extracted from a total of 227 individuals using the DNeasy Plant Mini Kit (QIAGEN Inc.), from 20 to 25 mg of silica-gel-dried leaves or herbarium leaf materials. For some species, DNA sequence data were retrieved from our previous study (Minaya et al., 2017). We included 21 additional *Festuca* and Loliinae species representing the sister South American (American II) clade of the afroalpine clade (following Minaya et al., 2017) that were used as alternative outgroups in the phylogenetic and biogeographic analyses. Additional details of the afroalpine *Festuca* species are explained in Supporting Information 'Expanded Material and Methods'.

2.2 | DNA sequencing and phylogenetic analysis

We selected the nuclear ribosomal (nrDNA) ITS region and two plastid intergenic spacer regions (*trnL-trnF* and *trnT-trnL*) for phylogenetic analysis. These markers have proven to be useful for inter-specific analyses in previous studies of Loliinae (Minaya et al., 2017). In all, we generated 78 new sequences for the afroalpine *Festuca*

(26 *trnL-trnF*, 26 *trnT-trnL*, 26 ITS). The population information of the material examined and GenBank accession numbers are detailed in Table S2.

Sequences for each plastid and nuclear region were aligned using MAFFT 6.814b (Katoh et al., 2002), implemented in the software GENEIOUS PRO 5.4.4. (Biomatters Ltd.). MRMODELTEST v.2.2 software (Nylander, 2004) was used to determine the best-fitting model of sequence evolution for each data partition. Phylogenetic analyses were performed as different data partitions, using each marker separately (ITS, cpDNA), before combining the data sets (ITS + cpDNA). The broad-leaved and fine-leaved *Festuca* and the American II group species were used as outgroups, rooting the tree with the basal broad-leaved *F. coerulescens*. Phylogenetic relationships were estimated using Bayesian inference implemented in MrBayes (Ronquist et al., 2012). Two independent runs of three Metropolis-coupled chains each were run for 10 – 20 million generations, sampling every 1000 generations. Convergence was assessed by monitoring cumulative split frequencies in MrBayes and the effective sampling size criterion (ESS >200) in Tracer v1.6 (Rambaut et al., 2013). After discarding the first 25% samples as burn-in, we pooled the remaining trees to construct a 50% majority rule consensus tree.

Lineage divergence times were estimated using a nested dating approach (Mairal et al., 2015; Pokorný et al., 2011), implemented in BEAST v.1.7 (Drummond & Rambaut, 2007). Due to the lack of fossil records for the afroalpine fescues, a more inclusive fine-leaved

TABLE 1 Descriptors of within-population genetic diversity in eastern African *Festuca abyssinica* populations and other afroalpine *Festuca* species estimated using AFLP markers. Due to the close geographic proximity, the Gahinga and Muhavura populations were united into a single 'sky-island'. Abbreviations: H_j, Nei's gene diversity; DW, frequency down-weighted value. The highest values observed for some parameters are indicated in bold.

	Sky-island	No. of populations	No of samples	H _j	No of private fragments	Proportion of variable markers (P)	DW
<i>F. abyssinica</i>							
	Simen	4	20	0.1531	1	0.5122	42.553
	Rwenzori	6	16	0.2376	11	0.7807	107.44
	Gahinga -Muhavura	3	8	0.2620	10	0.6684	54.843
	Elgon	7	33	0.1510	5	0.6140	90.623
	Bale	13	44	0.1743	5	0.7140	139.391
	Kenya	2	7	0.1642	1	0.3754	17.401
	Aberdare	3	10	0.1592	–	0.4263	21.816
	Kilimanjaro	5	11	0.1571	1	0.4859	43.717
	Meru	3	11	0.1176	–	0.3421	26.165
	Total	46	160		34		
<i>F. abyssinica</i> s. l. complex species							
	<i>F. gilbertiana</i> Simen	1	6	0.2466	–	0.5561	32.146
	<i>F. macrophylla</i> Simen	1	10	0.2257	–	0.6649	56.873
Other close afroalpine <i>Festuca</i> species							
	<i>F. obturbans</i> Kilimanjaro	1	4	0.1599	–	0.2912	11.88
	<i>F. pilgeri</i> Aberdare	20	20	0.1423	2	0.4947	60.491
	<i>F. pilgeri</i> Kenya	25	27	0.1245	2	0.4912	99.767
	Total	46	51		4		



Loliinae data set (31 samples, 2287 positions) was used to estimate divergence times within the afroalpine *Festuca* ingroup (22 samples, 2268 positions). The Loliinae tree was rooted with broad-leaved *F. pratensis*, *F. scariosa* and *F. coerulescens*. We calibrated (a) the crown node of the broad-leaved and fine-leaved Loliinae (22.5 Ma, standard deviation [SD] = 1); (b) the stem node of *Festuca-Wangenheimia* (11.47 Ma, SD = 1); (c) the stem node of *Aulaxyper-Vulpia* 2x (9.32 Ma, SD = 1); (d) the crown node of the American II and afroalpine clades (6.19 Ma, SD = 0.8) and (e) the crown node of the afroalpine clade (4.17 Ma, SD = 0.8). Choice of model priors was based on Bayes factor comparisons using the path sampling (PS) and stepping-stone (SS) sampling methods in BEAST (Baele et al., 2012). We used a uniform prior for the *ucl.d.mean* within values commonly observed in plant plastid markers (10^{-4} – 10^{-1} substitutions/site/Ma; Wolfe et al., 1987) and a default exponential prior for the standard deviation. Two MCMCs were run for 20 million generations, sampling every 1000th generation. We used Tracer v.1.6 (Rambaut et al., 2013) to monitor convergence and ESS values (>200) for all parameters, and TreeAnnotator v. 1.7 (Rambaut et al., 2013) to construct a maximum clade credibility tree from the posterior distribution after discarding 10% samples as burn-in.

To infer ancestral ranges and to trace the history of migration events in the *F. abyssinica* clade, we used the Discrete Phylogeographic Approach (DPA) of Lemey et al. (2009). To identify the rates (colonization routes) that are best supported by the data, we applied a continuous-time Markov chain (CTMC) model with a Bayesian stochastic search variable selection (Lemey et al., 2009) implemented in BEAST 1.7.5. We defined six discrete areas, grouping populations within the main geographical areas: (1) Harar Massif (Bale), (2) Abyssinian Massif (Simen), (3) Eastern Rift (Kenya, Kilimanjaro, Meru, Aberdare and Elgon), (4) Western Rift (Virunga, Rwenzori), (5) Cameroon and (6) Bioko. Migration rates among sky-islands were modelled using default gamma prior distributions.

2.3 | AFLP fingerprinting

For the AFLP analysis, we included five afroalpine *Festuca* species. For *Festuca abyssinica*, we analysed 160 individuals (46 populations) distributed in nine sky-islands and covering all the distribution of the species in Eastern Africa (Table 1) and one location in Western Africa (Cameroon; Figure 1). Additionally, we used six individuals of *F. gilbertiana* (1 population), 10 of *F. macrophylla* (1 population), 4 of *F. obturbans* (1 population) and 47 (20 from Aberdare and 27 from Kenya) of *F. pilgeri* (2 populations; Table 1). The AFLP fingerprinting followed the original procedure of Vos et al. (1995) with minor modifications and was implemented using the AFLP plant mapping kit (Applied Biosystems). Additional details are explained in Supporting Information 'Expanded Material and Methods'.

Reproducibility and processing of the AFLP fragments were performed using GeneMapper Software v4.1 (Applied Biosystems). To reduce potential errors associated with AFLP, we first discarded short fragments (length <100 bp) because the fragments of this

size class have a high chance of being non-homologous (Vekemans et al., 2002). AFLP profiles were scored as present or absent based on the selection of allele peaks with threshold intensity higher than 100 RFU (relative fluorescent units). Afterwards, we performed manual editing for each primer combination, checking the profile peak by peak to exclude shoulder peaks and unreliable loci (variation between replicates). We exported the peak height data and loaded them into the R package AFLPCORE version 1.4a (Whitlock et al., 2008). In this way, the AFLP profiles were scored and the error rates were estimated. The 10% of samples that were replicated to test for reproducibility were below the critical bound of 5% indicated in previous reports (Pompanon et al., 2005) for each primer combination. Additional data reliability was assessed through comparison of duplicates, from one or two individuals per population.

2.4 | AFLP data analyses

The resulting AFLP matrix was analysed using AFLP-SURV v.1.0 (Vekemans et al., 2002) to estimate demographic statistics such as Nei's gene diversity (H_j), proportion of variable markers and pairwise differentiation among subpopulations (F_{ST} ; Lynch & Milligan, 1994; Nei & Li, 1979). Ten thousand permutations were performed to calculate F_{ST} values. The *AFLPdat* R package (Ehrich, 2006) was used to determine the number of private fragments per population and to calculate the frequency-down-weighted values (DW) per population, equivalent to the weighted species value, using rarefaction analyses (Crisp et al., 2001; Schönswetter & Tribsch, 2005).

In order to explore the genetic clustering of populations of *Festuca*, we used different groups: (i) the afroalpine clade (*F. abyssinica*, *F. gilbertiana*, *F. macrophylla*, *F. obturbans*, *F. pilgeri*); (ii) the *F. abyssinica* s. l. group (*F. abyssinica*, *F. gilbertiana*, *F. macrophylla*) and (iii) *F. abyssinica* s.str. A pairwise similarity matrix using Dice's coefficient was constructed and subjected to a principal coordinate analysis (PCO) implemented in NTSYS v.2.1 (Rohlf, 1998). Genetic relationships among samples were visualized in SPLITSTREE v.4.10 (Huson & Bryant, 2005) using neighbor-net analysis with the split decomposition method. To quantify the amount of genetic differentiation attributable to geographical and population subdivision, a hierarchical Analysis of Molecular Variance (AMOVA) was performed using ARLEQUIN v.3.0 (Excoffier et al., 2005). For this, exploratory analyses were performed considering sky-islands as different units or grouping them according to geographical features (e.g. Gregory vs. Albertine Rift), or to previous bio/phylogeographic hypotheses (e.g. Mt Elgon as a crossroad and other paths; Mairal, Sanmartín, Herrero, et al., 2017). Bayesian clustering methods implemented in STRUCTURE v.2.3 (Falush et al., 2007; Pritchard et al., 2000) were used to assess genetic structure. Analyses were performed under admixture conditions and correlated allele frequencies between groups. A total of 500,000 MCMC generations with a burn-in of 100,000 were run for K values of 1–10, with 10 repetitions for each K . The most likely K value was determined using Evanno et al. (2005) ΔK , implemented in STRUCTURE HARVESTER (Earl, 2012). To

identify possible geographical locations acting as major genetic barriers among *F. abyssinica* populations, we computed barriers (using geographical coordinates of each sky-island; Table S3) on a Delaunay triangulation using Monmonier's algorithm in BARRIER v.2.2 (Manni et al., 2004). The significance was examined by means of 1000 bootstrapped distance matrices obtained using AFLPSURV.

2.5 | Phylogeographic hypothesis testing of the colonization routes

Based on the results from the above-mentioned analyses and on phylogeographic hypotheses for afroalpine organisms in the eastern African sky-islands (Mairal, Sanmartín, Herrero, et al., 2017), we tested the strength of alternative colonization routes of *F. abyssinica* populations against the null hypothesis of isolation by distance (IBD) – determined by the shortest pairwise linear distance connecting populations (Buckler et al., 2006). For this, we used our AFLP data set and the program PHYLOGEOGRAPHER 1.1 (Buckler, 1999; Buckler et al., 2006) to test simple correlations between pairwise genetic distances and geographical distances matrices among populations (Dietz, 1983; Smouse et al., 1986), assessing the significance with 10,000 permutations. For some models, we created intermedial virtual nodes in areas that have been postulated as phylogeographic canonical hypotheses for afromontane species (Kadu et al., 2011; Mairal, Sanmartín, Herrero, et al., 2017; White, 1981), like those in South Sudan and the Congo Basin.

Additionally, we tested phylogeographic hypotheses that take into account the potential existence of incomplete lineage sorting (ILS) using the chloroplast data set. For this, we performed Approximate Bayesian Computation techniques as implemented in the software DIYABC v1.0.4.46beta (Cornuet et al., 2008, 2010) to statistically evaluate alternative scenarios for the dispersal history of *F. abyssinica*. We clustered populations into five groups according to their plastid and nuclear geographical structure: Harar, Gregory, Albertine, Cameroon and Bioko (see Figure 4b). We considered six competing population history scenarios of colonization (Figure 4b): (1) stepping-stone dispersal from northeast to west; (2) stepping-stone dispersal from west to northeast; (3) ancestral colonization in Cameroon–Albertine Rift and dispersal to the east, with diversification on both sides of Africa; (4) ancestral colonization from the west to the east side of Africa, with a retro-colonization from east to west; (5) ancestral colonization from the east to the west side of Africa and (6) radiation. In a first analysis, the prior distribution of parameters had a larger interval due to the lack of ancestral information; they were subsequently corrected in a second analysis according to the values obtained after the first tests (Bobo-Pinilla et al., 2018). We assumed equal effective population sizes for each historical scenario (N_e) in the two tests. For the mutation model, we imposed a Uniform mutation rate ($\mu = 10^{-9}$ – 10^{-7}) for the plastid molecules. We simulated 10 million data sets for each scenario and summarized them using summary statistics (number of haplotypes, number of segregating sites, variance of pairwise differences and F_{ST}) to compute

measures of bias and precision for each scenario [95% high posterior density (HPD) credibility intervals] (Cornuet et al., 2008). We estimated the posterior probability of each scenario by weighted logistic regression, using the 1% of the simulated datasets with summary statistics that were closest to the observed values. We also estimated the posterior probability of the parameters from 1% of the best-simulated datasets, using local linear regression and logit transformation of parameters (Cornuet et al., 2010). Table S4 shows the prior distributions of the demographic and divergence parameters imposed for the six scenarios in the two consecutive searches and the estimated parameters' values.

2.6 | Ecological niche modelling and dispersal cost path analyses

We used a total of 305 occurrence records, 242 from our own field observations/collections, 16 from herbaria and 47 from Global Biodiversity Information Facility (GBIF; see Supporting Information 'Expanded Material and Methods'). Data were compiled in a matrix and records falling within 1 km distance from each other were removed. In order to perform the modelling analysis, three model algorithms varying in statistical properties were used to map the habitat suitability for *F. abyssinica*: (i) maximum entropy model (MaxEnt; Phillips et al., 2006), (ii) general additive model (GAM; Wood, 2011) and (iii) the gradient boosting model (GBM; Elith et al., 2008). Methods to generate and weight pseudo-absence records, and model fitting procedures and their evaluation are explained in Supporting Information 'Expanded Material and Methods'. We produced binary presence/absence maps of the *F. abyssinica* niche models using Maximum Sum Threshold (Maximum Sensitivity plus Specificity) criteria. We generated three binary maps for current potential distribution and for each of the LGM climate models. Then, we used an ensemble approach (Araújo & New, 2007) to assemble these binary maps and defined three habitat suitability classes for *F. abyssinica* based on agreements among the pixels in predicting habitat suitability (Chala et al., 2016): (i) unsuitable area with high certainty, when none of the maps from all the three algorithms predicted habitat suitability; (ii) uncertain habitat suitability, when only one of the three binary maps predicted suitability, and (iii) suitable habitat with high certainty, when two or three maps predicted suitability. We further divided the ensemble habitat suitability maps into two classes by assigning 1 to the pixels that were classified as suitable with high certainty and 0 to the rest to represent suitable and unsuitable habitats. Then, we overlaid the current and LGM versions of these *F. abyssinica* maps to detect temporal changes in habitat suitability and potential habitat corridors or suitable pockets between eastern and western African mountains during the LGM in ArcGIS environment.

To detect temporal changes in migration resistance, we derived pairwise least-cost path distances between the geographically closest eastern and western African high mountains, that is, between Gahinga/Muhavura and Cameroon, as well as between Simen and Cameroon, by using the habitat suitability maps as a

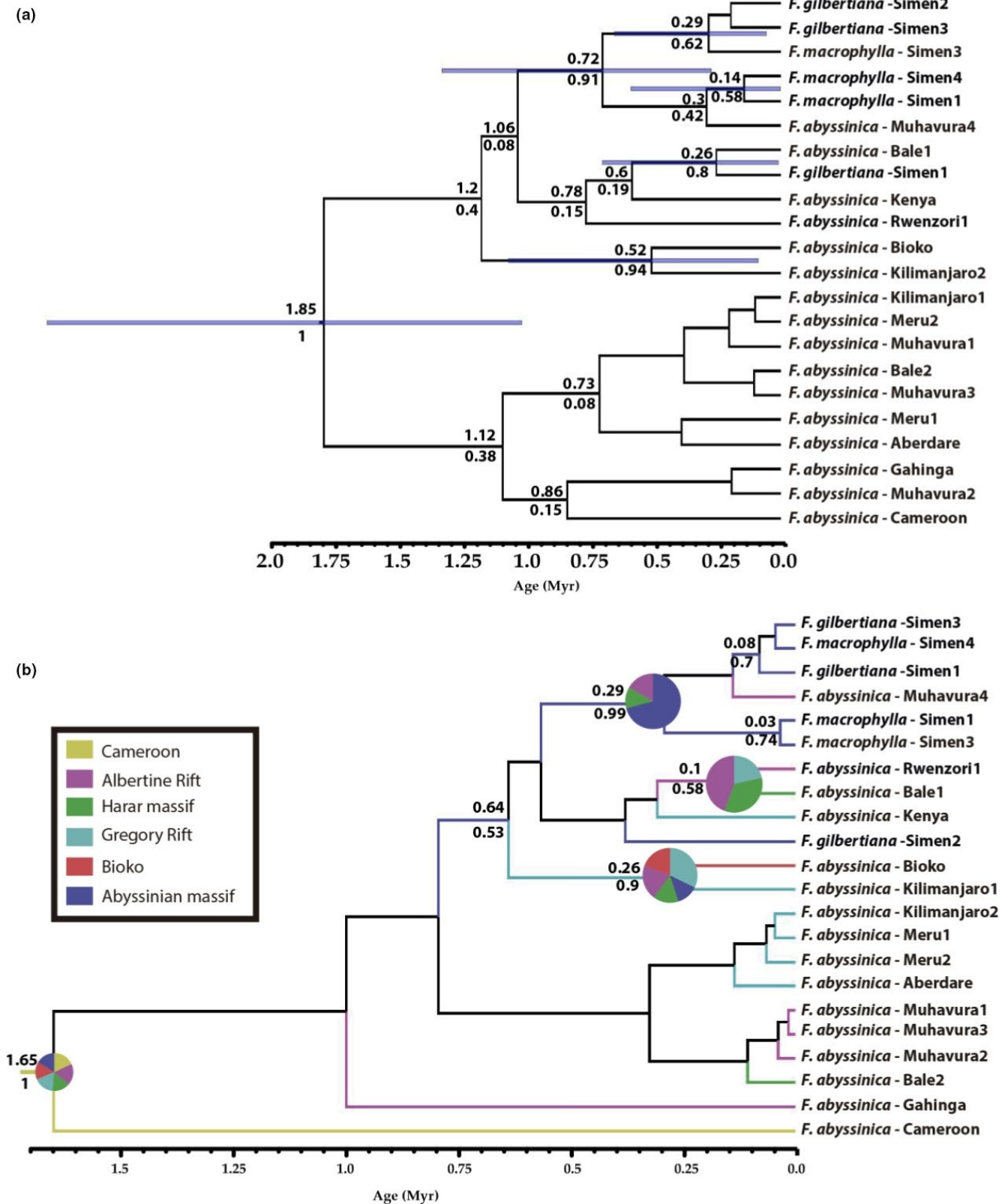


FIGURE 2 Phylogenetic analyses. (a) Maximum clade credibility (MCC) tree obtained from the BEAST analysis of the afroalpine *Festuca abyssinica* sensu lato species based on concatenated plastid and nuclear data set (*trnL-trnF*, *trnT-trnL* and ITS). (b) BEAST Maximum clade credibility (MCC) tree showing the Bayesian ancestral range reconstruction analysis of *Festuca abyssinica* s. lat. Coloured branches (see legend) represent the ancestral range with the highest posterior probability for each population (only branches with PP > 0.5 are coloured); node pie charts show marginal probabilities for alternative ancestral ranges (only shown in supported coloured branches). Numbers above branches are mean ages (Ma) and numbers below branches are Bayesian posterior probability support (PP).

cost raster. The cost raster was produced by assigning different cost values for the ensemble habitat suitability classes. Pixels which were unsuitable with high certainty were assigned a resistance cost of 10, pixels categorized into the uncertain suitability class a cost of 5 and pixels categorized into the certain suitability class a cost of 1.

3 | RESULTS

3.1 | Phylogenetic analysis

The *trnL-trnF*, *trnT-trnL* and ITS data sets consisted of 846, 739 and 587 nucleotide sites respectively. The concatenated pDNA matrix consisted of 22 sequences and 1585 sites, and the concatenated ITS + pDNA matrix of 22 sequences and 2,172 sites, of which 26 were polymorphic.

The plastid and nuclear Bayesian trees were congruent to each other (Figures S1 and S2). The 50% MR consensus tree of the combined ITS + pDNA data set resolved the afroalpine clade as

monophyletic with strong support (Figure S3). In this tree, the *F. abyssinica* sample from Cameroon was resolved as sister to *F. abyssinica* samples from Gahinga and Muhavura4 (Albertine Rift), whereas the *F. abyssinica* sample from Bioko was sister to a *F. abyssinica* sample from Kilimanjaro2 (Gregory Rift; Figure S3).

The birth–death (BD) and the strict-clock models were selected as best-fit models for divergence time estimation of lineages in BEAST (Table S5). The afroalpine *Festuca* samples selected for the BEAST dated tree (Figure 2a; Figure S4) showed the same resolution and high posterior probability values as those in the MrBayes trees. Split dates for the outgroup *Festuca* species agreed with those of Minaya et al. (2017), with the crown age of the afroalpine clade dated in the Pliocene (4.17 Ma, 95% HPD = 3.23–5.1; Figure S4). Our dating analysis inferred an early Pleistocene age for the strongly supported crown node of the *Festuca abyssinica* s. l. group (1.85 Ma, 95% HPD = 1.04–2.97; Figure 2a). Remarkably, a clade grouping *F. macrophylla*, *F. gilbertiana* and one individual of *F. abyssinica* from Muhavura4 (PP = 0.91) were dated 720 kya, and a clade of *F. abyssinica* samples from Kilimanjaro2 and Bioko (PP = 0.94) 520 kya. The relationship between the *F. abyssinica* samples from the

TABLE 2 Hierarchical analysis of molecular variance (AMOVA) for afroalpine *Festuca* species and exclusively for *Festuca abyssinica* based on percentages of allelic variation at different levels: (A) among groups, (B) among populations within groups and (C) within populations. Abbreviations indicating population codes of *F. abyssinica* are as follows: Ab (Aberdare), Ba (Bale), Ke (Kenya), Me (Meru), Ki (Kilimanjaro), Si (Simen), El (Elgon), Rw (Rwenzori), Mu (Muhavura) and Ca (Cameroon). Albertine Rift includes populations of Rwenzori, Gahinga and Muhavura; Gregory Rift includes populations of Aberdare, Elgon, Kenya, Kilimanjaro and Meru. Aby: *F. abyssinica*. All values have a significance of $p < 0.01$.

AMOVA groups	No of groups (K)	Levels			F-statistics		
		A	B	C	F_{sc}	F_{st}	F_{ct}
<i>Afroalpine Festuca</i>							
[pilgeri]/[obturans]/ [Aby(Albertine + Ca) + macrophylla + gilbertiana]/[Aby (Simen, Eastern Rift)]	4	26.88	9.17	63.95	0.12544	0.36052	0.2688
[abyssinica]/[macrophylla]/[gilbertiana]/[pilgeri]/ [obturans]	5	20.51	15.74	63.75	0.19806	0.3625	0.20506
[abyssinica + macrophylla + gilbertiana]/[pilgeri]/ [obturans]	3	21.15	16.97	61.88	0.21519	0.38121	0.21154
<i>Festuca abyssinica</i> group							
[macrophylla + gilbertiana + Aby(Albertine + Ca)]/[Aby (Simen, Eastern Rift)]	2	23.59	9.07	67.33	0.11874	0.32666	0.23593
[macrophylla + gilbertiana]/[abyssinica]	2	13.95	16.72	69.32	0.19436	0.30677	0.13953
<i>Festuca abyssinica</i>							
All populations	1	20.7	NA	79.3	NA	NA	NA
[Ab + Ba + Ke + Me + Ki + Si + El + Rw + Mu]/[Ca]	2	26.94	14.52	58.54	0.19875	0.41457	0.26936
[Ab + Ba + Ke + Me + Ki + Si + El]/[Rw + Mu + Ca]	2	22.88	10.27	66.85	0.13321	0.33151	0.22877
[Ca + Rw + Mu + Si]/[Ba + Ab + Ke + Me + Ki]	2	10.42	14.62	74.96	0.16318	0.25038	0.10421
All populations except Ca							
[Rw + Mu]/[Si + Ba + Ab + Ke + Me + Ki + El]	2	22.37	9.98	67.75	0.12857	0.32348	0.22367
[Rw + Mu + Si]/[Ba + Ab + Ke + Me + Ki + El]	2	9.79	14.13	76.07	0.15673	0.23931	0.09792
[Rw + Mu + Ca]/[Ba + Si]/[El + Ab + Ke + Me + Ki]	3	18.47	6.18	75.35	0.07575	0.24649	0.18473
[Rw + Mu + Si + El]/[Ba + Ab + Ke + Me + Ki]	2	-2.62	21.51	81.11	0.20962	0.18887	-0.2625
All populations except Ca and El							
[Rw + Mu + Si]/[Ba + Ab + Ke + Me + Ki]	2	6.68	15.63	77.69	0.16751	0.22308	0.06675



Albertine Rift (Gahinga, Muhavura²) and Cameroon dated 860 kya was, however, poorly supported (Figure 2a).

The Bayesian ancestral range reconstruction analysis showed nearly equal nodal probabilities for some of the potential ancestral ranges (Figure 2b). Nonetheless, the ancestral area with the highest support and highest probability was Cameroon, suggesting a potential origin in this area and an initial LDD of *F. abyssinica* from western to eastern Tropical Africa (Figure 2b). Interestingly, this analysis supported subsequent independent migrations: a first migration from the Abyssinian massif to the Albertine Rift (Muhavura) and a second migration event between eastern and western African sky-islands (from Kilimanjaro to Bioko) in the Pleistocene (Figure 2b), suggesting that the Bioko population is of recent origin.

3.2 | Population genetics and phylogeographic analysis

The final AFLP data set comprised 1,570 loci from 229 individuals and 53 populations of five afroalpine *Festuca* species. Despite the use of AFLP may have some biases, the large number of available AFLP loci in this study tends to reduce homoplasy and their widespread random distribution throughout the genome allows to balance these biases (Bonin et al., 2007). Additionally, our AFLP results are consistent with those of our DNA sequences (see below), and with numerous phylogeographic studies that have detected similar

phylogeographic patterns around the Rift Valley for angiosperms, vertebrates and insects (reviewed in Kostin et al., 2020; Mairal et al., 2015; Mankga et al., 2020), many of them using AFLP data (e.g. Kebede et al., 2007; Muchugi et al., 2008; Sertse et al., 2011; Tusiime et al., 2020; Wilfert et al., 2006; Wondimu et al., 2014).

Descriptors of within-population genetic diversity are summarized in Table 1. Within *F. abyssinica*, Nei's gene diversity, proportion of variable markers and the largest number of private fragments were highest in the Albertine Rift (Rwenzori and Muhavura), followed by the Harar Massif (Bale) and Elgon. The rarity index (DW) was highest for the Harar Massif (Bale), followed by the Western Rif (Rwenzori) and Elgon.

Hierarchical AMOVA (Table 2) and split networks recovered three main groups for the afroalpine *Festuca* species (Figure S5a): (i) *F. pilgeri* and *F. obturbans*; (ii) *F. gilbertiana* and the *F. macrophylla* populations from the Abyssinian Massif (Simen) plus the *F. abyssinica* populations from the Albertine Rift and Cameroon and (iii) the *F. abyssinica* populations from the Abyssinian Massif, Harar Massif and Gregory Rift. The subsequent network analysis using only the *F. abyssinica* s. l. group species (Figure 3a) differentiated two groups (ii and iii) fully congruent with the previous analysis. An additional network using only *F. abyssinica* sensu stricto samples (Figure S6a) differentiated two main groups, one of them grouping populations from Albertine Rift and Cameroon and the other containing the rest of the populations. Neighbor-joining trees of the afroalpine *Festuca* species (Figure S7) and the *F. abyssinica* populations (Figure S8) gave similar results.

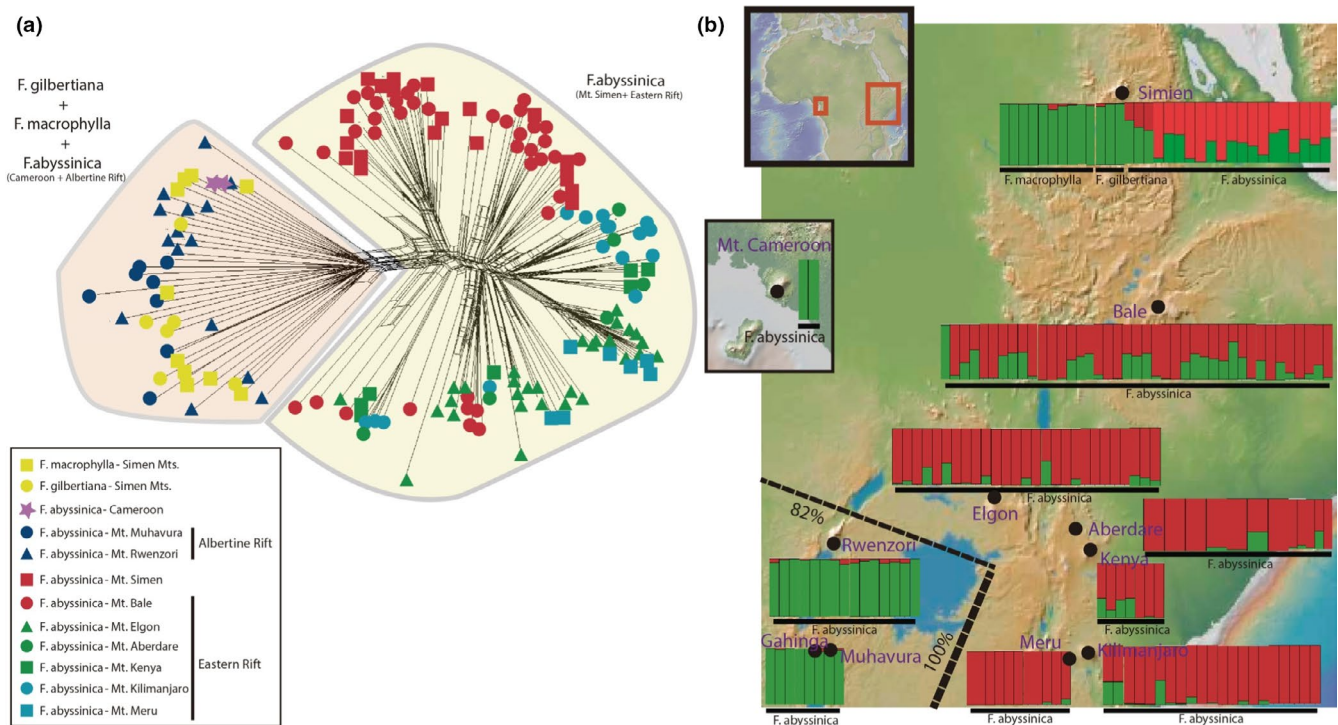


FIGURE 3 Population genetic analyses of *Festuca abyssinica* s.l. based on AFLP markers. (a) Split network with colours and symbols set according to taxonomy and location, as indicated in the chart. Codes for populations correspond to those indicated in Table S1. (b) Histograms showing the Bayesian clustering of individuals to genetic groups (STRUCTURE); colours represent the proportion of individual membership to each inferred Bayesian group. Dotted lines indicated barriers to gene flow for *Festuca abyssinica* sensu stricto and their percentage, as inferred by BARRIER.

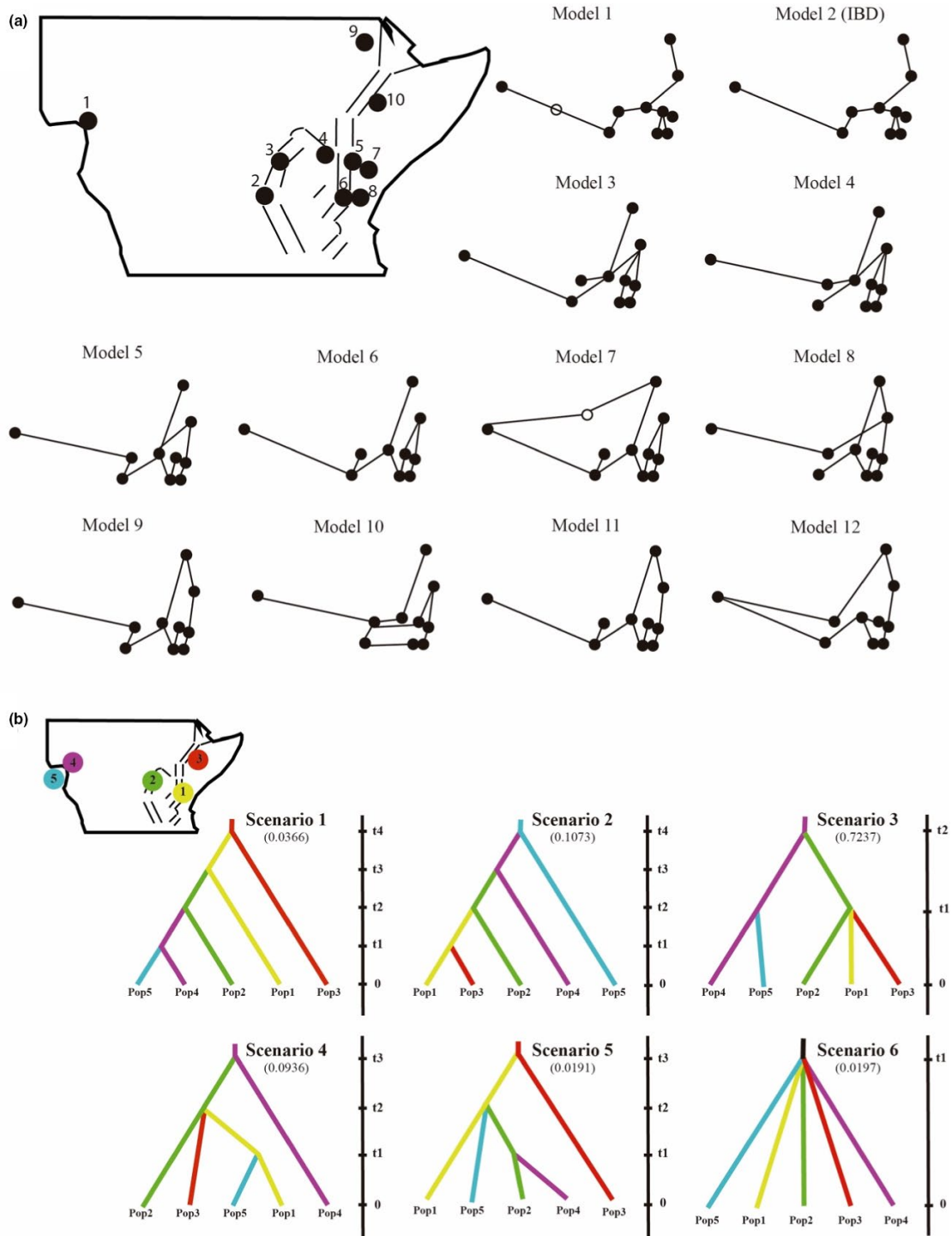


FIGURE 4 Dispersal model routes of *Festuca abyssinica* in the African sky-islands based on (a) AFLP markers analysed with PHYLOGEOGRAPHER, and (b) chloroplast markers using DIYABC. (a) Models are ordered from greater to lesser probability (Model 1 to Model 12). Connective lines represent dispersion paths. Only the 12 dispersal models with the highest correlation with genetic distance are shown (see Table S7). Model 1: 1-Node1//Node1-2-3-4//4-10-9//4-5//5-6//5-7//5-8; Model 2: 1-2-3-4//4-10-9//4-5//5-6//5-7//5-8; Model 3: 1-2-4//4-3//4-9//4-10//10-5-6//10-7-8; Model 4: 1-3-4//4-2//4-9//4-10//10-5-6//10-7-8; Model 5: 1-3-2-4//4-9//4-10-7-8//4-6-5; Model 6: 1-2//2-3//2-4//4-9//4-6-5-10-7-8; Model 7: 1//1-Node1-9//1-2//2-3//2-4//4-9//4-6-5-10-7-8; Model 8: 1-3-10//10-9-4//4-2//4-6-5//10-7-8; Model 9: 1-3-2-4//4-6-5//4-9-10-7-8; Model 10: 1-3//3-4-9//3-2-6//3-5-10-7-8; Model 11: 1-2//2-3//2-4//4-6-5//4-9-10-7-8 and Model 12: 1//1-2-4//4-5//4-6//1-3-9-10-7-8. The numbers represent sky-islands' populations: (1) Cameroon; (2) Gahinga-Muhavura; (3) Rwenzori; (4) Elgon; (5) Aberdare; (6) Meru; (7) Kenya; (8) Kilimanjaro and (9) Simen; (10) Bale. All connective paths (lines joining dots) are read from west to east. Double slashes represent principal bi- or trifurcations. (b) Graphical representation of the six scenarios simulating alternative dispersal routes analysed with DIYABC. The numbers represent populations groups: (1) Gregory Rift, (2) Albertine Rift, (3) Harar, (4) Cameroon and (5) Bioko. The right scale shows the states for the time parameter (t), setting the conditions $t_2 > t_1$, $t_3 > t_2$ and $t_4 > t_3$. The values indicate the probability of each scenario.

The Bayesian STRUCTURE analyses for the afroalpine *Festuca* species assigned individuals to three optimal clusters ($K = 3$; Figure S5b) that were similar to those found in previous analyses (Table 2; Figure S5a). *F. obturbans* and the Simen and Bale plants of *F. abyssinica* showed considerable admixture. Separate analyses of *F. abyssinica* s. l. (Figure 3b) and *F. abyssinica* s. str. (Figure S6b) recovered two optimal clusters ($K = 2$), congruent with the previous analyses.

Genetic differentiation among populations (F_{ST} values) was highest between Cameroon and the rest of populations, except for the Albertine Rift populations (Muhavura and Rwenzori), which in turn showed much lower values with respect to Cameroon than to the rest of populations (Table S6). The BARRIER analysis revealed a major boundary separating Albertine Rift plus Cameroon from the central and south Gregory Rift with a value of 100%, and a secondary boundary separating them from the Abyssinian, Harar and northern Gregory Rift populations with a value of 82% (Figure 3b).

3.3 | Colonization routes

The highest correlation of the tested colonization routes with PHYLOGEOGRAPHER using AFLP data corresponded to Model 1 ($r = 0.9832$; Table S7), which inferred a west-to-east plus Elgon-to-north and Elgon-to-south colonization, including the presence of a hypothetical, geographically intermediate node between the Cameroon and Albertine Rift populations (Figure 4a). The IBD model (Model 2) showed the next highest correlation ($r = 0.8981$). The model taking into account geographic barriers across the Rift Valley (Figure 3b) and having Mt. Elgon as a crossroad (Model 3) showed the next highest correlations (Figure 4a; Table S7). Additional models showed decreasing correlation values, with the model of go-and-back colonizations between western and eastern African mountains (Model 12) showing the lowest correlation value (Figure 4a; Table S7).

The DIYABC analysis supported scenario 3 (Figure 4b) of an ancestral split between Cameroon and the Albertine Rift, followed by diversification on both sides of Africa (colonization of Bioko from Cameroon and of Gregory Rift and Harar from Albertine Rift) as the most probable. The posterior probability of the logistic regression for this scenario was 72% [0.72; HPD: 0.71, 0.736], while the alternative hypotheses showed much lower probabilities (<11%; Figure 4b). The analysis estimated the ancestral Cameroon-Albertine Rift split 8.27^{04} generations ago, and the splits resulting in the five genetic groups 1.42^{04} generations ago.

3.4 | Niche modelling and dispersal cost path analyses

All models from the three algorithms had high performances, with area under the ROC curve (AUC) values greater than 0.98, and all predicted similar distribution of *F. abyssinica* under the current climate (Figures S9 and S10). The predictions for LGM were more variable, both among algorithms and climate models (Figures S11–13). Two of the LGM climate models, CC and ME, provided relatively

similar results showing presence of suitable habitat pockets between western and eastern African mountains across the eastern Democratic Republic of Congo, north-western Cameroon and Central Africa (Figure S14). All models showed that the habitat of *F. abyssinica* was highly expanded during the LGM (Figure S9), six to seven times larger on average compared to today (Figure 5b). Variations in predicted maps among different LGM climate models in Tropical Africa were also reported in an earlier study (Chala et al., 2017). The variation mainly emanates from strong disagreements among LGM global circulation models in predicted precipitation-related climate variables for the tropical regions (Varela et al., 2015).

Because of the habitat expansion during the LGM, the migration resistance estimates between Cameroon and Gahinga/Muhavura, and between Cameroon and north-western Ethiopia showed a strong reduction compared to today (Figure 5). The migration resistance between Cameroon and Gahinga/Muhavura was the lowest under both current and LGM climates. The potential migration path between Cameroon and north-western Ethiopia differed between the two climates. Under the current climate, stepwise migration was estimated to take place across the Central Republic of Africa and Sudan, but the cost accumulation was high. However, in all the LGM climate models, the eastern and central African mountains (i.e. the Gahinga/Muhavura pathway) were predicted to better bridge Cameroon and Ethiopia by stepwise migration through the shortest Central Africa–Sudan pathway (Figure 5). Alternatively, all the algorithms predicted that there was an extended, continuous suitable habitat south-westward to Angola and Namibia under all LGM climate models. However, our least-cost path analysis did not support that this potential route helped to improve migration between western and eastern Tropical African Mountains.

4 | DISCUSSION

4.1 | Inter- and intraspecies genetic diversity and structure of afroalpine fescues and implications with respect to their taxonomy

Our study demonstrates the importance of combining phylogenetic, phylogeographic and niche modelling approaches to increase our understanding of the histories of species in the Tropical African sky-islands. Sky-islands of similar size and age can be quite similar in species composition (Gehrke & Linder, 2014), but nevertheless show large differences among lineages (Ehrich et al., 2007; Mairal, Sanmartín, Herrero, et al., 2017).

Our phylogenetic analyses show that the populations of the widespread and morphologically polymorphic *F. abyssinica* s.str. form a monophyletic group together with accessions classified as *F. macrophylla* and *F. gilbertiana* based on morphology (here referred to as *Festuca abyssinica* s. lat.). Notably, *F. macrophylla* and *F. gilbertiana* from the Abyssinian Massif (Simen) formed a well-supported group with *F.*

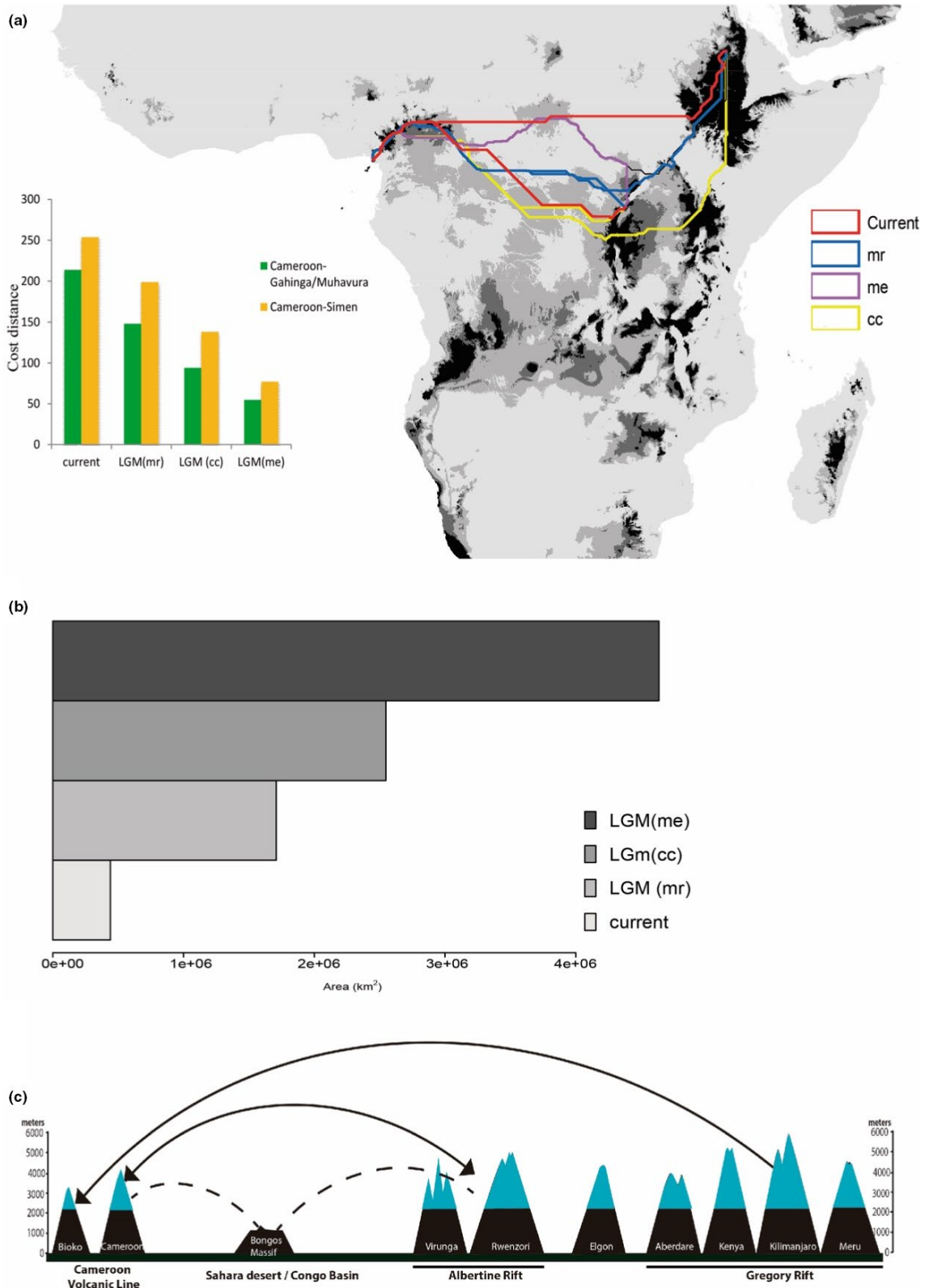


FIGURE 5 (a) Least-cost paths and least-cost path distances between western and eastern African high mountains under current and three LGM climate models [MIROC-ESM (MR), MPI-ESM-P (ME) and CCSM (CC)]. The paths are plotted on a habitat suitability map produced by averaging predicted probability values from nine LGM habitat suitability maps (three algorithms * three climate models), with increasing suitability indicated as increasing darkness of grey to black. (b) Spatial extent of suitable habitats for *F. abyssinica* under current and three LGM climate models [CCSM (LGM(cc)), MIROC-ESM (LGM(mr)) and MPI-ESM-P (LGM(me))]. (c) Schematic representation of the distribution of *F. abyssinica* during the glacial phases (in blue), showing the supported transcontinental dispersal events among the Tropical African sky-islands (continuous lines), as well as the hypothetical reconstructed pathway through Central Africa–Sudan (dashed line) in the Pleistocene.



abyssinica from the Albertine Rift, based both on our concatenated plastid and nuclear sequence data (Figure 2; Figure S3) and on our nuclear AFLP data (Figure S5; Figure 3). Our results suggest both a long history of isolation among the eastern African Great Rift populations, especially for *F. abyssinica*, as well as recent gene flow between the Abyssinian *F. macrophylla* and *F. gilbertiana* and the *F. abyssinica* populations of the Albertine Rift (Figure S5). These species are reported to differ in size traits (large panicle and spikelets in *F. macrophylla*, intermediate in *F. abyssinica*, smaller in *F. gilbertiana* and larger glumes and florets in *F. abyssinica* than in the other species; Clayton et al., 2006 onwards; Phillips, 1995a,b; Namaganda et al., 2007). However, these taxa could not be distinguished from the widespread *F. abyssinica* based on our genetic data, and we therefore merged these into '*F. abyssinica* s. lat.' to simplify the presentation of the results. The young ages inferred for the *F. macrophylla* and *F. gilbertiana* populations (300–290 kya; Figure 2a) may suggest a recent origin from *F. abyssinica* s. lat. ancestors (1.06–1.85 Ma; Figure 2a). The other afroalpine species included here (*F. pilgeri* and *F. obturbans*) are clearly divergent from the *F. abyssinica* clade (Figures S4, S5a and S7) and are also differentiated by their glabrous ovary tips and (for *F. pilgeri*) by a continuous leaf-blade section sclerenchyma ring (Clayton et al., 2006 onwards; Namaganda et al., 2007; Namaganda et al., 2009). Nevertheless, some individuals of *F. pilgeri* and most of *F. obturbans* show genetic admixture with the *F. abyssinica* s. l. genetic groups (Table 2; Figure S5b), suggesting introgression.

Within *F. abyssinica* s. lat., the strongest genetic structure was detected between two clusters (Figure 3; Figure S6) separated in a west–east distribution. These findings agree with previous studies that showed that genetic variation is strongly structured between the western and eastern sides of the Great Rift System (reviewed in Mairal, Sanmartín, Herrero, et al., 2017). In *F. abyssinica* s. lat., one genetic group includes the western populations (both the Albertine Rift in East Africa and Cameroon in West Africa), and the other group includes the remaining eastern African populations (Figures S5 and S6; Table 2). However, most individuals from the eastern (Ethiopian) massifs are admixed with the western group (Figures S5 and S6), and the inferred divergence ages of these populations are quite recent (~260 kys, Figure 2). These results suggest recent gene flow between the western and eastern genetic groups, as well as across the Rift Valley in Ethiopia within the eastern group. The genetic similarity among the populations of *Festuca* from the Simen and Bale mountains, across the Rift Valley, is in line with the regional floristic similarity patterns (Gehrke & Linder, 2014) and historical habitat connectivity between the two massifs (Figures S9 and S14; Chala et al., 2017).

Because the Pleistocene glaciations had limited effect at Tropical African latitudes, the sky islands may have acted as local refugia for the afroalpine vegetation during several glacial periods (Hewitt, 1996). Their rough topography provides high diversity of ecological niches, enabling them to act as long-term refugia with less drastic glacial climate effects (Ehrich et al., 2007; Tzedakis, 2009). In afroalpine *Festuca*, the highest genetic diversity is found in the Albertine Rift (Rwenzori and Muhavura) populations of *F.*

abyssinica (Table 1). Our results can reflect accumulation in long-term isolated populations, suggesting that the Albertine Rift mountains may represent the ancestral area for *F. abyssinica*. However, our limited sampling in Mt. Cameroon does not allow us to accurately infer potential ancestry. Nonetheless, Mt. Cameroon's 0.86 Ma divergence (Figure 2a), ancestral-most area (Figure 2b), its genetic similarity to the Albertine Rift populations (Figures S5b and S6) and its ancestral divergence in the most likely scenario 3 chosen by DIYABC (Figure 4b) indicate that this western African lineage may be old. Moderately high genetic diversity values are also found in *F. abyssinica* in the Harar Massif and Mt. Elgon. In the Harar Massif, successful conservation of the afroalpine forests may have favoured conservation of genetic diversity in plants since the LGM (Ayele et al., 2009; Kebede et al., 2007). In Mt. Elgon, high genetic diversity has been explained both by its antiquity and its geographical position at a crossroad between the west and the east in the Great Rift Valley (Mairal, Sanmartín, Herrero, et al., 2017). By contrast, the lower DW values in the other *F. abyssinica* populations, especially those in the Gregory Rift (e.g. Kenya, Aberdare and Meru; Table 1), suggest that they originated by recent dispersal.

4.2 | Mountain-hopping among close sky-islands and across Tropical Africa

It is remarkable that we found very close genetic relationships within each of two pairs of *F. abyssinica* populations growing at different sides of the continent (Albertine Rift and Mt. Cameroon; Kilimanjaro and Bioko), separated by great geographic distances including the currently inhospitable Sahara desert and the Congo Basin (Table 2; Figures 2 and 3; Figures S5 and S6). This finding contrasts with the strong genetic barriers detected within eastern Africa, between the Albertine Rift populations and the spatially close Gregory Rift and Mt. Elgon populations (Table S6; Figure 3b). The close relationships between the lineages from opposite sides of Africa could be interpreted as a result of two independent LDDs because their divergences (860 and 520 kya; Figure 2a) are much more recent than the formation of the Sahara and Congo Basin barriers (7–6 Ma and 13–12 Ma respectively; Pokorný et al., 2015). Despite the imprecision inherently associated with molecular dating, even the upper limit of the dated confidence intervals (1.35 Kya) shows them to be markedly younger. Several studies have already suggested predominance of LDD events in the colonization of eastern African sky-islands (Ayele et al., 2009; Wondimu et al., 2014). This implies, to some extent, stochasticity in the distribution of the different phylogroups. However, in our case, we found a strong west/east genetic structure, and our most likely dispersal models (using nuclear data) explain a colonization pathway between Cameroon and Albertine Rift through the existence of an ancestral intermediate population, now extinct (Model 1; Table S7; Figure 4a). Our selected scenarios using coalescence (cpDNA data, DIYABC) and correlations of genetic and geographic distances (AFLP data, PHYLOGEOGRAPHER) support this ancestral

relationship, although they differ in some more recent inferences, which may be a consequence of our limited cpDNA data for detecting the recent population dynamics with ABC. This hypothesis is further supported by our ecological niche modelling of *F. abyssinica* at the LGM, showing patches of habitat suitability (Figures S9 and S14) and reduced least-cost path distances (Figure 5) between these areas. One of the assumptions in distribution modelling is that the occurrence data used in models reflect the niche requirements of the modelled targets. In our case, we mainly relied on data from our own extensive field campaigns, with some supplementary data obtained from herbaria and GBIF, and we are confident that this data set is reasonably large and of high quality. We computed the least-cost path distance by negatively scaling cost values against habitat suitability. Although species do not necessarily disperse to all pixels that are suitable to them, the least-cost paths we computed were consistent with the genetic data and could explain the close genetic relationship we found between *F. abyssinica* populations from different sides of the continent, under all three LGM climate models.

Our results thus suggest a stepping-stone colonization model including previous existence of intermediate lower-altitude populations of *F. abyssinica* that connected the two sides of Tropical Africa and facilitated glacial transcontinental migration (notably, current populations of this species can be found down to 2,130 m; Namaganda, 2007).

Biotic connections between the two sides of Africa have been proposed to follow a Southern Migratory Tract (SMT; White, 1981), or a northern route through the mountain massifs in the middle of the Sahara (e.g. Hoggar and Tibesti; Désamonné et al., 2011; Mairal, Sanmartín, Herrero, et al., 2017). The northern track has been considered highly unlikely for afroalpine plants (Chala et al., 2017; Hedberg, 1970). Furthermore, the absence of both current populations and fossils records of *F. abyssinica* in North Africa and the less favourable cost path values (Figure 5) move us to disregard this hypothesis. Other studies have suggested mountains in Sudan as stepping stones (Kadu et al., 2011; Wickens, 1976). Our least-cost path analysis supports this Central Africa–Sudan pathway (Figure 5) as the optimal dispersal route. This shortest pathway is in concord with that supported by our ecological niche (Figures S9 and S14) and dispersal models (Figures 4 and 5). Our LGM niche reconstructions show suitable habitat pockets for *F. abyssinica* in the Marrah mountains (Sudan), Bongos massif (Central African Republic (CAR) and Adamawa plateau (Nigeria, Cameroon and CAR; Figures 1 and 5; Figures S9 and S14), favouring the potential existence of these abyssal stepping-stone populations between the high West and East African mountains. These hypothetical populations could have facilitated transcontinental dispersals during the LGM and other glacial periods, reinforcing the effectiveness of mountain hopping as a suitable dispersal model between the two sides of Africa for afroalpine plants. A postglacial climate-driven extinction due to gradual aridification of North and Central Africa (Mairal et al., 2015; Mairal, Sanmartín, & Pellissier, 2017) would have eliminated these intermediate *F. abyssinica* populations and left the species to track its remaining suitable climatic conditions, today restricted to the sky-islands at opposite sides of Africa.

5 | CONCLUSIONS

Biotic connections between afroalpine habitats on the two sides of Tropical Africa have been traditionally invoked to have resulted from long-distance dispersal (LDD) or from Mountain forest bridge scenarios. The study of a typical afroalpine grass (*Festuca abyssinica*) widely distributed at both sides of the continent has allowed us to test these hypotheses. Our phylogeographic data show high genetic similarities among populations from the two sides of the African continent, and together with niche modelling data, this suggests that migration occurred via direct or stepping-stone LDD. Specifically, our reconstructions show a much more congruent dispersal pathway favouring a mountain-hopping dispersal model, with migrations occurring between adjacent sky-islands in eastern Africa and through a Central Africa–Sudan pathway connecting the afroalpine habitats from the two sides of the continent throughout scattered suitable habitat pockets in Tropical Africa during the Last Glacial Maximum. Our evidence supports a stepping-stone transcontinental colonization scenario through a few intermediate lower-altitude populations, although we cannot exclude direct LDD.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Genbank (accession numbers of new trnT-trnL (MW201914–MW201935), trnL-trnF (MW201936–MW201957) and ITS (MW205795–MW205816) DNA sequences) and on Dryad <https://doi.org/10.5061/dryad.bcc2fqzb6> (original AFLP data matrix of Afroalpine *Festuca* species, ITS, trnLF and trnTL nexus alignments used in the phylogenetic analyses, and input/scripts files used to perform the dating and DIYABC analyses).

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REFERENCES

- Araújo, M. B., & New, M. (2007). Ensemble forecasting of species distributions. *Trends in Ecology & Evolution*, *22*, 42–47.
- Assefa, A., Ehrich, D., Taberlet, P., Nemomissa, S., & Brochmann, C. (2007). Pleistocene colonization of afro-alpine 'sky islands' by the arctic-alpine *Arabis alpina*. *Heredity*, *99*, 133–142. <https://doi.org/10.1038/sj.hdy.6800974>
- Ayele, T. B., Gailing, O., Umer, M., & Finkeldey, R. (2009). Chloroplast DNA haplotype diversity and postglacial recolonization of *Hagenia abyssinica* (Bruce) J.F. Gmel. in Ethiopia. *Plant Systematics and Evolution*, *280*, 175–185. <https://doi.org/10.1007/s00606-009-0177-5>
- Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M. A., & Alekseyenko, A. V. (2012). Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Molecular Biology and Evolution*, *29*(9), 2157–2167. <https://doi.org/10.1093/molbev/mss084>
- Bobo-Pinilla, J., Peñas de Giles, J., López-González, N., Mediavilla, S., & Martínez-Ortega, M. M. (2018). Phylogeography of an endangered disjunct herb: long-distance dispersal, refugia and colonization routes. *AoB Plants*, *10*(5), ply047. <https://doi.org/10.1093/aobpla/ply047>
- Bonin, A., Ehrich, D., & Manel, S. (2007). Statistical analysis of amplified fragment length polymorphism data: A toolbox for molecular ecologists and evolutionists. *Molecular Ecology*, *16*, 3737–3758. <https://doi.org/10.1111/j.1365-294X.2007.03435.x>
- Buckler IV, E. S. (1999). *Phylogeographer: A tool for developing and testing phylogeographic hypotheses*, 0.3 ed. Retrieved from <http://www.maizegenetics.net/phylogeographer>
- Buckler, E. S. IV, Goodman, M. M., Holtsford, T. P., Doebley, J. F., & Sánchez, G. (2006). Phylogeography of the wild subspecies of *Zea mays*. *Maydica*, *51*, 123–134.
- Chala, D., Brochmann, C., Psomas, A., Ehrich, D., Gizaw, A., Masao, C. A., Bakkestuen, V., & Zimmermann, N. E. (2016). Good-bye to tropical alpine plant giants under warmer climates? Loss of range and genetic diversity in *Lobelia rhynchopetalum*. *Ecology and Evolution*, *6*, 8931–9894.
- Chala, D., Zimmermann, N. E., Brochmann, C., & Bakkestuen, V. (2017). Migration corridors for alpine plants among the 'sky islands' of eastern Africa: do they, or did they exist? *Alpine Botany*, *127*, 133–144. <https://doi.org/10.1007/s00035-017-0184-z>
- Clayton, W. D. (1970). Flora of tropical East Africa. In E. Milne-Redhead & R. M. Polhill (Eds.), *Flora of Tropical East Africa. Gramineae Part 1*. (pp. 56–63). Crown Agents for Oversea Governments and Administrations.
- Clayton, W. D., Vorontsova, M. S., Harman, K. T., & Williamson, H. (2006, onwards). *GrassBase - The Online World Grass Flora*. Retrieved from <http://www.kew.org/data/grasses-db.html>
- Coetsee, J. A. (1964). Evidence for a considerable depression of the vegetation belts during the Upper Pleistocene on the East African Mountains. *Nature*, *204*, 564–566. <https://doi.org/10.1038/204564a0>
- Cornuet, J. M., Ravigné, V., & Estoup, A. (2010). Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1. 0). *BMC Bioinformatics*, *11*, 401.
- Cornuet, J.-M., Santos, F., Beaumont, M. A., Robert, C. P., Marin, J.-M., Balding, D. J., Guillemaud, T., & Estoup, A. (2008). Inferring population history with DIY ABC: A user-friendly approach to approximate Bayesian computation. *Bioinformatics*, *24*, 2713–2719.
- Crisp, M. D., Laffan, S., Linder, H. P., & Monro, A. (2001). Endemism in the Australian flora. *Journal of Biogeography*, *28*, 183–198.
- Désamoré, A., Laenen, B., Devos, N., Popp, M., González-Mancebo, J. M., Carine, M. A., & Vanderpoorten, A. (2011). Out of Africa: North-westwards Pleistocene expansions of the heather *Erica arborea*. *Journal of Biogeography*, *38*, 164–176.
- Dietz, J. (1983). Permutation tests for association between two distance matrices. *Systematic Zoology*, *32*, 21–26. <https://doi.org/10.2307/2413216>
- Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, *7*, 214. <https://doi.org/10.1186/1471-2148-7-214>
- Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*, 359–361. <https://doi.org/10.1007/s12686-011-9548-7>
- Ehrich, D. (2006). Aflpdat: A collection of r functions for convenient handling of AFLP data. *Molecular Ecology Notes*, *6*, 603–604. <https://doi.org/10.1111/j.1471-8286.2006.01380.x>
- Ehrich, D., Gaudeul, M., Assefa, A., Koch, M. A., Mummenhoff, K., Nemomissa, S., Consortium, I., & Brochmann, C. (2007). Genetic consequences of Pleistocene range shifts: Contrast between the Arctic, the Alps and the East African mountains. *Molecular Ecology*, *16*, 2542–2559.
- Elith, J., Leathwick, J. R., & Hastie, T. (2008). A working guide to boosted regression trees. *Journal of Animal Ecology*, *77*, 802–813. <https://doi.org/10.1111/j.1365-2656.2008.01390.x>
- Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*, 2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, *1*, 47.
- Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Molecular Ecology Notes*, *7*, 574–578. <https://doi.org/10.1111/j.1471-8286.2007.01758.x>
- Galley, C., Bytebier, B., Bellstedt, D., & Linder, H. P. (2007). The Cape element in the Afrotropical flora: from Cape to Cairo? *Proceedings of the Royal Society B: Biological Sciences*, *274*, 535–543. <https://doi.org/10.1098/rspb.2006.0046>
- Gehrke, B., & Linder, H. P. (2014). Species richness, endemism and species composition in the tropical Afroalpine flora. *Alpine Botany*, *124*, 165–177. <https://doi.org/10.1007/s00035-014-0132-0>
- Gizaw, A., Brochmann, C., Nemomissa, S., Wondimu, T., Masao, C. A., Tusiime, F. M., Abdi, A. A., Oxelman, B., Popp, M., & Dimitrov, D. (2016). Colonisation and diversification in the African 'sky islands': Insights from fossil-calibrated molecular dating of *Lychnis* (Caryophyllaceae). *New Phytologist*, *211*, 719–734.
- Gizaw, A., Kebede, M., Nemomissa, S., Ehrich, D., Bekele, B., Mirré, V., Popp, M., & Brochmann, C. (2013). Phylogeography of the heathers *Erica arborea* and *E. trimera* in the afro-alpine "sky-islands" inferred from AFLPs and plastid DNA sequences. *Flora*, *208*, 453–463. <https://doi.org/10.1016/j.flora.2013.07.007>
- Hall, J. B. (1973). Vegetational zones on the southern slopes of Mount Cameroon. *Vegetatio*, *27*, 49–69. <https://doi.org/10.1007/BF02389340>
- Hedberg, O. (1951). Vegetation belts of the East African mountains. *Svensk Botanisk Tidskrift Utgifven af Svenska Botaniska Foreningen. Stockholm*, *45*, 140–202.
- Hedberg, O. (1969). Evolution and speciation in a tropical high mountain flora. *Biological Journal of the Linnean Society*, *1*, 135–148. <https://doi.org/10.1111/j.1095-8312.1969.tb01816.x>
- Hedberg, O. (1970). Evolution of the Afroalpine Flora. *Biotropica*, *2*, 16. <https://doi.org/10.2307/2989783>
- Hewitt, G. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, *58*, 247–276. <https://doi.org/10.1006/bijl.1996.0035>
- Huson, D. H., & Bryant, D. (2005). Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, *23*, 254–267. <https://doi.org/10.1093/molbev/msj030>

- Kadu, C. A. C., Schueler, S., Konrad, H., Muluvi, G. M. M., Eyog-matig, O., Muchugi, A., Williams, V. L., Ramamonjisoa, L., Kapinga, C., Foahom, B., Katsvanga, C., Hafashimana, D., Obama, C., & Geburek, T. (2011). Phylogeography of the Afriomontane *Prunus africana* reveals a former migration corridor between East and West African highlands. *Molecular Ecology*, 20, 165–178.
- Katoh, K., Misawa, K., Kuma, K. I., & Miyata, T. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30, 3059–3066. <https://doi.org/10.1093/nar/gkf436>
- Kebede, M., Ehrich, D., Taberlet, P., Nemomissa, S., & Brochmann, C. (2007). Phylogeography and conservation genetics of a giant lobelia (*Lobelia giberroa*) in Ethiopian and Tropical East African mountains. *Molecular Ecology*, 16, 1233–1243.
- Kingdon, J. (1990). *Island Africa: the evolution of Africa's rare animals and plants*. Collins.
- Kostin, D. S., Martynov, A. A., Komarova, V. A., Alexandrov, D. Y., Yihune, M., Kasso, M., Bryja, J., & Lavrenchenko, L. A. (2020). Rodents of Choke Mountain and surrounding areas (Ethiopia): the Blue Nile gorge as a strong biogeographic barrier. *Journal of Vertebrate Biology*, 69(2), 1–12. <https://doi.org/10.25225/jvb.20016>
- Lemey, P., Rambaut, A., Drummond, A. J., & Suchard, M. A. (2009). Bayesian phylogeography finds its roots. *PLoS Computational Biology*, 5, e1000520. <https://doi.org/10.1371/journal.pcbi.1000520>
- Lynch, M., & Milligan, B. G. (1994). Analysis of population genetic structure with RAPD markers. *Molecular Ecology*, 3, 91–99. <https://doi.org/10.1111/j.1365-294X.1994.tb00109.x>
- Mairal, M., Pokorny, L., Aldasoro, J. J., Alarcón, M., & Sanmartín, I. (2015). Ancient vicariance and climate-driven extinction explain continental-wide disjunctions in Africa: The case of the Rand Flora genus *Canarina* (Campanulaceae). *Molecular Ecology*, 24, 1335–1354.
- Mairal, M., Sanmartín, I., Herrero, A., Pokorny, L., Vargas, P., Aldasoro, J. J., & Alarcón, M. (2017). Geographic barriers and Pleistocene climate change shaped patterns of genetic variation in the Eastern Afriomontane biodiversity hotspot. *Scientific Reports*, 7, e45749. <https://doi.org/10.1038/srep45749>
- Mairal, M., Sanmartín, I., & Pellissier, L. (2017). Lineage-specific climatic niche drives the tempo of vicariance in the Rand Flora. *Journal of Biogeography*, 44, 911–923. <https://doi.org/10.1111/jbi.12930>
- Mankga, L. T., Yessoufou, K., & Chitakira, M. (2020). On the origin and diversification history of the African genus *Encephalartos*. *South African Journal of Botany*, 130, 231–239. <https://doi.org/10.1016/j.sajb.2019.12.007>
- Manni, F., Guérard, E., & Heyer, E. (2004). *Barrier version 2.2 user's manual*. Population genetics team, Musée de l'Homme.
- McCormack, J. E., Huang, H., & Knowles, L. L. (2009). Sky islands. In R. G. Gillespie & D. A. Clague (Eds.), *Encyclopedia of Islands* (pp. 841–843). University of California Press.
- Minaya, M., Hackel, J., Namaganda, M., Brochmann, C., Vorontsova, M. S., Besnard, G., & Catalán, P. (2017). Contrasting dispersal histories of broad- and fine-leaved temperate Loliinae grasses: Range expansion, founder events, and the roles of distance and barriers. *Journal of Biogeography*, 44, 1980–1993.
- Muchugi, A., Muluvi, G. M., Kindt, R., Kadu, C. A., Simons, A. J., & Jamnadass, R. H. (2008). Genetic structuring of important medicinal species of genus *Warburgia* as revealed by AFLP analysis. *Tree Genetics & Genomes*, 4(4), 787–795.
- Namaganda, M. (2007). *A taxonomic review of the genus Festuca in Uganda: AFLP fingerprinting, chromosome numbers, morphology and anatomy* (PhD thesis). Norwegian University of Life Sciences. ISSN: 1503-1667. ISBN: 978-82-575-0782-4.
- Namaganda, M., Lye, K. A., Friebe, B., & Heun, M. (2006). AFLP-based differentiation of tropical African *Festuca* species compared to the European *Festuca* complex. *Theoretical and Applied Genetics*, 113, 1529–1538. <https://doi.org/10.1007/s00122-006-0400-5>
- Namaganda, M., Lye, K. A., Friebe, B., & Heun, M. (2009). Leaf anatomical characteristics of Ugandan species of *Festuca* L. (Poaceae). *South African Journal of Botany*, 75, 52–59.
- Namaganda, M., Lye, K. A., & Heun, M. (2007). The species distinction of the narrow-leaved *Festuca* from East Africa based on AFLP fingerprinting and morphology. *Nordic Journal of Botany*, 25, 85–95. <https://doi.org/10.1111/j.0107-055X.2007.00107.x>
- Nei, M., & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, 76, 5269–5273.
- Nylander, J. A. A. (2004). *MrModeltest v2*. Program distributed by the author. 2). Evolutionary Biology Centre, Uppsala University.
- Phillips, S. (1995a). Poaceae. In I. Hedberg & S. Edwards (Eds.), *Flora of Ethiopia and Eritrea* (Vol. 7, pp. 23–27). Addis Ababa.
- Phillips, S. (1995b). A new species of *Festuca* (Gramineae) from Ethiopia. *Nordic Journal of Botany*, 14, 649–651.
- Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, 190, 231–259. <https://doi.org/10.1016/j.ecolm.2005.03.026>
- Pokorny, L., Oliván, G., & Shaw, A. (2011). Phylogeographic patterns in two southern hemisphere species of *Calyptrochaeta* (Daltoniaceae, Bryophyta). *Systematic Botany*, 36, 542–553.
- Pokorny, L., Riina, R., Mairal, M., Meseguer, A. S., Culshaw, V., Cendoya, J., Serrano, M., Carbajal, R., Ortiz, S., Heuertz, M., & Sanmartín, I. (2015). Living on the edge: Timing of Rand Flora disjunctions congruent with ongoing aridification in Africa. *Frontiers in Genetics*, 6, 154.
- Pompanon, F., Bonin, A., Bellemain, E., & Taberlet, P. (2005). Genotyping errors: Causes, consequences and solutions. *Nature Reviews Genetics*, 6, 847. <https://doi.org/10.1038/nrg1707>
- Popp, M., Gizaw, A., Nemomissa, S., Suda, J., & Brochmann, C. (2008). Colonization and diversification in the African “sky islands” by Eurasian *Lychnis* L. (Caryophyllaceae). *Journal of Biogeography*, 35, 1016–1029.
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.
- Rambaut, A., Suchard, M. A., Xie, W., & Drummond, A. J. (2013). *Tracer v1.6*. Retrieved from <http://tree.bio.ed.ac.uk/software/tracer>
- Rohlf, F. J. (1998). *NTSYS-pc version 2.0. Numerical taxonomy and multivariate analysis system*. Exeter Software.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Schönswetter, P., & Tribsch, A. (2005). Vicariance and dispersal in the alpine perennial *Bupleurum stellatum* L. (Apiaceae). *Taxon*, 54, 725–732.
- Sertse, D., Gailing, O., Eliades, N. G., & Finkeldey, R. (2011). Anthropogenic and natural causes influencing population genetic structure of *Juniperus procera* Hochst. ex Endl. in the Ethiopian highlands. *Genetic Resources and Crop Evolution*, 58, 849–859.
- Sklenář, P., Hedberg, I., & Cleef, A. M. (2014). Island biogeography of tropical alpine floras. *Journal of Biogeography*, 41, 287–297. <https://doi.org/10.1111/jbi.12212>
- Smouse, P., Long, J., & Sokal, R. (1986). Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, 35, 627–632. <https://doi.org/10.2307/2413122>
- Tusiime, F. M., Gizaw, A., Gussarova, G., Nemomissa, S., Popp, M., Masao, C. A., Wondimu, T., Abdi, A. A., Mirré, V., Muwanika, V., Eilu, G., & Brochmann, C. (2020). Afro-alpine flagships revisited: Parallel adaptation, intermountain admixture and shallow genetic structuring

- in the giant senecios (*Dendrosenecio*). *PLoS One*, 15, e0228979. <https://doi.org/10.1371/journal.pone.0228979>
- Tzedakis, P. C. (2009). Museums and cradles of Mediterranean biodiversity. *Journal of Biogeography*, 36, 1033–1034.
- Varela, S., Lima-Ribeiro, M. S., & Terribile, L. C. (2015). A short guide to the climatic variables of the last glacial maximum for biogeographers. *PLoS One*, 10, e0129037. <https://doi.org/10.1371/journal.pone.0129037>
- Vekemans, X., Beuwers, T., Lemaire, M., & Roldán-Ruiz, I. (2002). Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular ecology*, 11(1), 139–151. <https://doi.org/10.1046/j.0962-1083.2001.01415.x>
- Vos, P., Hogers, R., Bleeker, M., Reijmans, M., Lee, T. V. D., Hornes, M., Friters, A., Pot, J., Paleman, J., Kuiper, M., & Zabeau, M. (1995). AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*, 23, 4407–4414. <https://doi.org/10.1093/nar/23.21.4407>
- Warren, B. H., Simberloff, D., Ricklefs, R. E., Aguilée, R., Condamine, F. L., Gravel, D., Morlon, H., Mouquet, N., Rosindell, J., Casquet, J., Conti, E., Cornuault, J., Fernández-Palacios, J. M., Heng, T., Norder, S. J., Rijdsdijk, K. F., Sanmartín, I., Strasberg, D., Triantis, K. A., ... Thébaud, C. (2015). Islands as model systems in ecology and evolution: prospects fifty years after MacArthur-Wilson. *Ecology Letters*, 18, 200–217. <https://doi.org/10.1111/ele.12398>
- White, F. (1981). The history of the Afrotropical archipelago and the scientific need for its conservation. *African Journal of Ecology*, 19, 33–54. <https://doi.org/10.1111/j.1365-2028.1981.tb00651.x>
- Whitlock, R., Hipperson, H., Mannarelli, M., Butlin, R. K., & Burke, T. (2008). An objective, rapid and reproducible method for scoring AFLP peak-height data that minimizes genotyping error. *Molecular Ecology Resources*, 8, 725–735. <https://doi.org/10.1111/j.1755-0998.2007.02073.x>
- Wickens, G. E. (1976). The flora of Jebel Marra (Sudan Republic) and its geographical affinities. *Kew Bulletin Additional Series*, 5, 1–368.
- Wilfert, L., Kaib, M., Durka, W., & Brandl, R. (2006). Differentiation between populations of a termite in eastern Africa: implications for biogeography. *Journal of Biogeography*, 33(11), 1993–2000. <https://doi.org/10.1111/j.1365-2699.2006.01556.x>
- Wolfe, K. H., Li, W. H., & Sharp, P. M. (1987). Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of the National Academy of Sciences USA*, 84(24), 9054–9058. <https://doi.org/10.1073/pnas.84.24.9054>
- Wondimu, T., Gizaw, A., Tusiime, F. M., Masao, C. A., Abdi, A. A., Gussarova, G., Popp, M., Nemomissa, S., & Brochmann, C. (2014). Crossing barriers in an extremely fragmented system: two case studies in the afro-alpine sky island flora. *Plant Systematics and Evolution*, 300, 415–430. <https://doi.org/10.1007/s00606-013-0892-9>
- Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, 73, 3–36. <https://doi.org/10.1111/j.1467-9868.2010.00749.x>

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Mario Mairal, Mary Namaganda, Abel Gizaw, Desalegn Chala and Christian Brochmann have specialized in phylogeographic, taxonomic and ecological analyses of afroalpine and afrotropical plants. Pilar Catalán is interested in systematic and evolutionary studies of grasses. All authors share a common interest in genetics, phylogeny and the spatio-temporal evolution of the African sky-islands angiosperms.

Author contributions: P.C., M.M. and C.B. designed the study. C.B., A.G. and D.C. collected samples in the field. P.C. obtained samples from herbaria. M.N. and P.C. generated the data. M.M. analysed the genetic data. D.C. performed the ENM analyses. M.M. and P.C. wrote the draft manuscript. All authors contributed to the writing of the final version of the manuscript.

SUPPORTING INFORMATION

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

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