MOLECULAR ECOLOGY

Molecular Ecology (2011) **20**, 165–178 doi: 10.1111/j.1365-294X.2010.04931.x

Phylogeography of the Afromontane *Prunus africana* reveals a former migration corridor between East and West African highlands

C. A. C. KADU,*[1] S. SCHUELER,*[1] H. KONRAD,* G. M. M. MULUVI,† O. EYOG-MATIG,‡ A. MUCHUGI,† V. L. WILLIAMS,§ L. RAMAMONJISOA,¶ C. KAPINGA,** B. FOAHOM,†† C. KATSVANGA,‡‡ D. HAFASHIMANA,§§ C. OBAMA¶¶ and T. GEBUREK*

**Federal Research Centre for Forests (BFW), Department of Genetics, Hauptstraße 7, A-1140 Vienna, Austria, †Kenyatta University, PO Box 43844, Nairobi, Kenya, ‡Bioversity International SSA, c/o CIFOR Regional Office, PO Box 2008, Messa, Yaounde, Cameroon, §School of Animal, Plant & Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, Johannesburg, South Africa, ¶Silo National des Graines Forestieres (SNGF), PO Box 5091, Antananarivo-101, Madagascar, **Tanzania Forestry Research Institute (TAFORI), PO Box 1854, Morogoro, Tanzania, ††Institute of Agricultural Research for Development (IRAD), PO Box 2123 or 2067, Yaounde, Cameroon, ‡‡Faculty of Agriculture and Environmental Science (Forestry Unit), Bindura University of Science Education, P. Bag 1020, Bindura, Zimbabwe, §§National Forestry Resources Research Institute (NaFORRI), PO Box 1752, Kampala, Uganda, ¶¶Coordinador Nacional de la COMIFAC Ministerio de Agricultura y Bosques BP 207, Bata, Equatorial Guinea*

Abstract

Scattered populations of the same tree species in montane forests through Africa have led to speculations on the origins of distributions. Here, we inferred the colonization history of the Afromontane tree *Prunus africana* using seven chloroplast DNA loci to study 582 individuals from 32 populations sampled in a range-wide survey from across Africa, revealing 22 haplotypes. The predominant haplotype, HT1a, occurred in 13 populations of eastern and southern Africa, while a second common haplotype, HT1m, occurred in populations of western Uganda and western Africa. The high differentiation observed between populations in East Africa was unexpected, with stands in western Uganda belonging with the western African lineage. High genetic differentiation among populations revealed using ordered alleles (N_{ST} = 0.840) compared with unordered alleles (G_{ST} = 0.735), indicated a clear phylogeographic pattern. Bayesian coalescence modelling suggested that 'east' and 'west' African types likely split early during southward migration of the species, while further more recent splitting events occurred among populations in the East of the continent. The high genetic similarity found between western Uganda and west African populations indicates that a former Afromontane migration corridor may have existed through Equatorial Africa.

Keywords: African cherry, Afromontane, evolutionary history, organelle marker, *Pygeum africanum*, Range-wide differentiation, rift valley

Received 8 April 2010; revision received 15 September 2010; accepted 26 September 2010

Introduction

The long-term environmental stability of Afromontane forest regions has been proposed as a mechanism for the accumulation and persistence of species during glacial periods, resulting in diverse species assemblages that are centres of endemism (Finch *et al.* 2009; Hall *et al.* 2009). Significant climate changes that occurred within Africa between the last glacial maximum (LGM) around 20 000 years ago and the present day have, however, caused considerable modifications in species ranges (e.g. Livingstone 1975; Castañeda *et al.* 2009). Pollen core

Correspondence: Thomas Geburek, Fax: (+43) (1) 87838 2250; E-mail: thomas.geburek@bfw.gv.at

[1]Contributed equally.

data suggest that during the LGM vegetation now classified as montane extended to wider areas of lower elevation in East, Central and West Africa (Van Zinderen Bakker & Clarke 1962; Caratini & Giresse 1979; Elenga 1987; Maley 1987, 1989). Later, during the early part of the Holocene, an increase in temperature caused an expansion of evergreen forests and a retraction of 'montane' species to current higher elevation distributions (Livingstone 1975; Maley 1991). High similarities between east and west African montane forests in their insect, bird and plant communities have long been evident and indicate connection between them, possibly via past lowland bridges (Moreau 1966; Hall 1973). The location of this connection and the time frame of its existence have been the subject of extensive discussion among paleoecologists (e.g. Hedberg 1969; Coetzee & Van Zinderen Bakker 1970; Wickens 1976). As the most recent connection, White (1981) proposed the southern migratory tract (SMT), where extant, patchily distributed Afromontane forests in the southern Democratic Republic of Congo, Zambia, northern Angola and Gabon could have acted as 'stepping stones' for various species (White 1983, 1993). Unfortunately, past vegetation patterns can only partly be reconstructed by pollen analysis because of a lack of lake sediments, particularly in Central Africa. Alternative methods to study past distributions, such as those based on phylogeographic reconstructions of typical representatives of the Afromontane flora based on DNA studies, can improve our knowledge of the historical processes responsible for determining current distributions and assist in predicting future responses to anthropogenic climate change.

Prunus africana (Hook.f.) Kalkman (synonym *Pygeum africanum* Hook.f.) (Rosaceae) is an evergreen tree typical of Afromontane forests. With the exception of some disjunct island populations in Madagascar, the Comoros and the Gulf of Guinea (Hall *et al*. 2000), *P. africana* is confined to the physiographic domain referred to as 'High Africa', extending from 34 °S to 12 °N. Within 'High Africa', the species occurs primarily in mountainous and volcanic highlands. In southern Africa, the range is between 600 and 1000 m in elevation, although in the Bloukrans River Gorge of South Africa it has been recorded to grow as low as 60 m (Geldenhuys 1981). Its occurrence below the montane zone is mainly in rocky areas and boulder accumulations or at drainage lines (Hall *et al*. 2000). In equatorial Africa, it is found at elevations ranging from 1000 to 3500 m; the distribution is generally typified by a relatively temperate climate and an annual rainfall from 500 to 700 mm at high latitudes to over 3000 mm at low latitudes. The species is a long-lived hermaphrodite tree that may grow to a height of more than 40 m and can attain a diameter greater than 1 m. It produces small white flowers in elongated clusters that are primarily insect pollinated. Flowering has been reported to start as early as age 5 and as late as age 20 (Hall *et al*. 2000). Its fleshy one-seeded fruits are dispersed by birds and mammals (Farwig *et al*. 2006). The species has been of considerable commercial interest in recent decades because bark extracts are used for the treatment of benign prostatic hyperplasia (Cunningham *et al*. 1997; Simons *et al*. 1998). This has led to significant international trade, overexploitation of natural populations and (since 1995) listing under Appendix II of CITES (http://www.cites.org/eng/prog/african_cherry.shtml).

There have been various speculations about the processes leading to the current distribution of *P. africana*, and several migration paths have been inferred based on extant stands (Aubréville 1976; Kalkman 1988). Aubréville (1976) suggested a Laurasian origin of *Prunus* (considering only subgenus Laurocerasus, which includes *P. africana*, in his work) with subsequent movement through the Middle East into northeast of Africa. On the other hand, Kalkman (1988) proposed a Gondwanian origin of species of the tribe Pruneae (containing the genera *Maddenia*, *Prinsepia* and *Prunus*), with northwards movement along a path starting in regions corresponding to Australia, South America and Africa. Another open question is the phylogeographic history of *P. africana* within Africa, which seems to be strongly connected with past vegetation dynamics and the distribution of Afromontane vegetation in general (White 1983). Based on a genetic study using random amplified polymorphic DNA (RAPD) markers, Muchugi *et al*. (2006) concluded that both long-distance seed dispersal and migration via the SMT could be responsible for the occurrence of *P. africana* in outlying islands and the distant West African mountain massifs. Because of the large genetic differences observed among western and eastern Kenyan populations, Muchugi *et al*. (2006) supported Aubréville's path of immigration (Aubréville 1976) and proposed the rift valley as a probable barrier to gene flow.

Organellar genome analysis has been intensively employed over the last two decades for phylogeographic studies in a wide range of plants, including within Rosaceae the tree genera *Sorbus* (Oddou-Muratorio *et al*. 2001), *Prunus* (Mohanty *et al*. 2001) and *Malus* (Vornam & Gebhardt 2000). In Rosaceae, chloroplast genomes are maternally inherited (e.g. Ishigawa *et al*. 1992; Matsumoto *et al*. 1997; Oddou-Muratorio *et al*. 2001), and markers therefore provide information on the spatial distribution of variation as a consequence of seed flows and founding events (Birky *et al*. 1989; McCauley *et al*. 2003). Chloroplast DNA markers are especially apt for investigating events that occurred in the more distant past (Petit *et al*. 1993a; Hewitt 2000). The approach we employed here, chloroplast simple sequence repeat

(cpSSR) analysis (McCauley 1995; Vendramin & Ziegenhagen 1997), has been widely adopted for phylogeographic study of tropical and subtropical tree genera such as *Hagenia* (Ayele *et al.* 2009), *Eucalyptus* (Nevill *et al.* 2010) and *Adansonia* (Pock Tsy *et al.* 2009).

Our aim in this study was to investigate the phylogeography of *P. africana* as a model to gain insights into past vegetation patterns of Afromontane forests. This involved testing cpSSR markers on a wide portion of the natural distribution of the species based on field sampling of material across nine African countries. This collection represents the most significant, extensive sampling of an Afromontane tree to be investigated in genetic studies. In particular, we were interested in the relationship between populations of east and west African montane forests and how observed patterns of variation relate to proposed migration routes. Using Bayesian coalescence modelling, we infer colonization dynamics and past vegetation history.

Materials and methods

Population sampling and DNA isolation

Leaf samples were obtained from 32 natural stands of *P. africana* covering the main block of the species' geographic range across sub-Saharan Africa (Fig. 1; Table 1). This included the West Africa region: Cameroon (three sites), Equatorial Guinea (Bioko) and Nigeria (one site each); the East African region: Uganda (four sites), Kenya (nine sites), Tanzania (six sites); and the southern Africa region: South Africa (two sites), Zimbabwe (three sites); and Madagascar (three sites). From each population, leaf samples from six to 36 trees were collected and dried with silica gel. Import, export and phytosanitary certificates were obtained according to national and CITES regulations. For each sampled tree, total genomic DNA was extracted from 40 to 60 mg of dry leaf material using the QIAGEN Plant mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions with slight modifications (Farwig *et al.* 2008). DNA was stored at 4 °C.

cpDNA amplification and genotyping

Chloroplast DNA variation was analysed with three microsatellite loci (cpSSR) originally characterized in Japanese plum (*Prunus salicinia*, primer pairs *TPSCP1*, *TPSCP5* and *TPSCP10*; Ohta *et al.* 2005) and two from *Sorbus* (primer pairs *rps16pm2* and *trnT-Lpm1*; Chester *et al.* 2007). Polymerase chain reaction (PCR) consisted of 1 μL (10–50 ng) total genomic DNA, 1× PCR buffer,

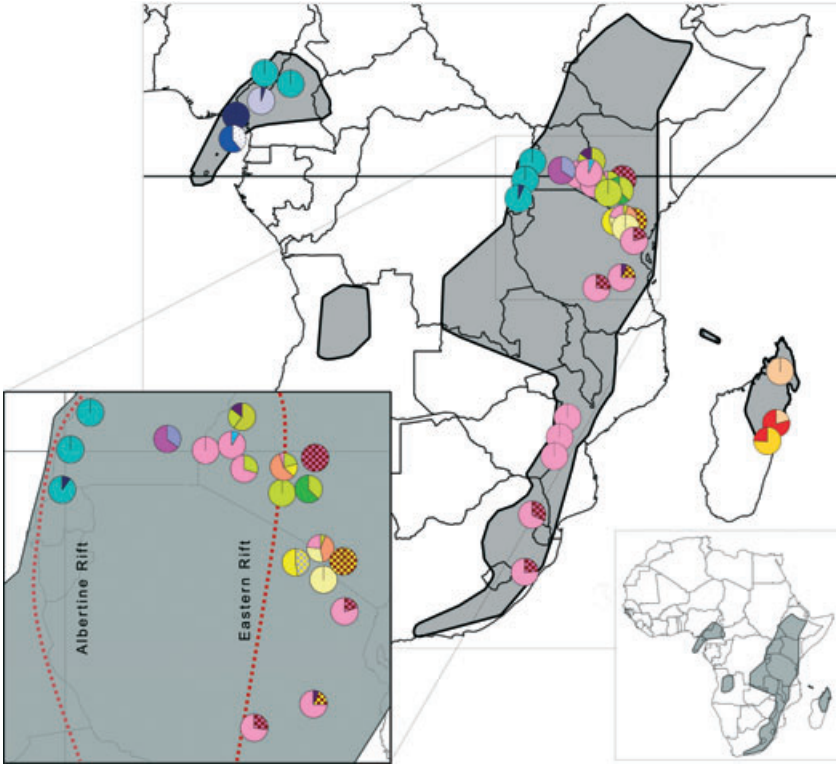

Fig. 1 Approximate geographic distribution of *Prunus africana* (grey shaded area), the origins of the populations used in the current study and haplotype distributions. Colour codes demonstrate the proportions of haplotypes within each population. Country boundaries are also shown.

Table 1 Description, location and haplotype diversity measures of investigated populations

Name of Population	Code	Country	Longitude	Latitude	*n*	N_a	N_e	N_p	H_e
1. Ngashie-Mt Oku	CA	Cameroon	10.5092	6.2048	19	2	1.11	1	0.105 (0.092–0.158)
2. Lower Mann's Spring, Mt Cameroon	CB	Cameroon	9.1192	4.1346	19	1	1	0	0.000 (0.000–0.000)
3. Mt Danoua	CC	Cameroon	11.9413	7.1871	20	1	1	0	0.000 (0.000–0.000)
4. Moka	GQ	Equatorial Guinea	8.6559	3.3625	18	2	1.91	2	0.503 (0.064–0.755)
5. Chuka, Central province	KA	Kenya	37.3645	−0.1798	20	1	1	0	0.000 (0.000–0.000)
6. Kinale, Central province	KB	Kenya	36.4151	−0.5278	19	4	2.46	0	0.626 (0.098–1.053)
7. Kapcherop, Cherangani Forest, Rift Valley	KC	Kenya	35.2161	1.0315	20	3	2.25	0	0.584 (0.091–0.955)
8. Kakamega Forest, Western Province	KD	Kenya	34.519	0.1415	20	1	1	0	0.000 (0.000–0.000)
9. Londiani, Rift Valley	KE	Kenya	35.1836	−0.2737	20	2	1.72	0	0.442 (0.087–0.663)
10. Ol Danyo Sambuk, Central Province	KF	Kenya	37.1501	−1.0744	19	2	1.87	1	0.491 (0.068–0.737)
11. Taita Hills, Coast Province	KG	Kenya	38.2088	−3.2462	20	1	1	0	0.000 (0.000–0.000)
12. Lari, Central Province	KT	Kenya	36.687	−1.0311	12	1	1	0	0.000 (0.000–0.000)
13. Kibiri forest, Western Province	KS	Kenya	34.8719	0.1502	16	2	1.13	1	0.125 (0.106–0.188)
14. Marovoay	MA	Madagascar	48.3069	−18.8023	5	2	1.47	0	0.400 (0.237–0.600)
15. Lakato forest	MB	Madagascar	48.2779	−19.1971	33	2	1.58	1	0.379 (0.079–0.568)
16. Antsahabiraoka	MC	Madagascar	49.2164	−14.4015	18	1	1	0	0.000 (0.000–0.000)
17. Ngel Nyaki Forest Reserve, Nigeria	NG	Nigeria	11.0271	7.066	9	1	1	0	0.000 (0.000–0.000)
18. Mpumalanga	SA	South Africa	30.7306	−25.0225	19	2	1.76	0	0.456 (0.085–0.684)
19. KwaZulu-Natal	SB	South Africa	30.2725	−29.2872	17	2	1.56	0	0.382 (0.113–0.574)
20. Meru Catchment Forest	TA	Tanzania	36.8074	−3.2925	19	2	1.99	1	0.526 (0.040–0.789)
21. Kilimanjaro Catchment Forest Reserve	TB	Tanzania	37.5237	−3.0036	17	4	3.18	0	0.728 (0.060–1.256)
22. Kindoroko Catchment Reserve	TC	Tanzania	37.6267	−3.7389	14	1	1	0	0.000 (0.000–0.000)
23. Shume Magamba Catchment Forest Reserve	TD	Tanzania	38.2521	−4.7544	20	2	1.47	0	0.337 (0.110–0.505)
24. Kidabaga	TE	Tanzania	35.9296	−8.1101	15	2	1.64	0	0.419 (0.113–0.629)
25. Udzungwa	TT	Tanzania	36.7791	−7.7668	16	3	1.66	0	0.425 (0.133–0.670)
26. Kibale Forest Natural Park	UA	Uganda	30.357	0.5644	20	1	1	0	0.000 (0.000–0.000)
27. Kalinzu Forest Reserve	UB	Uganda	30.1101	−0.3742	20	1	1	0	0.000 (0.000–0.000)
28. Bwindi Forest	UC	Uganda	29.7754	−1.0476	19	2	1.11	0	0.105 (0.092–0.158)
29. Mabira Forest	UD	Uganda	33.015	0.3806	20	2	1.83	2	0.479 (0.072–0.718)
30. Nyanga National Park	ZWA	Zimbabwe	32.7401	−18.2873	20	1	1	0	0.000 (0.000–0.000)
31. Cashel Valley Chimanimani	ZWB	Zimbabwe	32.8004	−19.5781	20	1	1	0	0.000 (0.000–0.000)
32. Chirinda forest Reserve Chipinge	ZWC	Zimbabwe	32.695	−20.409	19	1	1	0	0.000 (0.000–0.000)

n, number of individuals analysed; N_a, number of haplotypes; N_e, effective number of haplotypes; N_p, number of private haplotypes; H_e, haplotype diversity and standard error as calculated by rarefaction analysis.

0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.06–0.5 μM of fluorescence-labelled forward primer, the same amount of unlabelled reverse primer and 0.5 units of Platinum® Taq DNA Polymerase (Invitrogen, USA). Volumes were made up to 10 μL with autoclaved deionized water. Amplifications were run on a PTC-200 thermocycler (MJ Research) using the following heating profile: 3 min at 95 °C (initial denaturing step), followed by 35 cycles of 30–60 s at 94°C (denaturing step), 50 s at 50 °C (for *TPSCP5*, *rps16pm2*, *trnT-Lpm1*) or 60 s at 55 °C (for *TPSCP1*, *TPSCP10*) (annealing step), 1–2 min at 72 °C (extension step) and an additional 10 min at 72 °C (final extension step). PCR products were analysed using a CEQ8000 Beckmann Coulter Sequencer (Beckman-Coulter, USA) with an internal standard to measure the size of amplified bands. A volume of 1–2 μL of sample of each diluted PCR product was added to 0.4 μL of size standard in 35 μL sample loading solution. Differences in the sizes of products and the use of different fluorescent labels allowed the analysis of two to three microsatellites in a single run.

cpDNA variation and haplotype construction

To confirm the single nucleotide repeat motif of cpSSR variation and to avoid misinterpretations caused through size homoplasy, various alleles from each locus were sequenced. Sequence comparisons revealed single nucleotide steps for loci *TPSCP1*, *TPSCP5* and *TPSCP10*, whereas both of the loci *rps16pm2* and *trnT-Lpm1* were found to contain two indels of 5–26 base pairs in the flanking region. These indels were treated as two additional loci, namely *rps16pm2a* and *rps16pm2b*, and *trnT-Lpm1a* and *trnT-Lpm1b*. Additional length variation at the locus *rps16pm2* was not considered because it could not be resolved to unique mononucleotide repeat

variation. Sequences were deposited in GenBank under the accession numbers HM749746–HM749785.

Multilocus haplotypes were constructed by combining single loci. The number of haplotypes per population, the effective number of haplotypes, the number of private haplotypes and haplotype diversity were calculated using GenAlEx 6.0 (Peakall & Smouse 2006). To account for differences in sample size, rarefaction analysis of haplotype diversity was undertaken using CONTRIB (Petit *et al.* 1998), giving an improved estimate of haplotype diversity and its standard error. As mononucleotide microsatellites are potentially prone to homoplasy (i.e. alleles can be identical in state but not identical by descent owing to repeated forward and backward mutations), linkage disequilibrium (LD) among loci (including indels) was examined following Hale *et al.* (2004) using the statistic r_d implemented in the software MultiLocus 1.2 (Agapow & Burt 2001). If the multilocus LD is significantly high, we can expect that alleles identical in size are also identical in descent; phylogeographic inferences should then not be affected (Hale *et al.* 2004; Angioi *et al.* 2009).

Haplotype relationships were inferred by constructing a statistical parsimony network following a two-step strategy according to Bänfer *et al.* (2006). This procedure accounted for the (presumably) different mutation rates underlying indel and microsatellite variation. First, haplotype data from the indel variation were employed to construct a backbone network using the programme TCS 1.18 (Clement *et al.* 2000). Second, the network was enlarged by adding the variation at microsatellite loci manually at the respective positions of the backbone; mononucleotide microsatellites were coded according to a stepwise mutation model.

Population structure and phylogeography

Total gene diversity (h_t) and average within population gene diversity (h_s) were calculated according to Pons & Petit (1995, 1996) using the software PERMUT (http://www.pierroton.inra.fr/genetics/labo/Software). To test for the existence of phylogeographic patterns, we compared the two measures of differentiation N_{ST} and G_{ST}, which are based on ordered and unordered alleles, respectively. A significantly higher value for N_{ST} compared with G_{ST} indicates the existence of a phylogeographic structure (Pons & Petit 1996). Significance was tested based on 1000 permutations.

To analyse relatedness among populations without a priori hypotheses, a spatial analysis of molecular variance (SAMOVA) was applied according to Dupanloup *et al.* (2002). This method is based on a simulated annealing procedure where populations are assigned to a defined number (K) of groups. Groups are maximally differentiated from each other but include populations that are geographically proximate. Using pairwise differences among haplotypes, SAMOVA generates F-statistics (F_{SC}, F_{ST} and F_{CT}) following an AMOVA approach (Excoffier *et al.* 1992). By exploring the behaviour of the indices F_{CT} and F_{SC} for different values of K, it is possible to identify the optimum number of groups for a set of populations (Dupanloup *et al.* 2002). One hundred simulated annealing processes were used for each value of K, from $K = 2$ to 30.

Population history and coalescence

To relate the genealogical network to colonization history and the physical characteristics of Africa and its changes within the Holocene, we used Bayesian coalescence modelling of past population splitting events. The group structure defined by SAMOVA was used to identify the time and sequence of divergence among regional groups. The software BATWING generates random samples from posterior distributions of a variety of population genetic parameters, e.g. mutation rate, effective population size and growth rate, based on coalescence theory (Wilson *et al.* 2003). Because of a lack of detailed knowledge on population demographics, we used a simple model of constant population size, where the prior population size follows a uniform distribution within an interval of 10–10 000 individuals. Although diffuse, these size limits cover the most plausible values for N, if we consider neighbourhood sizes, gene flow distances and densities typical for tropical trees (Hardy *et al.* 2006) and other *Prunus* species (Schueler *et al.* 2006). Prior mutation rates were set according to previous reports of chloroplast loci (Provan *et al.* 1999). We specified two groups of loci, both following a gamma distribution: the indels *rps16pm2a*, *rps16pm2b*, *trnT-Lpm1a* and *trnT-Lpm1b* were set to priors of $\mu = 8 \times 10^{-5}$, while the mononucleotide microsatellites *TPSCP1*, *TPSCP5* and *TPSCP10* were set to priors of $\mu = 3.2 \times 10^{-5}$ (Afzal-Rafii & Dodd 2007) because they showed higher variation. With these priors, 55 000 samples in total were generated, from which the first 5000 were discarded as the 'burn-in'. Posterior parameter distributions for mutation rate, population size, splitting times and splitting sequence were estimated from the output of the remaining 50 000 samples.

Results

cpDNA variation and haplotype construction

Three cpDNA microsatellites and four cpDNA indels generated data for 582 individuals of *P. africana* from 32 populations. Data from all primers yielded 2–4

Table 2 Haplotype construction and frequency in 32 *Prunus africana* populations revealed by four cpDNA indels (Loci 1–4) and three cpDNA microsatellites (Loci 5–7). Alleles are represented as one for the presence of fragment and two for the absence of fragment in the case of the indels (Loci 1–4) and repeat numbers in the case of microsatellites (Loci 5–7)

Backbone Nt	1															2		3			4	5
Nt subdivision	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	a	b	a	b	c	a	a
Indels†																						
1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	2	2
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
MS‡																						
5	9	10	10	10	11	8	9	9	9	9	9	9	8	8	8	8	10	9	9	9	9	10
6	9	9	9	9	9	9	10	10	9	8	8	8	8	8	8	8	8	9	10	9	9	9
7	9	9	11	12	12	11	9	10	10	9	10	11	9	10	11	9	9	9	9	10	11	11
Population§																						
CA														1	18							
CB														19								
CC													20									
GQ											7	11										
KA							20															
KB	1			4		3		11														
KC		3	12	5																		
KD	20																					
KE	14			6																		
KF				7	12																	
KG									20													
KS	15									1												
KT				12																		
MA																		4		1		
MB																		8	25			
MC																				18		
NG													9									
SA	13						6															
SB	13						4															
TA						10																9
TB	4		1					7													5	
TC																					14	
TD	16						4															
TE	11						4															
TT	12	1							3													
UA													20									
UB													20									
UC													18	1								
UD																13	7					
ZWA	20																					
ZWB	20																					
ZWC	19																					
Total	178	4	13	34	12	13	38	18	23	1	7	11	87	21	18	13	7	12	25	19	19	9

†Indel loci 1,2,3,4 are rps16pm2a, rps16pm2b, trnT-Lpm1a, and trnT-Lpm1b.
‡MS-Chloroplast microsatellite loci 5, 6, 7 are TPSCP1, TPSCP5, and TPSCP10.
§Populations are Ngashie Mt. Oku (CA), Lower Mann's Spring, Mt. Cameroon (CB), Mt. Danoua (CC), Equatorial Guinea (GQ), Chuka (KA), Kinale (KB), Kapcherop (KC), Kakamega (KD), Londiani (KE), Ol Danyo Sambuk (KF), Taita (KG), Lari (KT), Kibiri forest (KS), Marovoay (MA), Lakato forest (MB), Antsahabiraoka (MC), Nigeria (NG), Mpumalanga (SA), Kwazulu Natal (SB), Meru (TA), Kilimanjaro (TB), Kindoroko (TC), Shume Magamba (TD), Kidabaga (TE), Udzungwa (TT), Kibale (UA), Kalinzu (UB), Bwindi (UC), Mabira (UD), Nyanga (ZWA), Chimanimani (ZWB) and Chipinge (ZWC).

alleles per locus and 19 alleles in total. The most variable cpSSR loci were *TPSCP1* and *TPSCP10* with four alleles each, followed by *TPSCP5* with three alleles. Overall multilocus LD was highly significant (r_d = 0.074, P < 0.002), indicating that homoplasy plays a minor role in explaining the observed phylogeographic pattern in comparison with migration.

Combination of loci resulted in 22 haplotypes in total (Table 2). The predominant haplotype HT1a (frequency 31%) occurred in 13 populations of East and southern Africa. HT1m, the second most frequent haplotype (frequency 14%), occurred only in populations of western Uganda and West Africa (Fig. 1). The genetic characteristics of chloroplast haplotypes in populations are shown in Table 1. The number of haplotypes (N_a) per population ranged from one to four with an average of two, with populations from the East African region harbouring the highest number of haplotypes. Nine haplotypes were private to single populations, while 13 occurred in two or more stands. The island of Bioko (GQ) and one population from Uganda (UD) each had two private haplotypes (GQ: HT1k and HT1l; UD: 2a and 2b) and did not share haplotypes with other populations. Fourteen populations revealed only one haplotype. The highest haplotype diversity (H_e) of 0.728 was found in the Tanzanian population from Mt. Kilimanjaro, followed by the Kenyan population Kinale (H_e = 0.626).

The relationship between haplotypes is demonstrated by the genealogical network given in Fig. 2. The backbone of the network consists of five main haplotypes (denoted HT1–HT5), of which HT1 is the dominant type on mainland Africa. Haplotypes of the HT3 family (3a–3c) form a geographically distinct group in Madagascar. The most common types HT1a and HT1m are separated only by two mutational steps but are geographically distributed either in the 'east' or 'west' African populations, respectively. HT1a and the haplotypes HT1b–HT1i can be found only in east (excluding Uganda) from here henceforth referred to as 'east' African populations and southern African populations, whereas populations from the eastern side of the Albertine Rift Valley in Uganda and the western part of Africa contain haplotypes related to HT1m (HT1j–HT1n) from here henceforth referred to as 'west' African populations.

Population structure and phylogeography

The total gene diversity (h_t) of 0.886 (SE = 0.0376) was more than three times higher than the mean within population diversity (h_s) of 0.234 (SE = 0.0432), suggesting high genetic differentiation among populations. This was also demonstrated by the high levels of differentiation of ordered (N_{ST}) and unordered (G_{ST}) alleles (0.840, SE = 0.0395 and 0.735, SE = 0.0481, respectively). N_{ST} was significantly higher than G_{ST} (P < 0.01), indicating a phylogeographic pattern for *P. africana*.

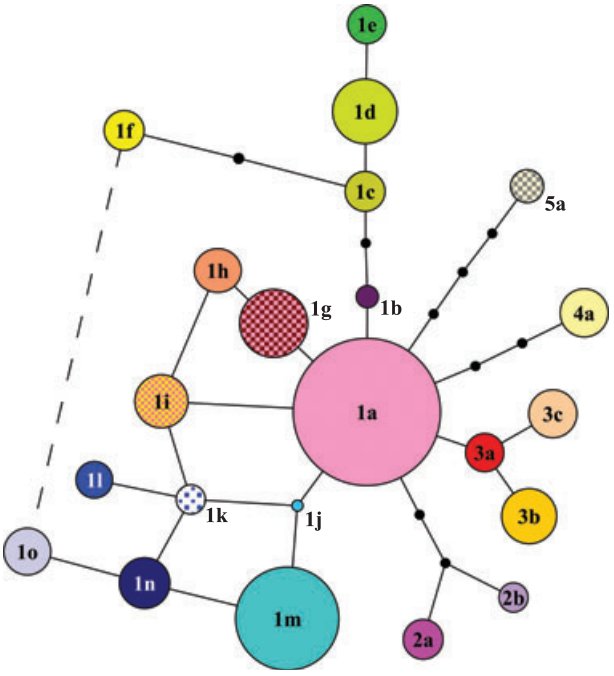

Fig. 2 Genealogical network revealing 22 haplotypes constructed from 19 alleles obtained from four cpDNA indels (Loci 1–4) and three cpDNA microsatellites (Loci 5–7) within 32 *Prunus africana* populations. Circle size is representative of the number of individuals having each haplotype (see Table 2).

Spatial analysis of molecular variance revealed a continuous increase in F_{CT} values from K = 2 to K = 20, again demonstrating the high differentiation among populations. F_{CT} values remained constant for K > 20 groups. Table 3 shows groupings for different values of K. Continuous bold lines depict barriers that remained constant from low to high numbers, while dotted bold lines depict barriers that occasionally disappear. Finer lines show boundaries that appear only at higher values of K (>14). The clearest spatial-genetic group is formed by the three populations from Madagascar, because their grouping is stable from K = 6 upwards. Interestingly, three populations from western Uganda group with the West Africa stands rather than other geographically proximate populations from East Africa. The East African region shows the highest heterogeneity, where for K = 2, a barrier was already found. The mainland southern region is closely related to the East African region, but within the mainland southern region populations are much less differentiated. The distribution of molecular variance to populations, populations within groups and groups is shown in Table 4; with increasing K, variation among populations within groups decreased and variation among groups increased.

Table 3 Genetic structure among *Prunus africana* populations as analysed by spatial analysis of molecular variance (SAMOVA). Increasing values of F_{CT} from $K = 2$ to 20 groups are shown. The groupings for different values of K are indicated by line demarcations. Indicated groupings relate to geographic origin

K	F_{CT}	West Africa Region and Uganda							Madagascar		Southern Region						East Africa Region excl. Uganda										
2	0.529	CA	CB	GQ	CC	UA	UB	UC	NG	UD	MB	MA	MC	SA	SB	ZWA	ZWB	ZWC	TD	TE	TT	KA	KD	KS	KE	KG	TB	KB	TC	TA	KC	KF	KT
4	0.595	CA	CB	GQ	CC	UA	UB	UC	NG	UD	MB	MA	MC	SA	SB	ZWA	ZWB	ZWC	TD	TE	TT	KA	KD	KS	KE	KG	TB	KB	TC	TA	KC	KF	KT
6	0.667	CA	CB	GQ	CC	UA	UB	UC	NG	UD	MB	MA	MC	SA	SB	ZWA	ZWB	ZWC	TD	TE	TT	KA	KD	KS	KE	KG	TB	KB	TC	TA	KC	KF	KT
8	0.700	CA	CB	GQ	CC	UA	UB	UC	NG	UD	MB	MA	MC	SA	SB	ZWA	ZWB	ZWC	TD	TE	TT	KA	KD	KS	KE	KG	TB	KB	TC	TA	KC	KF	KT
10	0.743	CA	CB	GQ	CC	UA	UB	UC	NG	UD	MB	MA	MC	SA	SB	ZWA	ZWB	ZWC	TD	TE	TT	KA	KD	KS	KE	KG	TB	KB	TC	TA	KC	KF	KT
12	0.745	CA	CB	GQ	CC	UA	UB	UC	NG	UD	MB	MA	MC	SA	SB	ZWA	ZWB	ZWC	TD	TE	TT	KA	KD	KS	KE	KG	TB	KB	TC	TA	KC	KF	KT
14	0.776	CA	CB	GQ	CC	UA	UB	UC	NG	UD	MB	MA	MC	SA	SB	ZWA	ZWB	ZWC	TD	TE	TT	KA	KD	KS	KE	KG	TB	KB	TA	TC	KC	KF	KT
16	0.793	CA	CB	GQ	CC	UA	UB	UC	NG	UD	MB	MA	MC	SA	SB	ZWA	ZWB	ZWC	TD	TE	TT	KA	KD	KS	KE	KG	TB	KB	TA	TC	KC	KF	KT
18	0.795	CA	CB	GQ	CC	UA	UB	UC	NG	UD	MB	MA	MC	SA	SB	ZWA	ZWB	ZWC	TD	TE	TT	KA	KD	KS	KE	KG	TB	KB	TA	TC	KC	KF	KT
20	0.825	CA	CB	GQ	CC	UA	UB	UC	NG	UD	MB	MA	MC	SA	SB	ZWA	ZWB	ZWC	TD	TE	TT	KA	KD	KS	KE	KG	TB	KB	TA	TC	KC	KF	KT

Boundaries stable
Boundaries change occasionally
Boundaries change at K>14

Table 4 Analysis of variance of population structure of 32 *Prunus africana* populations based on spatial analysis of molecular variance (SAMOVA) structure analysis using $K = 2$ to 20 groups

K	vg	vpg	vp	F_{SC}	F_{ST}	F_{CT}
2	52.89	38.6	8.47	0.82	0.92	0.53
4	59.49	28.2	12.3	0.7	0.88	0.6
6	66.71	20.1	13.2	0.6	0.87	0.67
8	70.02	16.7	13.3	0.56	0.87	0.7
10	72.71	13	14.3	0.48	0.86	0.73
12	74.17	10.9	14.9	0.42	0.85	0.74
14	78.05	7.01	15	0.32	0.85	0.78
16	79.27	5.45	15.3	0.26	0.85	0.79
18	79.51	4.91	15.6	0.24	0.84	0.8
20	82.5	1.91	15.6	0.11	0.84	0.83

K, number of groups; vg, variation within groups; vpg, variation of populations within groups; vp, variation within populations; F_{SC}, differentiation among populations within groups; F_{ST}, differentiation among populations; F_{CT}, differentiation among groups of populations.

Population history and coalescence

For coalescence modelling of *P. africana*, populations were aggregated into $K = 6$ regional groups according to the spatial-genetic structure observed in SAMOVA; this grouping provided an unbiased clustering of populations with a clear geographic distribution. To calculate estimates for the time of splitting events, we converted coalescent units given by BATWING into years by multiplying the population size given by the program with the generation time (GT) of *P. africana*, which was assumed to be 8 years at the lower limit and 15 years at the upper limit (Hall *et al.* 2000).

The two most recent splitting events with a node support of more than 80% were found to have occurred among populations of eastern and southern Africa (Fig. 3). The estimated confidence limit for the time of these population splitting events assuming a GT of 8 years ranged from 21 000 years before present (BP) to 1500 years BP for the second youngest split and from 6500 years BP until today for the youngest split, respectively (see Table 5 and Fig. 4). The splitting among 'west' African groups (including populations from Uganda) also received high node support (48%) and dates either to the second, third or fourth oldest splitting event to times between 1400 and 67 000 years BP (GT = 15: 2700–125 000 years). The split between Madagascar and all East and South African groups dates to the second and third oldest splitting events (from 7800 to 67 000 years BP using GT = 8 and from 14 600 to 125 000 years using GT = 15) and receives a node support of about 40%. Other potential splitting events at the second or third oldest splitting event receive a node

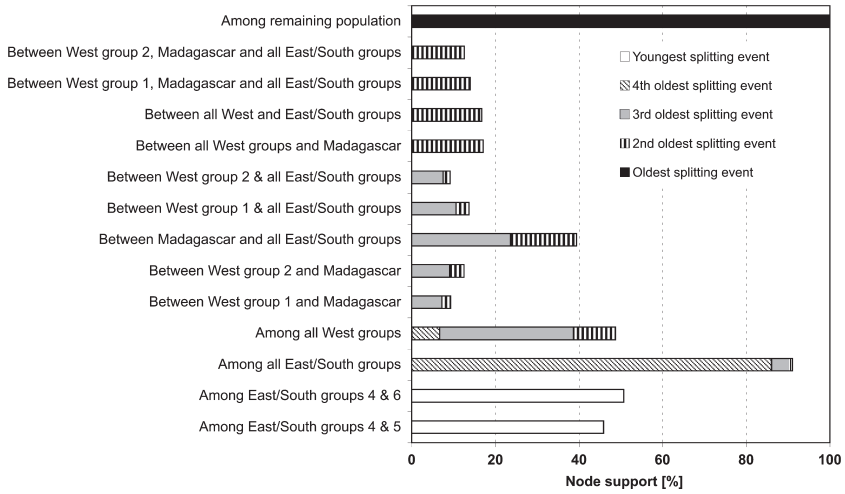

Fig. 3 Node support for various population splitting events identified by coalescence modelling using a Bayesian analysis. Only splitting events with a node support of more than 5% are shown.

Table 5 Results of coalescence modelling using a Bayesian analysis. Upper and lower 5% quantiles, mean and standard deviation of *a posteriori* estimates of ancestral population size (N) and mutation rates (μ) are given

	Quantile 5%	Mean	Quantile 95%	Standard deviation
Ancestral population size				
N	3807	6419	9290	1663
Posterior mutation rates				
μ (loci 1–4)	1.94×10^{-5}	3.57×10^{-5}	5.74×10^{-5}	1.18×10^{-5}
μ (loci 5–7)	8.10×10^{-5}	12.06×10^{-5}	16.93×10^{-5}	2.72×10^{-5}

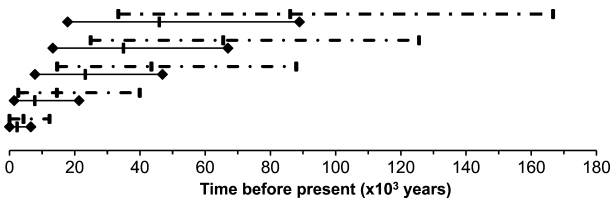

Fig. 4 Time of population splitting events identified by coalescence modelling using a Bayesian analysis for two estimates of generation time (8 years, solid line; 15 years, broken line). The graph gives the upper and lower 5% quantiles and the mean of the time estimates. In descending order, the upper line identifies the oldest event and the lowest one the youngest event.

support of <20%, suggesting branching among groups of 'east' and 'west' Africa occurred concurrently.

Discussion

In this study, we investigated the phylogeography of *P. africana* as a model species to derive vegetation history of Afromontane forests. We detected high population differentiation and significant phylogeographic structure within the species. Populations from West Africa were closely related to populations from Uganda. This finding provides strong evidence for a former migration corridor between 'east' and 'west' African populations.

Genetic diversity and population structure

Compared to other tree species from Africa, the observed differentiation among populations (G_{ST} = 0.735) is relatively high and comparable to that found in *Adansonia digitata* (G_{ST} = 0.97; Pock Tsy *et al.* 2009) and *Hagenia abyssinica* (G_{ST} = 0.899; Ayele *et al.* 2009), although the observed within population diversity is higher in *P. africana* (h_s = 0.237) than in either *Adansonia* (h_s = 0.017) or *Hagenia* (h_s = 0.079), maybe attributed to the lower sample size in these studies. Also, tropical species from South America show similar high patterns of differentiation, e.g. *Cedrela odorata* (G_{ST} = 0.96; Cavers *et al.* 2004) and *Vouacapoua americana* (G_{ST} = 0.89; Dutech *et al.* 2000).

In contrast, other range-wide cpDNA studies on Rosaceous trees (e.g. from Europe) show markedly lower genetic differentiation among populations, but higher within population diversity, e.g. *P. avium* (G_{ST} = 0.29; h_s = 0.33; Mohanty *et al.* 2001), *P. spinosa* (G_{ST} = 0.32; h_s = 0.49; Mohanty *et al.* 2002) and *Sorbus torminalis* (G_{ST} = 0.33, h_s = 0.39; Oddou-Muratorio *et al.* 2001). Differences may be explained by either different seed dispersal mechanisms or different population histories. Seed dispersal mechanisms play a crucial role in shaping distributions of haplotype diversity and genetic

differentiation (Petit *et al.* 1993b, 2003; Demesure *et al.* 1996; Dumolin-Lapegue *et al.* 1997). Farwig *et al.* (2006) listed 36 frugivorous birds and mammals with body sizes of 10–80 cm as seed dispersers for *P. africana*. These dispersers should facilitate short- and medium-distance seed dispersal at least as efficiently as seed dispersers of *Prunus* species in Europe. Therefore, we rather believe that differences in the population history of *P. africana* and European *Prunus* species are responsible for the stronger differentiation in the former. European Rosaceous tree populations have been affected by ice ages and recent (within the last 10 000 years) postglacial recolonization of central and northern European areas, whereas within the same period *P. africana* is likely to have experienced only smaller altitudinal range shifts. In comparison with latitudinal range shifts where a loss of genetic diversity attributed to repeated bottlenecks can occur (Hewitt 1996), range shifts in mountain regions occur over much shorter geographic distances, and population sizes may be more stable, thereby maintaining diversity (Ehrich *et al.* 2007). The isolation of mountain massifs from each other might limit gene flow and facilitate differentiation in local populations (Hewitt 2000). Thus, the history of single populations in Africa is doubtless much older than that of European Rosaceous tree populations. Similar strong differentiation patterns were found for Afroalpine populations of *Arabis alpina* in a comparison with European and Arctic populations (Ehrich *et al.* 2007).

Phylogeography of Prunus africana*—migration and dispersal within Africa*

Mountain ranges that provide habitats for Afromontane species today were formed within the late Pliocene and throughout the Pleistocene (Hedberg 1994). During the Pleistocene, several Afroalpine species colonized Africa from Asia through the coastal mountain ranges of the Arabian Peninsula (Koch *et al.* 2006). According to Aubréville (1976), *P. africana* could have followed a similar path based on the putative Laurasian origin of the genus *Prunus*. Our study was not able to provide data on the original immigration path of *P. africana* into the African continent as populations are not extant in the Arabian Peninsula and palaeoecological evidence is not available. However, our data provide the first significant insights into the population history of *P. africana* within mainland Africa and its neighbouring islands, as a significant signal for phylogeographic pattern was found.

Supported by Bayesian analysis where splitting events between Madagascar and other African populations were estimated to be among the oldest, populations from Madagascar were found to be genetically most distinct, suggesting an early split from the main lineage. As the separation of Madagascar from Africa was completed 100 Ma, (long before *P. africana* reached Africa), the colonization of Madagascar may reflect early and possibly unique dispersal events facilitated by birds or the southern Monsoon drift or the Mozambique current (Fig. 5).

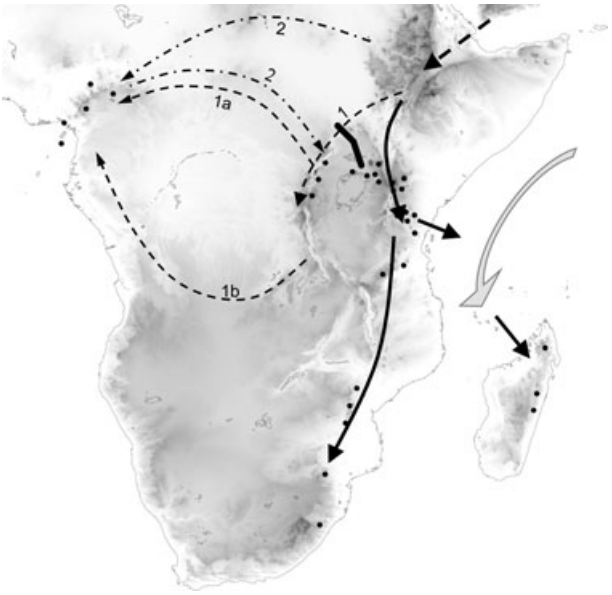

Fig. 5 Hypothesized migration route of *Prunus africana* as deduced from coalescence modelling and the observed phylogeographic—genetic structure. Black solid arrows indicate the hypothetical route according to the present study, the black broken arrow indicates Aubréville's path of immigration of the genus *Prunus* into Africa (Aubréville 1976). The putative migration paths to West Africa are shown by thin broken arrows: 1—split of eastern and western populations during southward migration at the southern fringe of the Ethiopian highlands, subsequently: 1a—migration from the Albertine Rift to West Africa via a northern connection; 1b—southern migratory tract (SMT) following White (1993); 2—independent colonization of West Africa via a northern migration corridor and subsequent colonization of the Albertine Rift from West Africa. The grey arrow indicates ocean currents involved in the possible dispersal to Madagascar. Black dots represent locations where population sampling was carried out in this study. The bold bar indicates the putative barrier between eastern and western lineage created by the upper river Nile and Lake Victoria basins.

On mainland Africa, a clear division between western populations (including Ugandan populations east of the Albertine Rift valley) and stands in eastern and southern Africa was detected. The haplotype sharing between West African and Ugandan populations suggests the former existence of a migration corridor from east to west. Although the exact migration routes to West Africa cannot to be determined at present, our data support two scenarios: (i) a split during southward migration of *P. africana* at the southern fringe of the

Ethiopian highlands (Fig. 5, route 1) with migration of Albertine Rift valley populations to West Africa (route 1a, 1b); or (ii) the independent colonization of West Africa via a northwestern migration corridor and the subsequent colonization of western Uganda from West Africa (route 2). Both scenarios imply that a strong barrier for gene flow has existed within East Africa and that the distribution range of *P. africana* included large areas of Central Africa at one time, where presently no populations exist (Hall *et al.* 2000).

The first scenario of colonization from the Albertine Rift to West Africa is supported by the relatively small genetic distances (<4 mutations) among the haplotypes found in West Africa, which suggests that all six observed haplotypes originate from the dominant type HT1m. In contrast, the various haplotypes found in East Africa have a deeper root with up to five mutations among them and include other main backbone lineages. Thus, the high haplotype diversity found in West Africa could be a result of younger differentiation events coupled with isolated mountain habitats that facilitated the fixation of newly mutated types. In contrast, further differentiation at the Albertine Rift may have been prevented by regular seed exchange. The colonization from East to West could have been through either the SMT as defined according to White (1981) or through a more northern direct connection. Our data suggest a more direct connection as the SMT, if it functioned as a bridge, must have originated south of Lake Tanganyika. Here, the Albertine Rift and the Eastern Rift meet and this should have provided chance for lineage mixing: i.e. populations from West Africa would then also be expected to contain haplotypes from the eastern lineage. On the other hand, the absence of such haplotypes in the west could be explained by the later splitting events of the east and southern populations compared with the splitting events between the 'east' and 'west' African populations. More data from populations sampled south of the Albertine Rift and following the SMT would be needed to resolve this definitively.

Under the second scenario, *P. africana* could have migrated directly from the Ethiopian highlands to West Africa, possibly using the Marra Mountains (Sudan) as stepping stones (cf. Wickens 1976). Thereafter, subsequent colonization of Uganda from West Africa could have occurred. This route would provide an alternative explanation for the lower diversity shown in the Albertine Rift valley populations. As there is no extant Afromontane forest or *P. africana* in the central African regions that must have been crossed, only an extensive phylogeographic study of populations from Ethiopian highlands could shed further light on this second hypothesis.

As key barriers to dispersal in the early population history of the species we suggest the upper river Nile basin and the Lake Victoria basin, because these areas experienced severe drought conditions before 12 000 BP (Livingstone 1975) and probably also earlier in the Pleistocene. According to pollen analysis and radiocarbon data of lake sediments, the water level of Lake Victoria at 14 000 BP was between 26 and 75 m lower than today, and the surrounding landscape was likely too dry for *P. africana* and characterized mainly by open vegetation (Livingstone 1975). About 10 000 years BP evergreen rainforest expanded into the region, which again likely did not provide suitable habitat. Today, the river Nile basin with Lake Kyoga and its surrounding extensive marshy areas are still not suitable habitat for Afromontane forest, although the climate has been somewhat dryer since 6000 years BP, facilitating more semideciduous forest (Livingstone 1975). Another reason for past absence of *P. africana* in this part of Uganda may be the relatively flat terrain, which did not allow for altitudinal range shifts during past climatic fluctuations. Within the adjacent rift valleys and mountain ranges, such range shifts might have supported the survival of populations during the unstable climatic history. Our data do not support the suggestion of Muchugi *et al.* (2006) who from a study based on RAPD markers of Cameroonian, Kenyan, and Ugandan populations of *P. africana* concluded that the Eastern Rift Valley acted as a barrier to gene flow, because in our case on either side of both branches of the Rift similar chloroplast haplotypes occurred: HT1a east and west of the Eastern Rift Valley, and HT1m east and west of the Albertine Rift.

Our data on the close relationship between Albertine Rift and west African populations of *P. africana* correspond with ecological comparisons between east and west African ornithological and entomological records. Already in the 1960s, Moreau (1966) suggested that montane vegetation belts were depressed to lower elevations connecting east and west African mountain regions during the last glacial period, although Livingstone (1975) doubted that such a belt existed. Based on data from palynological records from the last 15 000 years, Livingstone (1975) rather argued that forest trees were extremely rare in mountain regions and at lower altitudes during the LGM. Instead, he explained ecological affinities by the high migration capacity of birds and butterflies and suggested smaller isolated habitats to have served as stepping stones. Although *P. africana* is a typical species of Afromontane forest, it has also been reported to inhabit elevations below the montane zone (Hall *et al.* 2000). It is difficult to conclude whether such site conditions would have allowed spread across a distance of around 2000 km,

but future genetic studies of additional wide-spread Afromontane species should help to shed more light on past montane vegetation belts.

Our proposed migration paths and splits were well supported by both the genealogical network and the Bayesian simulations of coalescence. In general, the Bayesian simulations provided a broad estimate of splitting times for events. There is for example considerable overlap between the three oldest splitting events (Fig. 4). Although the uncertainties in estimating GT, population size and mutation rate limit the accuracy of the obtained splitting times, they elucidate the sequence of events. For example, the two most recent splits are sharply different from the older ones. Using the range of GTs given in the literature (Hall *et al*. 2000), the time of the population splits varies by a factor of 2–4. The overall time frame of the simulations shows that the complete colonization of the investigated distribution of *P. africana* has most likely occurred within the last 100 000–180 000 years. On a global scale, this time period is known as the last glaciation period, suggesting that the colonization of eastern and western Africa by *P. africana* occurred mainly during colder climatic episodes. This study has provided evidence on the phylogeography of *P. africana* and on a past migration corridor between Afromontane forests; to investigate further aspects in colonization dynamics, sampling is suggested in the Ethiopian highlands and in the southwestern/central African range.

Acknowledgements

We thank our national partners for carrying out the sample collections. This research was financed by the Austrian Development Agency through collaboration between BFW and Bioversity International. We kindly acknowledge colleagues in Cameroon, Alistair Jump, Stephen Cavers and Hazel Chapman for supplying us initial test samples and additional populations. We thank three anonymous referees and Ian Dawson for helpful comments on the content of a former version of this manuscript and improvements in style.

References

Afzal-Rafii Z, Dodd RS (2007) Chloroplast DNA supports a hypothesis of glacial refugia over postglacial recolonization in disjunct populations of black pine (*Pinus nigra*) in Western Europe. *Molecular Ecology*, **16**, 723–736.

Agapow P-M, Burt A (2001) Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes*, **1**, 101–102.

Angioi SA, Desiderio F, Rau D, Bitocchi E, Attene G, Papa R (2009) Development and use of chloroplast microsatellites in *Phaseolus* spp. and other legumes. *Plant Biology*, **11**, 598–612.

Aubréville A (1976) Centres tertiaires d'origine, radiations et migrations des flores angiospermiques tropicales. *Adansonia, serie 2*, **16**, 297–354.

Ayele TB, 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.

Bänfer G, Moog U, Fiala B, Mohamed M, Weising K, Blattner FR (2006) A chloroplast genealogy of myrmecophytic *Macaranga* species (Euphorbiaceae) in Southeast Asia reveals hybridization, vicariance and long-distance dispersals. *Molecular Ecology*, **15**, 4409–4424.

Birky CW, Fuerst P, Maruyama T (1989) Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. *Genetics*, **121**, 613–627.

Caratini C, Giresse P (1979) Contribution palynologique à la connaissance des environnements continentaux et marins du Congo à la fin du quaternaire. *Comptes Rendus de l'Académie des Sciences. Paris, série D*, **288**, 379–382.

Castañeda IS, Werne JP, Johnson TC, Filley TR (2009) Late Quaternary vegetation history of southeast Africa: the molecular isotopic record from Lake Malawi. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **275**, 100–112.

Cavers S, Navarro C, Lowe AJ (2004) Targeting genetic resource conservation in widespread species: a case study of *Cedrela odorata* L. *Forest Ecology and Management*, **197**, 285–294.

Chester M, Cowan RS, Fay MF, Rich TCG (2007) Parentage of endemic *Sorbus* L. (Rosaceae) species in the British Isles: evidence from plastid DNA. *Botanical Journal of the Linnean Society*, **154**, 291–304.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.

Coetzee JA, Van Zinderen Bakker EM (1970) Paleoecological problems of the Quaternary of Africa. *South African Journal of Science*, **66**, 78–84.

Cunningham M, Cunningham AB, Schippmann U (1997) *Trade in* Prunus africana *and the Implementation of CITES*. Bundesamt fur Naturschutz, Bonn, Germany.

Demesure B, Comps B, Petit RJ (1996) Chloroplast DNA phylogeography of the common beech (*Fagus sylvatica L.*) in Europe. *Evolution*, **50**, 2515–2520.

Dumolin-Lapegue S, Demesure B, Le Corre V, Fineschi S, Petit RJ (1997) Phylogeographic structure of white oaks throughout the European continent. *Genetics*, **146**, 1475–1487.

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define genetic structure of populations. *Molecular Ecology*, **11**, 2571–2581.

Dutech C, Maggia L, Joly HI (2000) Chloroplast diversity in *Vouacapoua americana* (Caesalpiniaceae), a neotropical forest tree. *Molecular Ecology*, **9**, 1427–1432.

Ehrich D, Gaudeul M, Assefa A *et al.* (2007) Genetic consequences of Pleistocene range shifts: contrast between the Arctic, the Alps and the East African mountains. *Molecular Ecology*, **16**, 2542–2559.

Elenga H (1987) *Les Plateaux Batéké (Congo). Paléoenvironnements Quaternaries d'après l'étude du sondage du bois de Bilanko. Diplôme d'Etudes Approfondies*, Université d'Aix-Marseille II, Marseille, 41 pp.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Farwig N, Böhning-Gaese K, Bleher B (2006) Enhanced seed dispersal of *Prunus africana* in fragmented and disturbed forests? *Oecologia*, **147**, 238–252.

Farwig N, Braun C, Böhning-Gaese K (2008) Human disturbance reduces genetic diversity of an endangered tropical tree, *Prunus africana* (Rosaceae). *Conservation Genetics*, **9**, 317–321.

Finch J, Leng MJ, Marchant R (2009) Late Quaternary vegetation dynamics in a biodiversity hotspot, the Uluguru Mountains of Tanzania. *Quaternary Research*, **72**, 111–122.

Geldenhuys CJ (1981) *Prunus africana* in the Bloukrans River George Southern Cape. *South African Forestry Journal*, **118**, 61–66.

Hale ML, Borland AM, Gustafsson MAG, Wolff K (2004) Causes of size homoplasy among chloroplast microsatellites in closely related *Clusia* species. *Journal of Molecular Evolution*, **58**, 182–190.

Hall JB (1973) Vegetational zones on the southern slopes of Mount Cameroon. *Vegetatio*, **27**, 49–69.

Hall JB, O'Brien EM, Munjuga M (2000) Ecology and biology. In: Prunus africana*: A Monograph. School of Agricultural and Forest Sciences*, vol. 18 (eds Hall JB, O'Brien EM, Sinclair FL), pp. 3–25. University of Wales, Bangor.

Hall J, Burgess ND, Lovett J, Mbilinyi B, Gereau RE (2009) Conservation implications of deforestation across an elevational gradient in the Eastern Arc Mountains, Tanzania. *Biological Conservation*, **142**, 2510–2521.

Hardy OJ, Maggia L, Bandou E *et al.* (2006) Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. *Molecular Ecology*, **15**, 559–571.

Hedberg O (1969) Evolution and speciation in a tropical high mountain flora. *Biological Journal of the Linnean Society*, **1**, 135–148.

Hedberg O (1994) Afroalpine region. East and northeast tropical Africa. In: *Centres of Plant Diversity: A Guide and Strategy for their Conservation* (eds Davis SW, Heywood VH, Hamilton AC), pp. 253–256. Information Press, Oxford.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Ishigawa S, Kato S, Imakawa S, Mikami T, Shimamoto Y (1992) Organelle DNA polymorphism in apple cultivars and rootstocks. *Theoretical and Applied Genetics*, **83**, 963–967.

Kalkman C (1988) The phylogeny of the Rosaceae. *Botanical Journal of the Linnean Society*, **98**, 37–59.

Koch MA, Kiefer C, Ehrich D, Vogel J, Brochmann C, Mummenhoff K (2006) Three times out of Asia Minor: the phylogeography of *Arabis alpina* L. (Brassicaceae). *Molecular Ecology*, **15**, 825–839.

Livingstone DA (1975) Late Quaternary climate change in Africa. *Annual Review of Ecology and Systematics*, **6**, 249–280.

Maley J (1987) Fragmentation de la forêt dense humide africaine et extension des biotopes montagnards au quaternaire récent: nouvelles données polliniques et chronologiques. Implications paléoclimatiques et biogéographiques. *Palaeoecology of Africa*, **18**, 307–334.

Maley J (1989) Late Quaternary climatic changes in the African rain forest: forest refugia and the major role of sea surface temperature variations. In: *Paleoclimatology and Paleometeorology: Modern and Past Patterns of Global Atmospheric Transport NATO Advances* Sc. Inst. Series, C, Math. & Phys. Sc., 282 (eds Leinen M, Sarnthein M), pp. 585–616. Kluwer Academic Publishers, Dordrecht.

Maley J (1991) The African Rain Forest vegetation and palaeoenvironments during late Quaternary. *Climatic Change*, **19**, 79–98.

Matsumoto S, Wakita H, Fukui H (1997) Molecular classification of wild roses using organelle DNA probes. *Scientia Horticulturae*, **68**, 191–196.

McCauley DE (1995) The use of chloroplast DNA polymorphism in studies of gene flow in plants. *Trends in Ecology and Evolution*, **10**, 198–202.

McCauley DE, Smith RA, Lisenby JD, Hsieh C (2003) The hierarchical spatial distribution of chloroplast DNA polymorphism across the introduced range of *Silene vulgaris*. *Molecular Ecology*, **12**, 3227–3235.

Mohanty A, Martín JP, Aguinagalde I (2001) A population genetic analysis of chloroplast DNA in wild populations of *Prunus avium* L. in Europe. *Heredity*, **87**, 421–427.

Mohanty A, Martín JP, Aguinagalde I (2002) Population genetic analysis of European *Prunus spinosa* (Rosaceae) using chloroplast DNA markers. *American Journal of Botany*, **89**, 1223–1228.

Moreau RE (1966) *The Birds Faunas of Africa and its Islands*. Academic Press, New York, 424 pp.

Muchugi A, Lengkeek AG, Kadu CAC, Muluvi GM, Njagi ENM, Dawson IK (2006) Genetic variation in the threatened medicinal tree *Prunus africana* in Cameroon and Kenya: implications for current management and evolutionary history. *South African Journal of Botany*, **72**, 498–506.

Nevill PG, Bossinger G, Ades PK (2010) Phylogeography of the world's tallest angiosperm, *Eucalyptus regnans*: evidence for multiple isolated Quaternary refugia. *Journal of Biogeography*, **37**, 179–192.

Oddou-Muratorio S, Petit RJ, Le Guerroue B, Guesnet D, Demesure B (2001) Pollen versus seed-mediated gene flow in a scattered woody species, *Sorbus torminalis* L. *(Crantz). Evolution*, **55**, 1123–1135.

Ohta S, Nishitani C, Yamamoto T (2005) Chloroplast microsatellites in *Prunus*, Rosaceae. *Molecular Ecology Notes*, **5**, 837–840.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Petit RJ, Kremer A, Wagner DB (1993a) Finite island model for organelle and nuclear genes in plants. *Heredity*, **71**, 630–641.

Petit RJ, Kremer A, Wagner DB (1993b) Geographic structure of chloroplast DNA polymorphisms in European oaks. *Theoretical and Applied Genetics*, **87**, 122–128.

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conservation Biology*, **12**, 844–855.

Petit RJ, Aguinagalde I, de Beaulieu J-L *et al.* (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, **300**, 1563–1565.

Pock Tsy JML, Lumaret R, Mayne D *et al.* (2009) Chloroplast DNA phylogeography suggests a West African centre of origin for the baobab, *Adansonia Digitata* L. (Bombacoideae, Malvaceae). *Molecular Ecology*, **18**, 1707–1715.

Pons O, Petit RJ (1995) Estimation, variance and optimal sampling of gene diversity. I. Haploid locus. *Theoretical and Applied Genetics*, **90**, 462–470.

Pons O, Petit RJ (1996) Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics*, **144**, 1237–1245.

Provan J, Soranzo N, Wilson NJ, Goldstein DB, Powell W (1999) A low mutation rate for chloroplast microsatellites. *Genetics*, **153**, 943–947.

Schueler S, Tusch A, Scholz F (2006) Comparative analysis of the within-population genetic structure in wild cherry (*Prunus avium* L.) at the self-incompatibility locus and nuclear microsatellites. *Molecular Ecology*, **15**, 3231–3243.

Simons AJ, Dawson IK, Duguma B, Tchonndjeu Z (1998) Passing problems: prostrate and *Prunus*. *Herbalgram*, **43**, 49–53.

Van Zinderen Bakker EM, Clarke JD (1962) Pleistocene climates and cultures in North Eastern Angola. *Nature*, **196**, 639–642.

Vendramin GG, Ziegenhagen B (1997) Characterisation and inheritance of polymorphic plastid microsatellites in *Abies*. *Genome*, **40**, 857–864.

Vornam B, Gebhardt K (2000) PCR-based markers reveal genetic identity and diversity in subset collections of wild and cultivated apple. *Acta Horticulturae*, **530**, 463–467.

White F (1981) The history of the Afromontane archipelago and the scientific need for its conservation. *African Journal of Ecology*, **19**, 33–54.

White F (1983) Long-distance dispersal and the origins of the Afromontane flora. *Sonderbände des Naturwissenschaftlichen Vereins in Hamburg*, **7**, 87–116.

White F (1993) Refuge theory, ice-age aridity and the history of tropical biota: an essay in plant geography. *Fragmenta Floristica et Geobotanica Supplement*, **2**, 385–409.

Wickens GE (1976) Speculations on long distance dispersal and the flora of Jebel Marra, Sudan Republic. *Kew Bulletin*, **31**, 105–150.

Wilson IJ, Weale ME, Balding DJ (2003) Inferences from DNA data: population histories, evolutionary processes and forensic match probabilities. *Journal of the Royal Statistical Society A*, **166**, 155–201.

CK is interested in the population genetics and phylogeography of forest trees. This work constitutes part of her PhD carried out at BFW and Kenyatta University, Nairobi, Kenya. SS, HK and TG from the Department of Genetics at the BFW have long standing interests in plant evolutionary biology. GM is a professor at Kenyatta University in Biochemistry and Biotechnology and has interests in population genetics. OE-M works on forest plant genetic resources. AM has interests in agroforestry species as well as tree population genetics and biotechnology, VW has interests in Ethnobotany and Indigenous Plant Use and LR, CK, BF, CK, DH and CO are interested in management and conservation of African tree species.
