Complex ancient genetic structure and cultural transitions in southern African populations

Francesco Montinaro[1†], George BJ Busby[2], Miguel Gonzalez-Santos[1], Ockie Oosthuitzen[3], Erika Oosthuizen[3], Paolo Anagnostou[4,5], Giovanni Destro-Bisol[4,5], Vincenzo L Pascali[5], and Cristian Capelli[1,†]

[1]Department of Zoology,University of Oxford, South Parks Road, Oxford,OX1 3PS, UK
[2]Wellcome Trust Centre for Human Genetics, Oxford, OX3 7BN, UK
[3]School of Medicine, University of Namibia, Windhoek, Namibia
[4]Dipartimento di Biologia Ambientale, Università "La Sapienza", Rome, Italy
[5] Istituto Italiano di Antropologia 00185, Rome, Italy
[6]Institute of Public Health, Catholic University, Largo Francesco Vito 1 00168 Roma
[†]corresponding authors: francesco.montinaro@gmail.com; cristian.capelli@zoo.ox.ac.uk

March 14, 2016

Abstract

The characterization of the structure of southern Africa populations has been the subject of numerous genetic, medical, linguistic, archaeological and anthropological investigations. Current diversity in the subcontinent is the result of complex episodes of genetic admixture and cultural contact between the early inhabitants and the migrants that have arrived in the region over the last 2,000 years, with some of the variation present in the past being now lost as the result of cultural and demographic assimilation by surrounding populations. Here we analyze 1,856 individuals from 91 populations, comprising novel and available genotype data to characterize the genetic ancestry profiles of southern African populations. Combining local ancestry and allele frequency analyses we identify a tripartite, ancient, Khoesan-related genetic structure, which correlates with geography, but not with linguistic affiliation or subsistence strategy. The fine mapping of these components in southern African populations reveals admixture dynamics and episodes of cultural reversion involving several Khoesan groups and highlights different mixtures of ancestral components in Bantu speakers and Coloured individuals.

Introduction

Southern Africa is characterised by substantial spatial and diachronic cultural variation. Archaeologically, the prehistory of this part of the African continent has been characterized by extended regional variation in lithic industries at the interface between the Middle and Later Stone Ages [Mitchell, 2002]. The different stone-based technologies dated to between 19 and 12 thousand years ago (Kya) have been interpreted in the context of long term isolation, while similarities observed among various Robberg industry stone bladelets found in southern Africa suggested some long-range contact [Mitchell, 2002]. The more recent appearance of pastoralism and agriculture has further complicated the cultural profile of this region. Human and livestock remains documented the arrival of herders in the region less than 2 Kya and several disciplines have mapped the local dispersal of agro-pastoralist Bantu speaking populations during the last few centuries. The arrival of European colonists and the subsequent relocation of groups from Asia have added additional complexity to the history of the region. Extended variation can be also observed from a linguistic point of view. Bantu languages are the most commonly spoken in southern Africa, where they have been subdivided into Western and Southern in relation to their geographical distribution. Some of the non-Bantu languages spoken in southern Africa are characterised by click-sounds and are often referred to as Khoesan (here intended as a non-genealogical group of click-containing languages spoken by a variety of southern African herders and hunter-gatherers [Guldemann and Fehn, 2014]. These languages are classified into three major families [Blench, 2006, Guldemann and Fehn, 2014]: the Kx'a, the Taa and the Khoe-Kwadi, and are characterized by broad and overlapping geographic distributions. This cultural complexity extends also to the different subsistence economies implemented by groups who reside in this region, which include hunter-gathering, animal husbandry and agriculture, plus various combinations of these strategies [Barnard, 1992, Murdock, 1981].

From a genetic point of view, Africa hosts most of the worldwide genomic variability [Campbell and Tishkoff, 2010], and some of the earliest branching Y chromosome and mitochondrial DNA lineages are located in the Southern part of the continent [Tishkoff et al., 2007, Rosa and Brehem, 2011, Barbieri et al., 2013b]. Due to their potential significance for the origin of modern humans, groups residing in southern Africa have attracted the attention of both academic researchers and the general public [Batini et al., 2011, Schlebusch et al., 2012, Pickrell et al., 2012, Barbieri et al., 2013b, Gurdasani et al., 2015]. Such interest has capitalized on the advent of new analytical tools which have contributed to a better characterization and understanding of the history of southern African populations [Henn et al., 2012, Schlebusch et al., 2012, Pickrell et al., 2012, 2014, Kim et al., 2014]. Model-based analyses have demonstrated that populations located north of the Kalahari desert, such as Jul'Hoan and !Xun, are characterized by a so called *Northern* component, which is substantially different from that characterizing populations located to the south of the Kalahari (referred to as the *Southern* component; [Schlebusch et al., 2012, Pickrell and Pritchard, 2012]). However, in-depth analyses of Khoesan genetics have suggested a greater degree of complexity within Khoesan-speaking populations. For example, Schlebusch et al. [2012] highlighted the genetic peculiarity of Glui and Gllana individuals when compared with Northern and Southern Khoesan (here referring to the geographic location of Khoesan speaking groups), while Petersen and collaborators [Petersen et al., 2013] suggested additional structure among Northern Khoesan

populations. In addition to this early structure, a signal of West Eurasian ancestry, which predates the arrival of Bantu-speaking farmers, has also been detected [Schlebusch et al., 2012, Pickrell et al., 2014].

Despite several investigations conducted in the past few years, we are still far from a detailed dissection of the genomic structure related to Khoesan speaking populations. Its exhaustive characterization is challenging due to the fact that various ancestral groups have overlapped over the last millennia and that gene-flow has probably been common among groups. In this context, the legacy left by Khoesan populations in highly admixed groups such as southern African Bantu and Coloured populations is far from clear, which makes the design and the interpretation of regional genome-wide association studies challenging [Rosenberg et al., 2010, Price et al., 2010]. The reconstruction of the ancestry profiles of these populations is further complicated by the fact that groups speaking different languages and implementing different lifestyles have been in contact for extended periods of time, prompting genetic and cultural exchange. The ability to rapidly switch to different cultural packages is potentially an effective solution for surviving in challenging environments [Kinahan, 2001].

Here, to further dissect and clarify the genomic stratification of southern African populations, we analyzed 1,856 individuals from 91 populations using a combination of novel and published genome-wide SNP data. By applying a local ancestry deconvolution approach we highlight previously unobserved complexity in the Khoesan-related ancestry components and generate novel insight into the genetic history of the region. We provide evidence for the presence of at least three distinct Khoesan ancestral components and reveal a substantial degree of admixture between Khoesan groups. Our fine dissection of the Khoesan-related legacy in highly admixed populations also reveals slight, but significant genetic structure between Coloured and Bantu-speaking populations, suggesting different admixture histories in these two groups. Finally, we demonstrate that Khoesan-related ancestry structure is highly correlated with the geographic location of populations but not with linguistic affiliations or subsistence strategies.

Results

Complex population structure and mixed ancestry in southern Africa

We described population structure in southern Africa using the "Unlinked Dataset" (See Methods) comprising 1,856 individuals from 91 populations genotyped at 63,767 autosomal markers (Fig.1, Fig. S1). After kinship analysis, 25 individuals were removed dur to the existence of 25 pairs of highly related individuals, ten of which contained individuals genotyped in different studies. We identified 2 Bantu South Africa from the HGDP [Li et al., 2008] with a high kinship index with 2 Herero from Schlebusch et al. [2012], 3 and 5 pairs among the Jul'Hoan (from Pickrell and Pritchard [2012], Petersen et al. [2013] and Schlebusch et al. [2012]) and the Khomani (from Henn et al. [2011] and Schlebusch et al. [2012]), respectively.

To visualize the evolutionary relationships among the analyzed individuals, we used ADMIXTURE [Alexander et al., 2009], varying the prescribed number of clusters, K, from 2 to 20 (Fig. 1, Fig. S2, Fig. S3). At $K = 6$, all African populations are mostly characterized as a mixture of four African-specific components defined by language, geography or ethnicity, representing Khoesan, Niger-Congo,

East African, and rainforest Hunter-Gatherer (Pygmies) populations. Interestingly, the latter is also present in Western and Eastern Bantu populations, and in the Hadza, Sandawe and Maasai from East Africa, possibly reflecting admixture and/or the existence of a geographically extended Pygmy-related ancestral component [Destro-Bisol et al., 2004, Patin et al., 2014].

Among Khoesan groups, signatures of admixture and possible cultural transition are evident in most of the populations. For example the Damara show a high fraction of Bantu-like ancestry components (Fig. 1, Fig. S2, Fig. S3). More generally, almost all of the Khoesan populations show a non-negligible fraction of ancestry components which are modal in East Africa and Europe, consistent with ancient and recent migrations from these regions [Tishkoff et al., 2009, Schlebusch et al., 2012, Pickrell et al., 2014]. At $K = 8$ the Khoesan ancestry component splits into two. One is modal in all the K'xa speaking populations (less in the =Hoan), while the other is common in all the other Khoesan populations, reaching the highest frequency in the Nama and Khomani. At $K = 9$ and $K = 10$ additional Eurasian components emerge, which differentiate from Afro-Asiatic ancestries.

We noted that although the smallest estimated cross-validation (CV) values are found for $K = 9$ and 10 (Fig. S4), analyses performed at higher values of K provide insights into the genomic history and substructure of populations, so we describe these results below. At $K = 12$, the component common in Niger-Congo speaking populations splits into two, one common in Western and Central African populations and in Western Bantu speakers, the other more common in Southeastern Bantu speakers, consistent with these representing the last stage of the Bantu migration process [González-Santos et al., 2015]. Interestingly, this Southeastern Bantu component is present in most Khoesan populations from Botswana and South Africa, providing evidence for admixture during the expansion of Bantu-speaking populations [Marks et al., 2015, González-Santos et al., 2015]. The presence of ancestry related to Western Bantu speakers in some of the Western Khoesan populations such as the Khoe, the Haillom and the Nama, is consistent with their current geographic position and could be interpreted as a signature of admixture events. At $K = 13$, an ancestry component present only in the recently admixed population of South Africa and Namibia (Coloured and Basters) emerges: F_{ST} values of this component suggests genetic similarity with European populations. However, the genetic distance between this ancestry component and the other African components is consistently smaller than the values estimated when using European populations, which suggests that the admixture between African and Eurasian populations generated a novel combination of allele frequencies that is captured by this component (Fig.1, Fig. S2, Fig. S3). At $K = 14$, a component which almost exclusively characterizes Western Bantu populations becomes evident. Interestingly, this component is modal in the Damara, Herero and Himba populations, providing evidence for a common origin [Barbieri et al., 2013a]. In addition, this same ancestral component is at high frequency in all the other Bantu populations, where it is complemented by the presence of the Southeastern Bantu component. At $K = 15$, the Sandawe population differentiates from other groups from Tanzania, Hadza and Maasai.

Local ancestry analysis reveals three distinct Khoesan-related ancestries

Ancestry analysis of Khoesan populations is complicated by the fact that the genomes of most contemporary populations are a mosaic of multiple ancestries [Pickrell et al., 2014, Marks et al., 2015, Busby et al., 2016]. For this reason we performed a Local Ancestry Multi-Dimensional-Scaling (LAMDS) analysis using only genomic fragments assigned with high confidence to Khoesan ancestry ("Khoesan Ancestry dataset", see Methods). Similar methods have previously been successful in assessing the continental legacy of American populations [Moreno-Estrada et al., 2013]. However, such methods rely on a large set of reference populations that can be used as a scaffold for local PCA visualization, which were not available here. We therefore applied a new LAMDS approach, in which an IBS-based distance matrix is generated by comparing only those variants on chromosomal segments identified as being of Khoesan ancestry. This analysis has the advantage of using chromosomes instead of individuals and allows one to plot admixed populations even when there is not a comprehensive reference dataset.

Previous analyses of Khoesan populations suggested the existence of two distinct ancestries, although subsequent investigation has suggested more complex underlying structure [Schlebusch et al., 2012, Pickrell and Pritchard, 2012, Pickrell et al., 2014]. Our LAMDS analysis reveals three main groups of Khoesan-related ancestry (Fig.2A). Specifically, the first group (*Northern* Khoesan) is composed of all the K'xa speaking populations (Jul'Hoan and !Xun) with the exception of the =Hoan. Notably, all these populations live in an area located at the North of the Kalahari. The remaining two groups separate the Nama, Khomani and Karretjie (*Southern* Khoesan) from the remaining Khoesan populations, which define a *Central* Khoesan ancestry component.

We further investigate the presence of the described Khoesan-related three-way genetic structure with a series of analyses of the "Unlinked Dataset". First, we built Maximum Likelihood (ML) trees from allele frequencies using TREEMIX, and tested their robustness with bootstrapping. Khoesan populations (with the exception of the Damara, who were excluded due to their low Khoesan ancestry as reported in Fig. 1B) are separated in three groups, a pattern consistently found across tree bootstraps. The three-way partition described by TREEMIX broadly mirrors the clustering patterns suggested by the ancestry-based analysis (Fig. 2B). The Karretjie seem to be more related to the Khomani than to the Nama, with the Naro acting as an outgroup to these two branches, probably as a result of their own complex pattern of Khoesan ancestry. Similarly, Haillom and Shua form a distinct branch that splits from the other Southern Khoesan. However, the poor support for these branches highlights their complex genetic make-up, which could be the result of interactions and admixture between different Khoesan populations. On the other hand, the split which separates the !Xun samples from the Jul'Hoan is well supported, providing evidence for genetic distinctiveness between these two K'xa populations, an observation which is further suggested by the third component of the PCA analysis of individuals with more than 80% of Khoesan ancestry (see below; Fig. 2B,C).

PCA using individuals with at least 80% Khoesan ancestry provides additional evidence for the Khoesan ancestry tripartition (Fig. 2C). Specifically, the three vertices of the plot recapitulate the ADMIXTURE and TREEMIX analyses, with the three clusters composed by populations defined by different amounts of *Northern*, *Central* and *Southern* Khoesan ancestries. Finally, in the ADMIXTURE analysis

described above, at $K = 16$ three Khoesan-related components emerge, separating all the populations from the central Kalahari area (Botswana) speaking Taa, K'xa and Khoe-Kwadi (*Central* Khoesan) from the K'xa in the North (*Northern* Khoesan) and the Nama, Khomani and Karratije in the South (*Southern* Khoesan). Notably, the F_{ST} values between these three components are similar, suggesting either a deep split (possibly followed by admixture) and/or drastic demographic events, such as bottlenecks or founder effects. Using F_{ST} and sample sizes for Jul'Hoan and Taa [Kim et al., 2014], we estimate a split time of ∼33 Kya (25-43 Kya). It is interesting to note that among the Basters and the Coloured, the *Southern* Khoesan component represents most of the Khoesan-like ancestry, whilst, conversely, in South African and Lesotho Bantu-speaking populations the majority component is *Central* Khoesan. Furthermore, a substantial number of Khoesan populations show a combination of these three ancestries, suggesting extensive admixture in the history of these populations.

Contemporary Khoesan populations contain a mixture of Khoesan-related ancestries

Our LAMDS analysis offers further insight into the relationships between Khoesan groups (Fig. 2A). In fact, chromosomes from several populations seem to be scattered between different clusters, potentially as a result of admixture. For example, Khomani individuals are spread towards groups enriched in *Central* Khoesan ancestry, the Naro and the Jul'Hoan occupy a position intermediate between populations characterized mostly by *Central* and *Northern* components and the Haillom are scattered between individuals with *Northern* and *Southern* Khoesan genetic profiles. With the exception of the Haillom (where all individuals have less than 80% Khoesan ancestry), these results are consistent with our PCA analyses based on the subset of individuals in each group with more than 80% Khoesan ancestry. Moreover, PCA confirms similar patterns to those described above for the Khomani and Naro, which are spread towards groups rich in *Central* and *Northern* Khoesan ancestry, respectively (Fig. 2C). We formally tested for admixture between populations applying, the f_3 analysis on the same dataset, and reported the two most significant comparison for each population in Fig. 2C (all the comparison are reported in Table S3). Significant f_3 statistics provide evidence that these mixed ancestries are the result of admixture between different ancestral Khoesan populations (Table S3, Fig.2C). In the Khomani, for example, the lowest f_3 values are found when considering Taa populations (Table S3, Fig.2C). The Naro show evidence of admixture involving populations close to Jul'Hoan and a central Khoesan population, such as Taa and Glui. The Jul'Hoan also show significant f_3 values when tested against !Xun (*Northern* Khoesan) and Naro (*Central* Khoesan). Similarly, the !Xun also show evidence for admixture with the Jul'Hoan.

Khoesan-related genetic structure in admixed populations

To better visualize the relationships between Khoesan and non-Khoesan populations, including individuals with less than 80% Khoesan ancestry, we plotted all southern African groups with 90% utilization distribution density kernels for the three ancestries, estimated using the KernelUD function in the *adehabitatHR* package [Calenge, 2006]. This approach allows us to explore which of the three ancestry components is present in Bantu-speaking, admixed, and Khoesan groups with high Bantu ancestry, such

as the Khwe and the Damara. The Khwe cluster with the sympatric Jul'Hoan and !Xun populations, although some individuals are located closer to populations mostly containing a *Central* Khoesan component, potentially reflecting a non-negligible degree of admixture. The Damara, conversely, seem to be genetically closer to the Khomani and Nama, although they are scattered towards the K'xa populations in the North, in accordance with their geographic location. Interestingly, we identified two Owambo individuals with genomic features related to Jul'Hoan and !Xun populations.

All of the other Bantu-speaking groups – with the exception of the Kwangali, who are closer to Taa and K'xa speaking groups from Botswana (*Central* Khoesan) – are genetically related to the cluster defined by Nama, Karretjie and Khomani (*Southern* Khoesan). We noted that admixed individuals mapping to this cluster appear to highlight a partly structured distribution, since the Bantu populations are located on the upper side of the distribution, while Coloured and Basters are on the lower side (Fig. 3A). To test this hypothesis we used *mclust* to explore the most supported number of clusters, from 1 to 9 inclusive, using either the MDS coordinates or the IBS distance matrix. We inferred 7 clusters using the MDS coordinates and 9 with the distance matrix and the average probability for each population are shown in Fig. 3B and Fig.S7. The two groups of populations are mostly defined by the same cluster affiliation, although present in different proportions. An additional minor cluster, related to Bantu-speaking populations from Botswana, present in the Southern Bantu populations, is absent in the Coloured and Basters. Such differences are still evident when the complete distance matrix is considered (Fig.S7).

Khoesan-related genetic structure and geography, language and subsistence

We performed a Procrustes analysis to test the relationship between genetic and geographic distances and found a statistically significant correlation (Procrustes correlation= 0.65, $p<0.001$), as previously observed by Schlebusch et al. [2012]. We noted that these authors tested this correlation across a small subset of Khoesan populations. Here we extended this analysis to include not only a larger dataset of Khoesan populations but also Khoesan fragments in highly admixed groups such as southern African Bantu-speaking populations and Coloured. To further investigate the association between geography with the observed Khoesan-related structure and to explore the correlation with cultural variables, we evaluated the power of models predicting the positioning of individuals along the two dimensions of the Khoesan-ancestry IBS-based MDS plot (Fig. 3C) for geography, language, and subsistence. Major reductions in model predictive error, which is indicative of better model fit, are only observed when variables are considered in relation to MDS dimension 1 (Fig.3C), while dimension 2 shows some degree of model prediction reduction only when geography is considered (Fig. 3C). Geography shows the smallest predictive error, and therefore best model-fit, when each variable is singularly considered (geography: 0.000201, language: 0.0007, subsistence1: 0.0007, subsistence2: 0.000652). Although the predictive power of the analysis is improved when multiple variable are considered, the reduction of cross validation error is minimal (Geography + Language: 0.0002, Geography + Subsistence1: 0.000199 , Geography + Subsistence2: 0.000179, Geography + Language + Subsistence1: 0.000192 , Geography + Language + Subsistence2: 0.000177). These results hint at the existence of a geographically-described ancient complexity in Khoesan ancestry which likely pre-dates the arrival of Bantu and European populations,

and which is only marginally captured by current ethno-linguistic population descriptors.

Discussion

The genetic characterization of populations from the African continent is crucial from an epidemiological, pharmacological, anthropological and evolutionary perspective. Within the continent, southern Africa displays an impressive degree of genetic and cultural diversity, this being a region where groups speak several different languages and implement a variety of different subsistence strategies. From a linguistic point of view, Khoesan languages are unique to this region and are divided in three major families: K'xa, Khoe-Kwadi, and Taa. While the separate grouping of K'xa and Taa speakers has reached a consensus among linguists, the internal structure of the Khoe-Kwadi family is still debated. The most heterogeneous of the three linguistic groups, Khoe-Kwadi, is usually classified into three subgroups; East (spoken by Thswa and Shua), West (Khwe, Glui,Gllana and Naro) and Khoekhoe, which is currently spoken by the Nama, Damara, and Haillom populations [Guldemann and Fehn, 2014]. The history of Khoekhoe populations still remains unresolved; for example, the Haillom and Damara have previously been classified as 'other bushmen' given their phenotypic and/or linguistic/cultural peculiarities. The Haillom live in Northern Namibia, and they are thought to be !Xun individuals that have recently acquired the Nama language. The Damara – who were sometimes referred to as BergDama or BergDamara – live in Northern Namibia and their origins are also unclear. Including both herders and foragers, the ancestral population probably arrived in the area before the Nama and Western Bantu populations, such as Herero and Owambo. The arrival of the Nama pastoralists in the Namibia region from an area in the South African Northern Cape (Namaqualand) is a recent event dating to the end of the 19th century [Barnard, 1992]. The first pastoralist populations described by Dutch colonists in the 17th Century – initially referred to as "Hottentots" – were Khoekhoe-speakers. They are usually referred to as the Cape-Khoekhoe and !Ora people (who were previously indicated as Korana [Barnard, 1992]) but their genetic relationships with other extant populations are obscure, as they became "extinct" soon after the arrival of the Europeans. Little is also known about the Taa speaking populations that inhabited the Southernmost area of southern Africa such as the /Xam, the /Xegwi and the Baroa (the latter sometimes referred to as the Mountain bushmen, located in and around the Maloti/Drakensberg mountain range in South Africa/Lesotho), which probably spoke a language similar to the Khomani (of the !Ui group) and who were soon assimilated into Bantu populations who settled in the area. The Karretjie people of South Africa are often considered as the descendants of the /Xam. Given this complex process of contacts and admixture, it is expected that the analysis of admixed populations may help to revive the genetic ancestry of such "vanished" communities and therefore provides a description of the genomic landscape pre-dating the arrival of Bantu speaking populations and European colonists.

Our analysis provides crucial insights into the unsolved histories described above, and more generally on the populations living in the region. Firstly, all of the approaches exploited here point to the existence of an ancient tripartite genetic structure in southern Africa populations, dating back to around 33 Kya (25-43 Kya, Fig. 1B,Fig. 2, Fig. 3,Fig. S4); these dates are in line with previous estimates for the separation of the two Khoesan components reported earlier [Schlebusch et al., 2012, Pickrell et al.,

2012, Kim et al., 2014] and close to the start of Marine Isotope Stage 2 and the beginning of the Last Glacial Maximum, whose impact on the distribution of resources might have triggered such differentiation [Mitchell, 2002]. The first ancestry that we identified – *Northern* Khoesan – mainly comprises Jul'Hoan and !Xun individuals, who live in the Northern Kalahari area. Our TREEMIX and PCA analyses suggest that the two populations are modestly genetically distinct, underlining further structure within this component (Fig. 2B,C). Interestingly, the Khoe-Kwadi speaking Khwe, whose genetic ancestry is mostly Bantu-related, and the Haillom, share Khoesan genetic affinity with these populations, as expected given their geographic proximity (Fig. 3A). Their genomes also contain the *Central* Khoesan component, which suggests that further admixture with populations with such ancestry may have occurred.

The second ancestry component is common across all the populations in the central Kalahari area (indicated here as the *Central* Khoesan component), and includes all the Taa populations, with the exception of the Khomani (*Southern* Khoesan), but including West and East Khoe-Kwadi speakers and the K'xa speaking population =Hoan, which once again highlights the mismatch between genetics and linguistic affiliation in populations from the region [Schlebusch et al., 2012]. This component has not been reported before, although Schlebusch et al. [2012] noticed the unique behavior of Glui and Gllana indviduals. The inclusion of a more representative set of populations in the current analysis, a few of which are characterized by this Khoesan component, together with a focus on the Khoesan-specific genetic components has led to the secure identification and further characterization of this key element of Khoesan-related ancestry.

The third *Southern* Khoesan component is mainly represented by a set of linguistically heterogeous but geographically proximate populations: the Khomani (Taa speakers), Karretjie, and Nama (Khoe-Kwadi). All of these populations are thought to have originated in the Northern Cape [Barnard, 1992]. Barnard considered "the Khoekhoe and the Bushmen [of the Cape area] as members of a single regional unit, separate from the other (black and white) peoples of the subcontinent" [Barnard, 1992]. This is in agreement with our findings of substantial genetic similarities between these groups, despite their different cultural affiliations. Taken together, this suggests that cultural diffusion – in the absence of significant gene-flow – might have played an important role in the spread of pastoralism and possibly Khoe languages in southern Africa [Sadr, 1998, Barnard, 2007, Barham and Mitchell, 2008, Schlebusch et al., 2012]. The Khoesan-like genetic ancestry of the Khoe-Kwadi speaking Damara maps to this component, which is consistent with their long-term interaction with the Nama, who speak a very similar language [Guldemann and Fehn, 2014], possibly coupled with admixture with K'xa populations living in the same area (as suggested by the occurrence of the *Northern* Khoesan component in their genetic make-up). All the Coloured and the Bantu populations from the Southernmost part of the continent (South Africa and Lesotho) are characterized by the *Southern* Khoesan component (Fig. 3A), suggesting an overall broad homogeneity in Khoesan ancestry over this specific region. However, it is worth noting that several Bantu individuals in the LAMDS plot are slightly deviated toward central Khoesan populations, and that Bantu populations show substantial differences when compared to Coloured individuals, as our cluster analyses based on MDS and IBS distances suggest. Given their different geographical distribution, such observations could be explained by the existence of additional Khoesan structure in the region and the past presence of differentiated groups around the Lesotho/Drakensberg area (assimilated by local

Bantu speaking groups), or by admixture between Bantu speaking population and groups characterized by *Central* and *Southern* Khoesan ancestries [Busby et al., 2016].

Interestingly, the tripartition observed in the Khoesan ancestry does not recapitulate cultural affiliation (Fig. 3C). As described above, we in fact identified several instances of populations speaking related languages grouping with speakers of unrelated languages and groups of populations that are characterized by different lifestyles. When we predicted genetic similarity among individuals from geography, predictive error was substantially lower than that of subsistence strategy or linguistic affiliation, both marginally improving the predictive power when considered together with geography. Extensive admixture and cultural transition appears to have characterized populations from this area. Similar scenarios have been proposed also for Europe [Lazaridis et al., 2014, Haak et al., 2015] and Madagascar [Pierron et al., 2014], suggesting a common process across human populations.

Our ADMIXTURE analysis of Niger-Congo-speaking populations (which includes Bantu speakers) identified five different ancestral components broadly consistent with their geographic location (Fig. 1B). Specifically, beside the two non-Bantu Niger-Congo related ancestries, which separate Nigeria from Senegal and populations from The Gambia, we identified three Bantu components that are represented in Eastern, South-Eastern and Western Africa. Interestingly, the latter is modal in the Damara, and in the pastoralist Bantu-speaking Herero and Himba, but not in other Bantu-speaking groups of the region (Mbukushu, Owambo and Kwangali). This component is slightly more related to West Africa than the Eastern and South-Eastern Niger-Congo components, which may be explained by different waves of Bantu colonists into southern Africa, as suggested in a recent survey of African genetic history based on haplotype analyses [Busby et al., 2016] and possibly related to the Early Iron Age settlers who arrived in the mid-1st millennium AD [Diamond, 1997].

Conclusions

The genetic structure of southern African populations is complicated by the existence of ancient population structure, onto which several layers of additional genetic ancestries have been overimposed over the last few centuries. Here, we demonstrate that local ancestry approaches can be used to tease apart the genetic structure of such ancient components, characterizing their relationships and current distribution, further supporting a role for widespread admixture in human history [Patterson et al., 2012, Hellenthal et al., 2014, Busby et al., 2015, Montinaro et al., 2015]. Further insights are expected to be collected by the molecular investigation of archaeological human remains [Llorente et al., 2015, Morris et al., 2014]. Beyond the obvious historical and archaeological implications for the reconstruction of the subcontinent dynamics, these observations are of relevance for anthropological studies as well as for epidemiological and translational applications (for example, in the design of genome-wide association studies).

Materials and Methods

Datasets

New data

We generated novel genotype data for 60 individuals from seven southern African populations collected in Namibia and Lesotho. Forty-four of these individuals from four Bantu speaking groups [MbukushuM, OvamboM, Kwangali, Sotho] and a Khoesan-speaking group [NamaM] have been published previously [González-Santos et al., 2015]. Eight individuals each from the Damara and Haillom, collected in the Khorixas and Etosha areas of Namibia, respectively, are presented here for the first time. Detailed information about the collecting process and samples are available elsewhere [Marks et al., 2012, 2015, González-Santos et al., 2015]. Full ethical approval for the collections was provided by the Oxford Tropical Research Ethics Committee (OxTREC), the Lesotho Ministry of Health and Social Welfare, the Lesotho Ministry of Local Government, the Lesotho Ministry of Tourism, Environment and Culture, and the Namibian Ministry of Health and Social Services. The Nama, Owambo and Sotho populations were genotyped on the Illumina Human 610-Quad BeadChip (Illumina, San Diego, CA, USA), while the Haillom, Kwangali, Damara and Mbukushu were genotyped on the Human Omni5-Quad BeadChip (Illumina, San Diego, CA, USA). All the data are available at (`https://capelligroup.wordpress.com/data/`).

Existing datasets

Our analyses focus on southern African populations. We therefore merged our data with an additional 31 Khoesan-speaking and 20 "admixed" and Bantu-speaking populations from Li et al. [2008], Consortium [2010], Henn et al. [2012], Schlebusch et al. [2012], Pickrell et al. [2012], Petersen et al. [2013], Pickrell et al. [2014], Lazaridis et al. [2014] (Fig. 1,Fig. S1,Table S1). Additional data from outside of southern Africa were taken from populations with European, African and Middle East ancestry, genotyped on different Illumina platforms and the Affymetrix Axiom Genome-Wide Human Origins 1 array (Fig. 1, Fig. S1, Table S1) [Li et al., 2008, Consortium, 2010, 2012, Patterson et al., 2012, May et al., 2013]. Our final dataset comprised 1,856 individuals from 91 populations (Fig. 1a, Table S1).

Process for merging datasets

Because the genotype data described above came from multiple different platforms and studies, we performed a systematic pipeline for merging the data, keeping the Illumina and Affymetrix data separated. Each dataset was pre-processed removing markers and individuals with a missing rate higher than 10% using PLINK 1.9 [Chang et al., 2015]. Marker positions were lifted to build 37 human genetic map using data provided by Illumina and Affymetrix and all the non-autosomal markers were excluded from the analysis. Specifically, we first merged all the datasets genotyped on the same platform, discarding individuals and markers with a call rate lower than 98% and excluding SNPs with G/C or A/T mutations, which could lead to errors in the merging procedure. We next used the KING software to infer

kinship [Manichaikul et al., 2010], and randomly removed one individual from pairs with a kinship rate higher than 0.0884. The resulting platform-specific datasets comprise 250,547 (Illumina) and 498,140 (Affymetrix) markers, respectively.

The Unlinked Dataset

To maximise the number of populations analyzed (at the expense of SNP density) we merged all data collected from all studies into a large dataset, which we refer to as the "Unlinked Dataset". The two platform-specific datasets, comprising 250,547 (Illumina) and 498,140 (Affymetrix) markers, were merged on physical position to avoid unnecessary loss of markers due to mismatching IDs on different platforms. Following this merge, we again performed the same quality control and removal of relatives described above obtaining a final dataset containing 1,856 individuals genotyped at 63,767 SNPs.

The Khosean Ancestry dataset

To maintain a high density of SNPs for local ancestry analyses, we analyzed the quality controlled Illumina and Affymetrix datasets separately. For each of the two platform-based datasets described above, we computationally phased the genotype data to generate haplotypes using SHAPEITv2 [Delaneau et al., 2012, 2013] with the human genome build 37 recombination map downloaded from the SHAPEIT website `https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html#gmap`. We generated a second dataset ("The Khosean Ancestry dataset"), by initially removing from each platform-specific dataset (Illumina and Affymetrix) Non-Khoesan genomic fragments as identified using PCAdmix [Henn et al., 2012]. In brief, PCAdmix builds a PCA space based on reference panels, and projects tested genomic chunks on it. Subsequently, the probability of a given ancestry for a selected chromosomal chunk is estimated from PC loadings, and a Hidden Markov Model is then applied to refine them. In the current context, we estimated local ancestry likelihoods in 1 cM windows, using Yoruba, Jul'Hoan, and CEU individuals as ancestry donors. Given the recent West Eurasian genomic component documented in the Jul'Hoan populations as the result of admixture with non-Khoesan populations [Pickrell and Pritchard, 2012, Hellenthal et al., 2014, Busby et al., 2016], only individuals with more than 99% of the "Khoesan component" – as estimated by the $K = 3$ ADMIXTURE run described above – were considered as donors, with the remaining individuals used as target individuals. The final number of Jul'Hoan individuals used as ancestry donors was 28 and 26 in the Illumina and Affymetrix datasets, respectively. To minimise the impact of chunks with mixed ancestry, we post-processed inferred local ancestry estimates by retaining only those windows with a ancestry probability >99%. In addition, we only analyzed individuals characterized genome-wide by more than 35% of the tested ancestry (as for ADMIXTURE analysis for $K = 3$; see below). We used a custom-made PYTHON script (*MaskMix*, available at: `https://capelligroup.wordpress.com/scripts/`), to extract the Khoesan Specific Fragments (KSF) inferred from the post-processing described above. *MaskMix* considers each individual as homozygous and composed by one chromosome per pair only, from which high confidence KSFs were extracted and analysed. To allow the comparison between individuals genotyped using arrays from different providers, the resulting two datasets were pruned to retain markers which overlapped between the Illumina and

Affymetrix datasets and that were located on khoesan-specific genomic fragments. Finally, we removed all the individuals for which less than 10% of the total number of overlapping SNPs were retained. The resulting dataset is composed of a total of 63,767 markers and 787 individuals. Given the variation in Khoesan ancestry in different individuals, the average number of retained SNPs per individual was 22,442 (median 19,887; range 5,457-50,643). We refer to this final set of SNPs selected as described above as the "Khoesan Ancestry dataset".

Statistical Analyses

Population structure

We applied both model-based and non-parametric clustering approaches to describe population structure in the Unlinked Dataset. First, we used the ADMIXTURE [Alexander et al., 2009] maximum likelihood algorithm to estimate the individual-level ancestry, applying the author's cross validation procedure and a random seed, for all values of $K = \{2...20\}$. After post-processing the ADMIXTURE output with DISTRUCT [Rosenberg, 2004], we plotted the results using the R statistical programming software and a modified version of *polarHistogram* function from the *phenotypic phorest* package (`http://chrisladroue.com/phorest/`). For $K = 20$, we computed pairwise F_{ST} [Holsinger and Weir, 2009] for each of the K ancestral components as implemented by ADMIXTURE, visualizing the distances between the components with a heatmap using the *pheatmap* [Kolde, 2015] package. Ancestry components were additionally clustered through a complete hierarchical approach [Everitt and Britain), 1980]. We estimated splitting time between the three Khoesan components using F_{ST} and effective population size using the following formula[Holsinger and Weir, 2009, Henn et al., 2012]: $1 - F_{ST} = (1 - \frac{1}{2Ne})^t$.

Principal Components Analysis (PCA) was performed using PLINK 1.9 [Chang et al., 2015]. To focus on the structure of Khoesan populations, we selected only those individuals characterized by more than 80% of the "Khoesan" ancestral component as estimated from the $K = 3$ ADMIXTURE analysis described above; we define the "Khoesan" component as the major ancestry present in Jul'Hoan individuals. We refer to this dataset at the "80% Khoesan dataset". Populations containing the same subset of individuals were tested for admixture using f_3 statistics [Reich et al., 2010, Patterson et al., 2012] considering all three-populations combinations (3990 combinations, Table S3).

TREEMIX Analysis

A maximum likelihood tree describing the relationships between Khoesan populations was inferred using allele frequency distributions implemented in the TREEMIX software [Pickrell and Pritchard, 2012, Pickrell et al., 2012]. Given the high complexity of the original dataset, we selected 35 populations to represent all the Khoesan populations and a subset of African and European populations ("TreeMix populations"). We used a chimpanzee outgroup using genome data available in [Patterson et al., 2012], and accounted for LD by jack-knifing over blocks of 500 SNPs, as suggested by the authors in [Pickrell and Pritchard, 2012]. The robustness of the resulting tree was tested by performing 100 bootstrap runs and estimating branch support using DENDROPY software [Sukumaran and Holder, 2010].

We performed ten different runs using different random seeds (Fig.S6), and report the tree with the maximum support in Fig.2B. To visualize only the Khoesan ancestry and to remove the confounding factors of admixture, we informed TREEMIX of existing relationships between Khoesan and non Khoesan populations using the cor_mig and climb commands (Table S2). The amount of genetic ancestry shared between populations was estimated through the $K = 3$ ADMIXTURE run described above. It is important to note that these estimates are not fixed values, but are used by the algorithm as starting points to infer the maximum likelihood estimates. [Pickrell et al., 2014].

Population structure inference using the Khosean Ancestry dataset

Referring to the Khosean Ancestry dataset, we estimated pairwise (1-IBS) genetic distance with PLINK v1.9, correcting for missing data, and summarized relationships with a Multi-Dimensional Scaling (MDS) plot, using the *cmdscale* function in *R*(Fig. 2A, Fig. 3A). We corrected the inferred IBS-based distances using the formula $\frac{ibs}{1-\sum md}$, where md is the number of missing data in the pair of individuals analyzed. Furthermore, after visual inspection, we removed 25 outlier chromosomes (1 chromosome each from AmaXhosa, Basters, ColouredEC, ColouredWellington, Khwe; 2 from SouthAfricaBantuS and Sotho; 3 from Tswana and Kgalagadi, 8 from SouthAfricaBantuMa). Distances are computed using the number of SNPs shared across pairs of individuals, which differs across pairs given the variation across individuals in the number of SNP markers found on khoesan genomic fragments. The average number of markers used for individual to individual comparisons is 9,310 (median= 6404, range= 111-46,419).

Structure and distribution of Khoesan ancestry in southern African populations

To assess the presence of ancient structure in Khoesan speaking populations and the existence of different Khoesan ancestry in admixed Bantu and Coloured individuals, we explored the MDS coordinates and IBS distance matrix. Firstly, we used the MDS coordinates and IBS distances to group all individuals into N different clusters, for values of $N = \{1...9\}$. We next used the average probability that each individual from the different groups belonged to one of the 7 and 9 inferred most supported number of clusters, respectively, and visualized the probabilities into population- and affiliation-based barplots. Finally, to test for a correlation between genetic and geographic distances, we performed a Procrustes test [Peres-Neto and Jackson, 2001] as implemented in the package *ade4* [Dray and Dufour, 2007, Dray et al., 2007], with 1000 bootstrap iterations between geographic and MDS (genetic) coordinates.

Estimating predicting power for geographic location, linguistic affiliation and type of subsistence

In order to assess the role of geography, subsistence and language on genetic variation of Khoesan populations, we performed regression analysis of LAMDS first two components versus all the other variables, singularly or combined ("Geography + Language", "Geography + Subsistence" and "Geography + Language + Subsistence"; Table S4). All the combinations were tested trough a 5-folded cross validation analysis in which the dataset was split in 5 random subset. Each of these subsets has been then tested against the other four and the combined error recorded and show in a barplot (Fig. 3C). Two alternative

subsistence affiliation lists were used to take into account uncertainty in designation or the co-existence of multiple subsistence strategies. In details "Subsistence 1" has been annotated according to Schlebusch et al. [2012] and Barnard [1992]. In order to take into account the multiple and/or uncertain subsistences in Damara and admixed populations, we used an alternative list ("Subsistence 2") in which these groups were indicated as Hunter-Gatherer/Herder and farmers, respectively (Table S4).

Acknowledgments

This work was funded by Leverhulme Trust Research Project Grant and supported by the Wenner-Gren Foundation, the University of Oxford Boise Fund, the John Fell Oxford University Press (OUP) Research Fund, and Fundação para a Ciência e Tecnologia (grant number SFRH/BD/90648/2012 to M.G.-S.). We thank all the people who donated their DNA samples making this work possible and all the various people and institutions that helped with the organization of the fieldwork and the collection of the samples. We are grateful to Joe Pickrell and David Reich for the sharing of southern Africa genetic data; Hie Lie Kim and Stephan C. Schuster for making available their PMSC results. We are grateful to Peter Mitchell for the suggestions on archaeological patterns in southern Africa.

Authors contributions

F.M. and C.C. conceived the study; F.M., G.B.J.B and M.G.S. performed the analyses; O.O. and E.O. provided support for the collection of DNA samples; P.A., G.D.B. and V.P. contributed to the genotyping of a subset of the novel samples presented here; F.M, G.B.J.B and C.C. wrote the manuscript with the contribution of all the other authors. All the authors read and approved the manuscript.

References

David H. Alexander, John Novembre, and Kenneth Lange. Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, July 2009. ISSN 1088-9051, 1549-5469. doi: 10.1101/gr.094052.109.

Chiara Barbieri, Anne Butthof, Koen Bostoen, and Brigitte Pakendorf. Genetic perspectives on the origin of clicks in Bantu languages from southwestern Zambia. *European Journal of Human Genetics*, 21(4):430–436, April 2013a. ISSN 1018-4813. doi: 10.1038/ejhg.2012.192.

Chiara Barbieri, Mário Vicente, Jorge Rocha, Sununguko W. Mpoloka, Mark Stoneking, and Brigitte Pakendorf. Ancient substructure in early mtDNA lineages of southern Africa. *American Journal of Human Genetics*, 92(2):285–292, February 2013b. ISSN 1537-6605. doi: 10.1016/j.ajhg.2012.12.010.

Lawrence Barham and Peter Mitchell. *The First Africans: African Archaeology from the Earliest Toolmakers to Most Recent Foragers*. Cambridge University Press, June 2008. ISBN 9780521847964.

Alan Barnard. *Hunters and Herders of Southern Africa: A Comparative Ethnography of the Khoisan Peoples*. Cambridge University Press, February 1992. ISBN 978-0-521-42865-1.

Alan Barnard. *Anthropology and the Bushman*. Berg, June 2007. ISBN 978-1-84520-429-7.

Chiara Batini, Gianmarco Ferri, Giovanni Destro-Bisol, Francesca Brisighelli, Donata Luiselli, Paula Sánchez-Diz, Jorge Rocha, Tatum Simonson, Antonio Brehm, Valeria Montano, Nasr Eldin Elwali, Gabriella Spedini, María Eugenia D'Amato, Natalie Myres, Peter Ebbesen, David Comas, and Cristian Capelli. Signatures of the preagricultural peopling processes in sub-Saharan Africa as revealed by the phylogeography of early Y chromosome lineages. *Molecular Biology and Evolution*, 28(9):2603–2613, September 2011. ISSN 1537-1719. doi: 10.1093/molbev/msr089.

R. Blench. *Archaeology, Language, and the African Past*. Rowman Altamira, 2006. ISBN 978-0-7591-0466-2.

George Busby, Gavin Band, Quang Si Le, Muminatou Jallow, Edith Bougama, Valentina Mangano, Lucas Amenga-Etego, Anthony Emil, Tobias Apinjoh, Carolyne Ndila, Alphaxard Manjurano, Vysaul Nyirongo, Ogobara Doumbo, Kirk Rockett, Domnic Kwiatkowski, Chris Spencer, and The Malaria Genomic Epidemiology Network. Admixture into and within sub-Saharan Africa. *bioRxiv*, page 038406, February 2016. doi: 10.1101/038406.

George B. J. Busby, Garrett Hellenthal, Francesco Montinaro, Sergio Tofanelli, Kazima Bulayeva, Igor Rudan, Tatijana Zemunik, Caroline Hayward, Draga Toncheva, Sena Karachanak-Yankova, Desislava Nesheva, Paolo Anagnostou, Francesco Cali, Francesca Brisighelli, Valentino Romano, Gerard Lefranc, Catherine Buresi, Jemni Ben Chibani, Amel Haj-Khelil, Sabri Denden, Rafal Ploski, Pawel Krajewski, Tor Hervig, Torolf Moen, Rene J. Herrera, James F. Wilson, Simon Myers, and Cristian Capelli. The Role of Recent Admixture in Forming the Contemporary West Eurasian Genomic Landscape. *Current biology: CB*, 25(19):2518–2526, October 2015. ISSN 1879-0445. doi: 10.1016/j.cub.2015.08.007.

Clément Calenge. The package "adehabitat" for the R software: A tool for the analysis of space and habitat use by animals. *Ecological Modelling*, 197(3–4):516–519, August 2006. ISSN 0304-3800. doi: 10.1016/j.ecolmodel.2006.03.017. URL `http://www.sciencedirect.com/science/article/pii/S0304380006001414`.

Michael C. Campbell and Sarah A. Tishkoff. The Evolution of Human Genetic and Phenotypic Variation in Africa. *Current biology : CB*, 20(4):R166–R173, February 2010. ISSN 0960-9822. doi: 10.1016/j.cub.2009.11.050.

Christopher C. Chang, Carson C. Chow, Laurent CAM Tellier, Shashaank Vattikuti, Shaun M. Purcell, and James J. Lee. Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience*, 4:7, 2015. ISSN 2047-217X. doi: 10.1186/s13742-015-0047-8.

The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1,092 human genomes. *Nature*, 491(7422):56–65, November 2012. ISSN 0028-0836. doi: 10.1038/nature11632.

The International HapMap 3 Consortium. Integrating common and rare genetic variation in diverse human populations. *Nature*, 467(7311):52–58, September 2010. ISSN 0028-0836. doi: 10.1038/nature09298.

Olivier Delaneau, Jonathan Marchini, and Jean-François Zagury. A linear complexity phasing method for thousands of genomes. *Nature Methods*, 9(2):179–181, 2012. ISSN 1548-7091. doi: 10.1038/nmeth.1785.

Olivier Delaneau, Jean-Francois Zagury, and Jonathan Marchini. Improved whole-chromosome phasing for disease and population genetic studies. *Nature Methods*, 10(1):5–6, January 2013. ISSN 1548-7091. doi: 10.1038/nmeth.2307.

Giovanni Destro-Bisol, Valentina Coia, Ilaria Boschi, Fabio Verginelli, Alessandra Cagliá, Vincenzo Pascali, Gabriella Spedini, and Francesc Calafell. The analysis of variation of mtDNA hypervariable region 1 suggests that Eastern and Western Pygmies diverged before the Bantu expansion. *The American naturalist*, 163(2):212–226, February 2004. ISSN 0003-0147. doi: 10.1086/381405.

Jared M Diamond. *Guns, germs, and steel: the fates of human societies*. W.W. Norton & Co., New York, 1997. ISBN 0393038912 9780393038910 0393317552 9780393317558.

S. Dray and A.B. Dufour. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22(4):1–20, 2007.

S. Dray, A.B. Dufour, and D. Chessel. The ade4 package-II: Two-table and K-table methods. *R News*, 7 (2):47–52, 2007.

Brian Everitt and Social Science Research Council (Great Britain). *Cluster analysis*. published on behalf of the Social Science Research Council by Heinemann Educational Books, August 1980. ISBN 978-0-470-26991-6.

Miguel González-Santos, Francesco Montinaro, Ockie Oosthuizen, Erica Oosthuizen, George B. J. Busby, Paolo Anagnostou, Giovanni Destro-Bisol, Vincenzo Pascali, and Cristian Capelli. Genome-Wide SNP Analysis of Southern African Populations Provides New Insights into the Dispersal of Bantu-Speaking Groups. *Genome Biology and Evolution*, 7(9):2560–2568, September 2015. ISSN , 1759-6653. doi: 10.1093/gbe/evv164.

Tom Guldemann and Anne-Maria Fehn, editors. *Beyond 'Khoisan': Historical relations in the Kalahari Basin*. John Benjamins Publishing Company, Amsterdam ; Philadelphia, August 2014. ISBN 978-90-272-4849-7.

Deepti Gurdasani, Tommy Carstensen, Fasil Tekola-Ayele, Luca Pagani, Ioanna Tachmazidou, Konstantinos Hatzikotoulas, Savita Karthikeyan, Louise Iles, Martin O. Pollard, Ananyo Choudhury, Graham R. S. Ritchie, Yali Xue, Jennifer Asimit, Rebecca N. Nsubuga, Elizabeth H. Young, Cristina Pomilla, Katja Kivinen, Kirk Rockett, Anatoli Kamali, Ayo P. Doumatey, Gershim Asiki, Janet Seeley, Fatoumatta Sisay-Joof, Muminatou Jallow, Stephen Tollman, Ephrem Mekonnen, Rosemary Ekong, Tamiru Oljira, Neil Bradman, Kalifa Bojang, Michele Ramsay, Adebowale Adeyemo, Endashaw Bekele, Ayesha Motala, Shane A. Norris, Fraser Pirie, Pontiano Kaleebu, Dominic Kwiatkowski, Chris Tyler-Smith, Charles Rotimi, Eleftheria Zeggini, and Manjinder S. Sandhu. The African Genome Variation Project shapes medical genetics in Africa. *Nature*, 517(7534):327–332, January 2015. ISSN 0028-0836. doi: 10.1038/nature13997.

Wolfgang Haak, Iosif Lazaridis, Nick Patterson, Nadin Rohland, Swapan Mallick, Bastien Llamas, Guido Brandt, Susanne Nordenfelt, Eadaoin Harney, Kristin Stewardson, Qiaomei Fu, Alissa Mittnik, Eszter Bánffy, Christos Economou, Michael Francken, Susanne Friederich, Rafael Garrido Pena, Fredrik Hallgren, Valery Khartanovich, Aleksandr Khokhlov, Michael Kunst, Pavel Kuznetsov, Harald Meller, Oleg Mochalov, Vayacheslav Moiseyev, Nicole Nicklisch, Sandra L. Pichler, Roberto Risch, Manuel A. Rojo Guerra, Christina Roth, Anna Szécsényi-Nagy, Joachim Wahl, Matthias Meyer, Johannes Krause, Dorcas Brown, David Anthony, Alan Cooper, Kurt Werner Alt, and David Reich. Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature*, 522(7555):207–211, June 2015. ISSN 0028-0836. doi: 10.1038/nature14317.

Garrett Hellenthal, George B. J. Busby, Gavin Band, James F. Wilson, Cristian Capelli, Daniel Falush, and Simon Myers. A Genetic Atlas of Human Admixture History. *Science*, 343(6172):747–751, February 2014. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.1243518.

Brenna M. Henn, Christopher R. Gignoux, Matthew Jobin, Julie M. Granka, J. M. Macpherson, Jeffrey M. Kidd, Laura Rodríguez-Botigué, Sohini Ramachandran, Lawrence Hon, Abra Brisbin, and others. Hunter-gatherer genomic diversity suggests a southern African origin for modern humans. *Proceedings of the National Academy of Sciences*, 108(13):5154–5162, 2011.

Brenna M. Henn, Laura R. Botigué, Simon Gravel, Wei Wang, Abra Brisbin, Jake K. Byrnes, Karima Fadhlaoui-Zid, Pierre A. Zalloua, Andres Moreno-Estrada, Jaume Bertranpetit, Carlos D. Bustamante,

and David Comas. Genomic Ancestry of North Africans Supports Back-to-Africa Migrations. *PLoS Genet*, 8(1):e1002397, January 2012. doi: 10.1371/journal.pgen.1002397.

Kent E. Holsinger and Bruce S. Weir. Genetics in geographically structured populations: defining, estimating and interpreting FST. *Nature Reviews Genetics*, 10(9):639–650, September 2009. ISSN 1471-0056. doi: 10.1038/nrg2611.

Hie Lim Kim, Aakrosh Ratan, George H. Perry, Alvaro Montenegro, Webb Miller, and Stephan C. Schuster. Khoisan hunter-gatherers have been the largest population throughout most of modern-human demographic history. *Nature Communications*, 5:5692, December 2014. doi: 10.1038/ncomms6692.

John Kinahan. *Pastoral Nomads of the Namib Desert: The People History Forgot*. Namibia Archaeological Trust, 2001. ISBN 978-99916-779-1-0.

Raivo Kolde. pheatmap: Pretty Heatmaps, December 2015.

Iosif Lazaridis, Nick Patterson, Alissa Mittnik, Gabriel Renaud, Swapan Mallick, Karola Kirsanow, Peter H. Sudmant, Joshua G. Schraiber, Sergi Castellano, Mark Lipson, Bonnie Berger, Christos Economou, Ruth Bollongino, Qiaomei Fu, Kirsten I. Bos, Susanne Nordenfelt, Heng Li, Cesare de Filippo, Kay Prüfer, Susanna Sawyer, Cosimo Posth, Wolfgang Haak, Fredrik Hallgren, Elin Fornander, Nadin Rohland, Dominique Delsate, Michael Francken, Jean-Michel Guinet, Joachim Wahl, George Ayodo, Hamza A. Babiker, Graciela Bailliet, Elena Balanovska, Oleg Balanovsky, Ramiro Barrantes, Gabriel Bedoya, Haim Ben-Ami, Judit Bene, Fouad Berrada, Claudio M. Bravi, Francesca Brisighelli, George B. J. Busby, Francesco Cali, Mikhail Churnosov, David E. C. Cole, Daniel Corach, Larissa Damba, George van Driem, Stanislav Dryomov, Jean-Michel Dugoujon, Sardana A. Fedorova, Irene Gallego Romero, Marina Gubina, Michael Hammer, Brenna M. Henn, Tor Hervig, Ugur Hodoglugil, Aashish R. Jha, Sena Karachanak-Yankova, Rita Khusainova, Elza Khusnutdinova, Rick Kittles, Toomas Kivisild, William Klitz, Vaidutis Kučinskas, Alena Kushniarevich, Leila Laredj, Sergey Litvinov, Theologos Loukidis, Robert W. Mahley, Béla Melegh, Ene Metspalu, Julio Molina, Joanna Mountain, Klemetti Näkkäläjärvi, Desislava Nesheva, Thomas Nyambo, Ludmila Osipova, Jüri Parik, Fedor Platonov, Olga Posukh, Valentino Romano, Francisco Rothhammer, Igor Rudan, Ruslan Ruizbakiev, Hovhannes Sahakyan, Antti Sajantila, Antonio Salas, Elena B. Starikovskaya, Ayele Tarekegn, Draga Toncheva, Shahlo Turdikulova, Ingrida Uktveryte, Olga Utevska, René Vasquez, Mercedes Villena, Mikhail Voevoda, Cheryl A. Winkler, Levon Yepiskoposyan, Pierre Zalloua, Tatijana Zemunik, Alan Cooper, Cristian Capelli, Mark G. Thomas, Andres Ruiz-Linares, Sarah A. Tishkoff, Lalji Singh, Kumarasamy Thangaraj, Richard Villems, David Comas, Rem Sukernik, Mait Metspalu, Matthias Meyer, Evan E. Eichler, Joachim Burger, Montgomery Slatkin, Svante Pääbo, Janet Kelso, David Reich, and Johannes Krause. Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature*, 513(7518):409–413, September 2014. ISSN 0028-0836. doi: 10.1038/nature13673.

Jun Z. Li, Devin M. Absher, Hua Tang, Audrey M. Southwick, Amanda M. Casto, Sohini Ramachandran, Howard M. Cann, Gregory S. Barsh, Marcus Feldman, Luigi L. Cavalli-Sforza, and Richard M. Myers.

Worldwide Human Relationships Inferred from Genome-Wide Patterns of Variation. *Science*, 319 (5866):1100–1104, February 2008. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.1153717.

M. Gallego Llorente, E. R. Jones, A. Eriksson, V. Siska, K. W. Arthur, J. W. Arthur, M. C. Curtis, J. T. Stock, M. Coltorti, P. Pieruccini, S. Stretton, F. Brock, T. Higham, Y. Park, M. Hofreiter, D. G. Bradley, J. Bhak, R. Pinhasi, and A. Manica. Ancient Ethiopian genome reveals extensive Eurasian admixture in Eastern Africa. *Science*, 350(6262):820–822, November 2015. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.aad2879. URL `http://science.sciencemag.org/content/350/6262/820`.

Ani Manichaikul, Josyf C. Mychaleckyj, Stephen S. Rich, Kathy Daly, Michèle Sale, and Wei-Min Chen. Robust relationship inference in genome-wide association studies. *Bioinformatics (Oxford, England)*, 26(22):2867–2873, November 2010. ISSN 1367-4811. doi: 10.1093/bioinformatics/btq559.

Sarah J. Marks, Hila Levy, Conrado Martinez-Cadenas, Francesco Montinaro, and Cristian Capelli. Migration distance rather than migration rate explains genetic diversity in human patrilocal groups. *Molecular Ecology*, 21(20):4958–4969, October 2012. ISSN 1365-294X. doi: 10.1111/j.1365-294X.2012.05689.x.

Sarah J. Marks, Francesco Montinaro, Hila Levy, Francesca Brisighelli, Gianmarco Ferri, Stefania Bertoncini, Chiara Batini, George B. J. Busby, Charles Arthur, Peter Mitchell, Brian A. Stewart, Ockie Oosthuizen, Erica Oosthuizen, Maria Eugenia D'Amato, Sean Davison, Vincenzo Pascali, and Cristian Capelli. Static and Moving Frontiers: The Genetic Landscape of Southern African Bantu-Speaking Populations. *Molecular Biology and Evolution*, 32(1):29–43, January 2015. ISSN 0737-4038, 1537-1719. doi: 10.1093/molbev/msu263.

Andrew May, Scott Hazelhurst, Yali Li, Shane A. Norris, Nimmisha Govind, Mohammed Tikly, Claudia Hon, Keith J. Johnson, Nicole Hartmann, Frank Staedtler, and Michèle Ramsay. Genetic diversity in black South Africans from Soweto. *BMC Genomics*, 14:644, 2013. ISSN 1471-2164. doi: 10.1186/1471-2164-14-644.

Peter Mitchell. *The Archaeology of Southern Africa*. Cambridge University Press, Cambridge ; New York, December 2002. ISBN 978-0-521-63389-5.

Francesco Montinaro, George B. J. Busby, Vincenzo L. Pascali, Simon Myers, Garrett Hellenthal, and Cristian Capelli. Unravelling the hidden ancestry of American admixed populations. *Nature Communications*, 6:6596, March 2015. doi: 10.1038/ncomms7596.

Andrés Moreno-Estrada, Simon Gravel, Fouad Zakharia, Jacob L. McCauley, Jake K. Byrnes, Christopher R. Gignoux, Patricia A. Ortiz-Tello, Ricardo J. Martínez, Dale J. Hedges, Richard W. Morris, Celeste Eng, Karla Sandoval, Suehelay Acevedo-Acevedo, Paul J. Norman, Zulay Layrisse, Peter Parham, Juan Carlos Martínez-Cruzado, Esteban González Burchard, Michael L. Cuccaro, Eden R. Martin, and Carlos D. Bustamante. Reconstructing the Population Genetic History of the Caribbean. *PLoS Genet*, 9(11):e1003925, November 2013. doi: 10.1371/journal.pgen.1003925.

Alan G. Morris, Anja Heinze, Eva K.F. Chan, Andrew B. Smith, and Vanessa M. Hayes. First Ancient Mitochondrial Human Genome from a Prepastoralist Southern African. *Genome Biology and Evolution*, 6(10):2647–2653, September 2014. ISSN 1759-6653. doi: 10.1093/gbe/evu202. URL `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4224329/`.

George Peter Murdock. *Atlas of World Cultures*. University of Pittsburgh Pre, May 1981. ISBN 978-0-8229-7631-8.

Etienne Patin, Katherine J. Siddle, Guillaume Laval, Hélène Quach, Christine Harmant, Noémie Becker, Alain Froment, Béatrice Régnault, Laure Lemée, Simon Gravel, Jean-Marie Hombert, Lolke Van der Veen, Nathaniel J. Dominy, George H. Perry, Luis B. Barreiro, Paul Verdu, Evelyne Heyer, and Lluís Quintana-Murci. The impact of agricultural emergence on the genetic history of African rainforest hunter-gatherers and agriculturalists. *Nature Communications*, 5:3163, February 2014. doi: 10.1038/ncomms4163.

Nick J. Patterson, Priya Moorjani, Yontao Luo, Swapan Mallick, Nadin Rohland, Yiping Zhan, Teri Genschoreck, Teresa Webster, and David Reich. Ancient Admixture in Human History. *Genetics*, page genetics.112.145037, January 2012. ISSN 0016-6731, 1943-2631. doi: 10.1534/genetics.112.145037.

Pedro R. Peres-Neto and Donald A. Jackson. How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia*, 129(2):169–178, October 2001. ISSN 0029-8549, 1432-1939. doi: 10.1007/s004420100720.

Desiree C. Petersen, Ondrej Libiger, Elizabeth A. Tindall, Rae-Anne Hardie, Linda I. Hannick, Richard H. Glashoff, Mitali Mukerji, Pedro Fernandez, Wilfrid Haacke, Nicholas J. Schork, Vanessa M. Hayes, and Indian Genome Variation Consortium. Complex Patterns of Genomic Admixture within Southern Africa. *PLoS Genet*, 9(3):e1003309, March 2013. doi: 10.1371/journal.pgen.1003309.

Joseph K. Pickrell and Jonathan K. Pritchard. Inference of Population Splits and Mixtures from Genome-Wide Allele Frequency Data. *PLoS Genet*, 8(11):e1002967, November 2012. doi: 10.1371/journal.pgen.1002967.

Joseph K. Pickrell, Nick Patterson, Chiara Barbieri, Falko Berthold, Linda Gerlach, Tom Güldemann, Blesswell Kure, Sununguko Wata Mpoloka, Hirosi Nakagawa, Christfried Naumann, Mark Lipson, Po-Ru Loh, Joseph Lachance, Joanna Mountain, Carlos D. Bustamante, Bonnie Berger, Sarah A. Tishkoff, Brenna M. Henn, Mark Stoneking, David Reich, and Brigitte Pakendorf. The genetic prehistory of southern Africa. *Nature Communications*, 3:1143, 2012. ISSN 2041-1723. doi: 10.1038/ncomms2140.

Joseph K. Pickrell, Nick Patterson, Po-Ru Loh, Mark Lipson, Bonnie Berger, Mark Stoneking, Brigitte Pakendorf, and David Reich. Ancient west Eurasian ancestry in southern and eastern Africa. *Proceedings of the National Academy of Sciences*, 111(7):2632–2637, February 2014. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.1313787111.

Denis Pierron, Harilanto Razafindrazaka, Luca Pagani, François-Xavier Ricaut, Tiago Antao, Mélanie Capredon, Clément Sambo, Chantal Radimilahy, Jean-Aimé Rakotoarisoa, Roger M. Blench, Thierry

Letellier, and Toomas Kivisild. Genome-wide evidence of Austronesian–Bantu admixture and cultural reversion in a hunter-gatherer group of Madagascar. *Proceedings of the National Academy of Sciences*, 111(3):936–941, January 2014. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.1321860111. URL `http://www.pnas.org/content/111/3/936`.

Alkes L. Price, Noah A. Zaitlen, David Reich, and Nick Patterson. New approaches to population stratification in genome-wide association studies. *Nature Reviews Genetics*, 11(7):459–463, July 2010. ISSN 1471-0056. doi: 10.1038/nrg2813.

David Reich, Richard E. Green, Martin Kircher, Johannes Krause, Nick Patterson, Eric Y. Durand, Bence Viola, Adrian W. Briggs, Udo Stenzel, Philip L. F. Johnson, Tomislav Maricic, Jeffrey M. Good, Tomas Marques-Bonet, Can Alkan, Qiaomei Fu, Swapan Mallick, Heng Li, Matthias Meyer, Evan E. Eichler, Mark Stoneking, Michael Richards, Sahra Talamo, Michael V. Shunkov, Anatoli P. Derevianko, Jean-Jacques Hublin, Janet Kelso, Montgomery Slatkin, and Svante Pääbo. Genetic history of an archaic hominin group from Denisova Cave in Siberia. *Nature*, 468(7327):1053–1060, December 2010. ISSN 0028-0836. doi: 10.1038/nature09710.

Alexandra Rosa and António Brehem. African human mtDNA phylogeography at-a-glance. *Journal of anthropological sciences = Rivista di antropologia: JASS / Istituto italiano di antropologia*, 89:25–58, 2011. ISSN 2037-0644. doi: 10.4436/jass.89006.

Noah A. Rosenberg. distruct: a program for the graphical display of population structure. *Molecular Ecology Notes*, 4(1):137–138, March 2004. ISSN 1471-8286. doi: 10.1046/j.1471-8286.2003.00566.x.

Noah A. Rosenberg, Lucy Huang, Ethan M. Jewett, Zachary A. Szpiech, Ivana Jankovic, and Michael Boehnke. Genome-wide association studies in diverse populations. *Nature Reviews. Genetics*, 11(5): 356–366, May 2010. ISSN 1471-0064. doi: 10.1038/nrg2760.

Karim Sadr. The First Herders at the Cape of Good Hope. *The African Archaeological Review*, 15(2): 101–132, 1998. ISSN 0263-0338. URL `http://www.jstor.org/stable/25130648`.

Carina M. Schlebusch, Pontus Skoglund, Per Sjödin, Lucie M. Gattepaille, Dena Hernandez, Flora Jay, Sen Li, Michael De Jongh, Andrew Singleton, Michael G. B. Blum, Himla Soodyall, and Mattias Jakobsson. Genomic variation in seven Khoe-San groups reveals adaptation and complex African history. *Science (New York, N.Y.)*, 338(6105):374–379, October 2012. ISSN 1095-9203. doi: 10.1126/science.1227721.

Jeet Sukumaran and Mark T. Holder. DendroPy: a Python library for phylogenetic computing. *Bioinformatics*, 26(12):1569–1571, June 2010. ISSN 1367-4803, 1460-2059. doi: 10.1093/bioinformatics/btq228.

Sarah A. Tishkoff, Mary Katherine Gonder, Brenna M. Henn, Holly Mortensen, Alec Knight, Christopher Gignoux, Neil Fernandopulle, Godfrey Lema, Thomas B. Nyambo, Uma Ramakrishnan, Floyd A. Reed, and Joanna L. Mountain. History of click-speaking populations of Africa inferred from mtDNA and Y chromosome genetic variation. *Molecular Biology and Evolution*, 24(10):2180–2195, October 2007. ISSN 0737-4038. doi: 10.1093/molbev/msm155.

Sarah A. Tishkoff, Floyd A. Reed, Françoise R. Friedlaender, Christopher Ehret, Alessia Ranciaro, Alain Froment, Jibril B. Hirbo, Agnes A. Awomoyi, Jean-Marie Bodo, Ogobara Doumbo, Muntaser Ibrahim, Abdalla T. Juma, Maritha J. Kotze, Godfrey Lema, Jason H. Moore, Holly Mortensen, Thomas B. Nyambo, Sabah A. Omar, Kweli Powell, Gideon S. Pretorius, Michael W. Smith, Mahamadou A. Thera, Charles Wambebe, James L. Weber, and Scott M. Williams. The Genetic Structure and History of Africans and African Americans. *Science (New York, N.Y.)*, 324(5930):1035–1044, May 2009. ISSN 0036-8075. doi: 10.1126/science.1172257.

List of Figures

List of Supplementary Figures

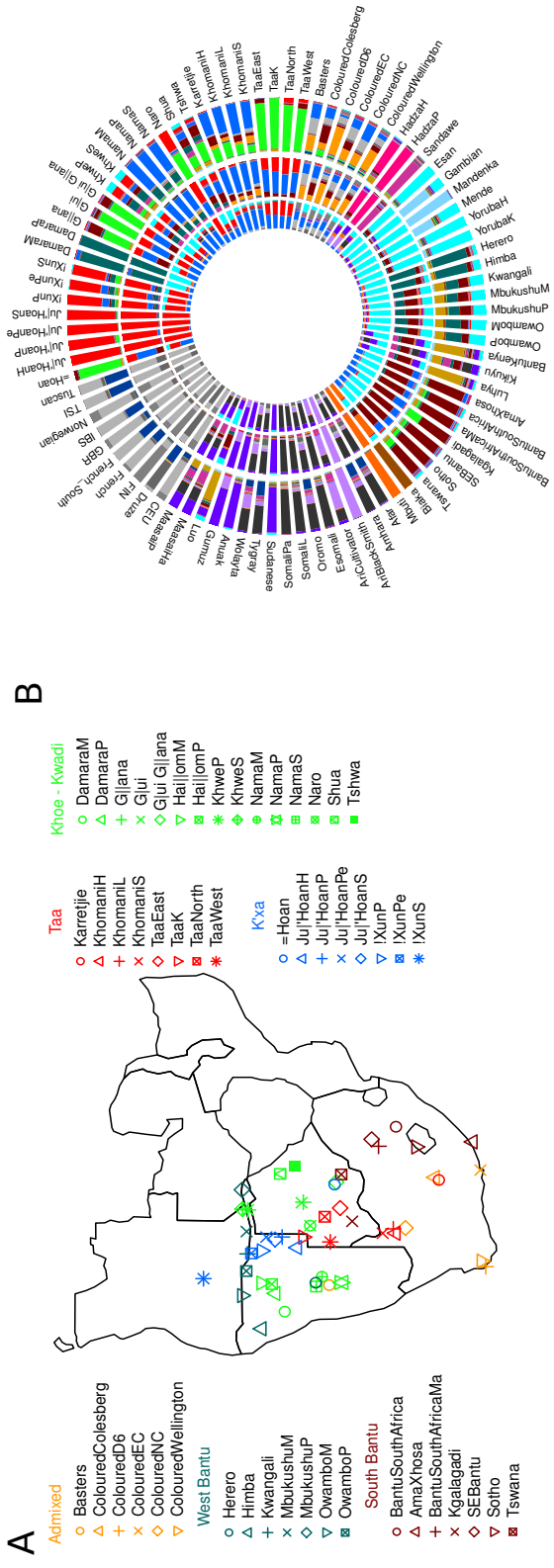


Figure 1: **The genetic structure of southern Africa populations. A.** southern Africa populations analyzed in this study. Different colors are associated with different language/ethnic affiliation. The complete dataset used for analysis is shown in Fig. S1, and Table S1. **B.** Admixture results for $K = 5, 10, 15$ (from the inner to the outer circle). We analyzed 1856 individuals for 91 populations and summarized the results in a population based barplot. The full set of results ($K = \{1..20\}$) for individuals and populations are reported in Fig. S2 and Fig. S3.

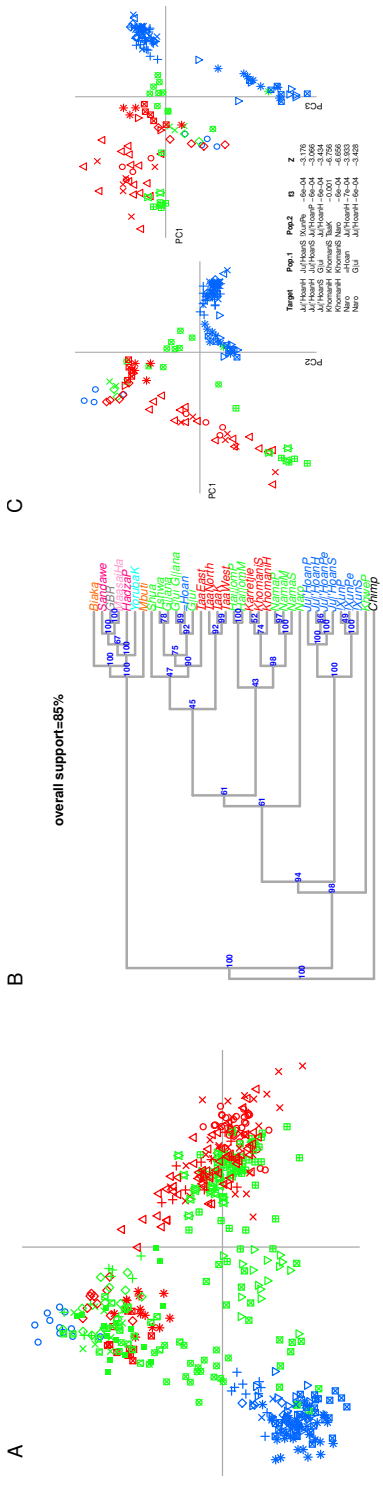


Figure 2: **Local Ancestry deconvolution reveals complex Khoesan-related structure. A.** Principal Component Analyses of Khoesan specific fragments. We extracted fragments with high (>99%) probability to be derived from Khoesan populations and visualized it in a Multi-Dimensional Scaling Plot, as described in Methods section. **B.** Maximum likelihood tree of Khoesan populations. We selected all the Khoesan populations and added 7 African and European populations. We performed ten different run and assessed the support of each tree trough 100 bootstraps (Fig. S6). Colours key are as in Fig.1a and Fig S1. **C.** Principal component analysis of individuals with more than 80% of Khoesan-related genetic ancestry. We used the K=3 ADMIXTURE run to select individuals characterised by at least 80% of Khoesan genetic ancestry and performed a Principal component Analysis as described in Methods section. The two most significant f_3 between "Target" and sources ("P1" and "P2") populations, including standard deviation (sd) and Z-score, are reported.

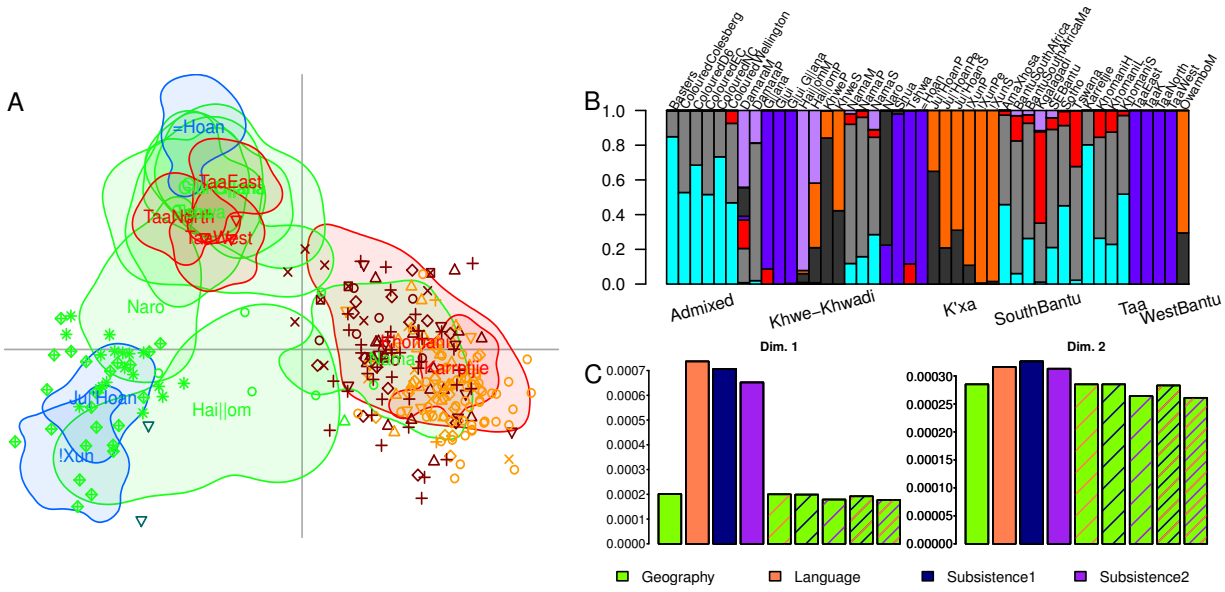
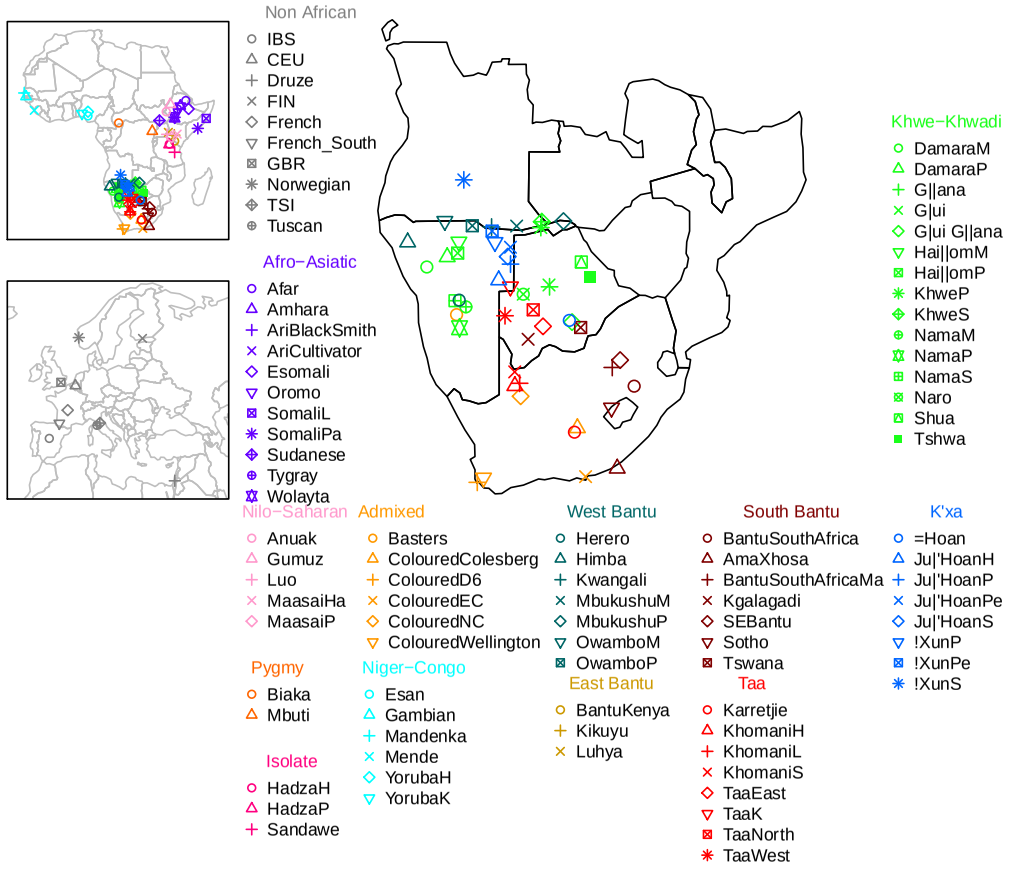
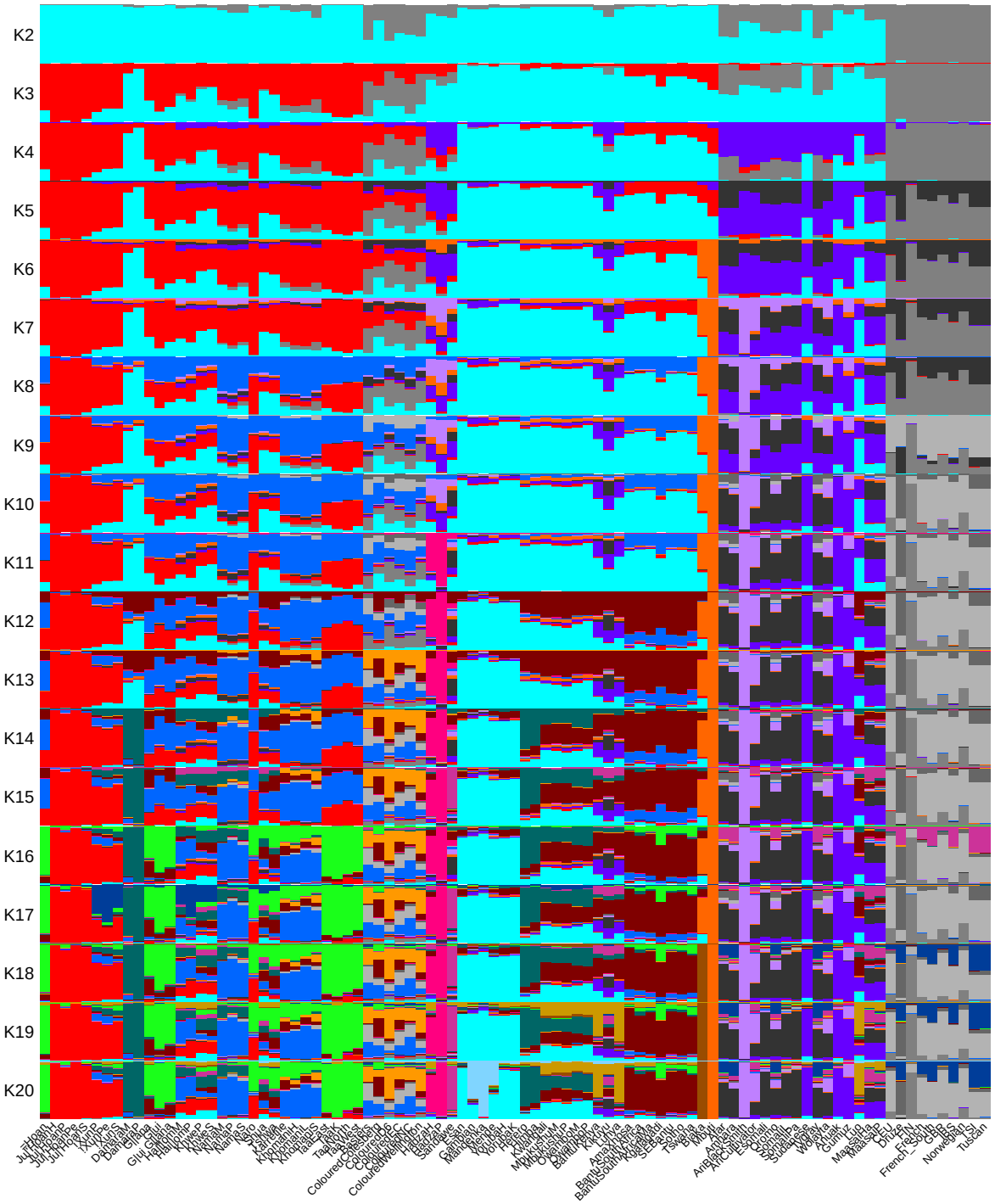


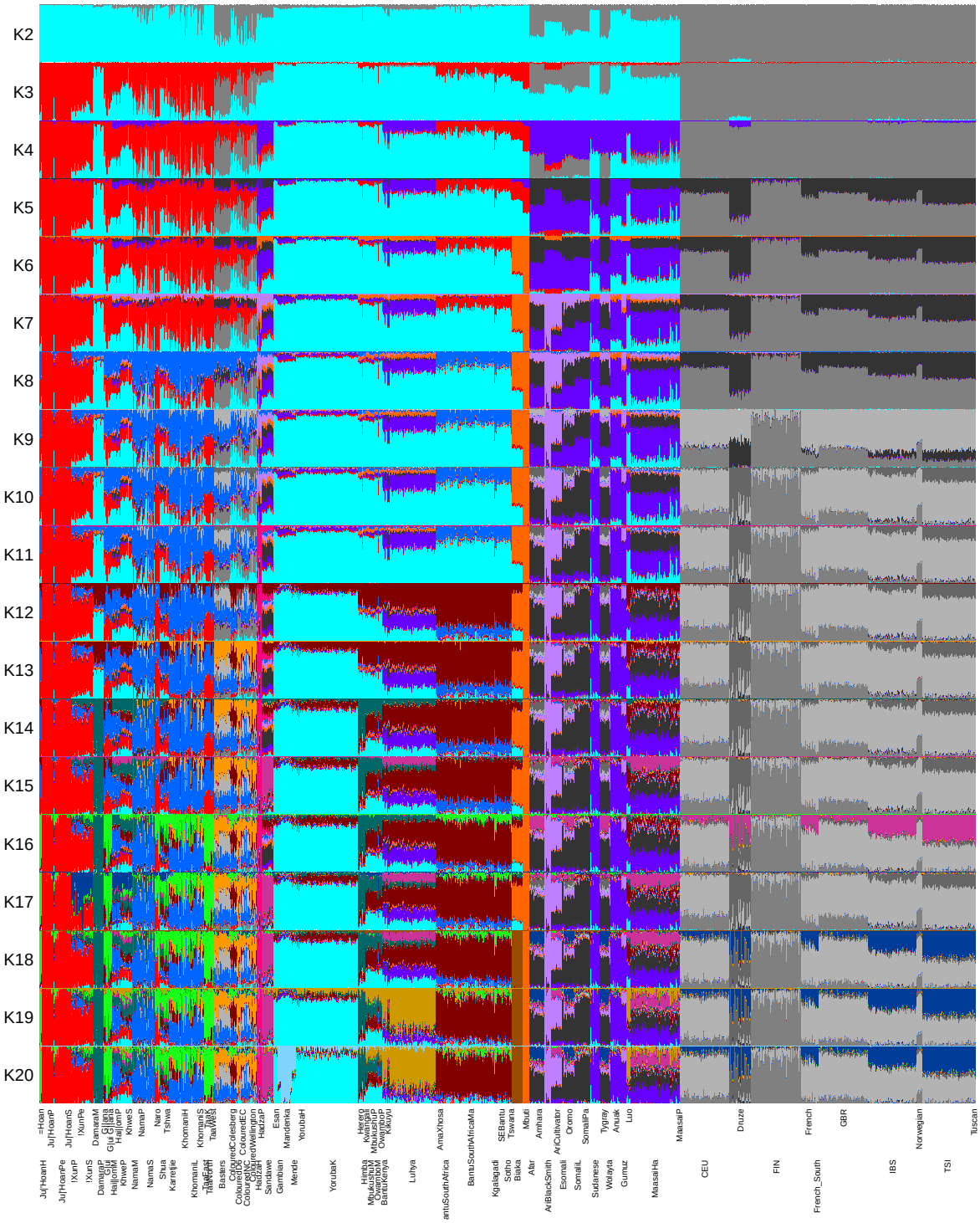
Figure 3: **A.** Genetic structure of admixed southern African populations. We estimated the 90% utilization kernel of all the Khoesan populations (except Damara and Khwe, see text), and plotted the highly admixed individuals. **B.** Cluster Analysis of genomic fragments. We grouped all the individuals in 7 clusters, as inferred by mclust package (see methods) and visualized the results in barplot according to populations and language/ethnic affiliation. The results highlight the large heterogeneity in populations sharing the same affiliation and the existence of a slight but significant substructure between Bantu and Coloured populations. **C.** Predictive errors of genetic components for geographic, linguistic and subsistence affiliation. Geography better predicts genetic ancestries, though adding new covariates slightly decrease the predictive error.



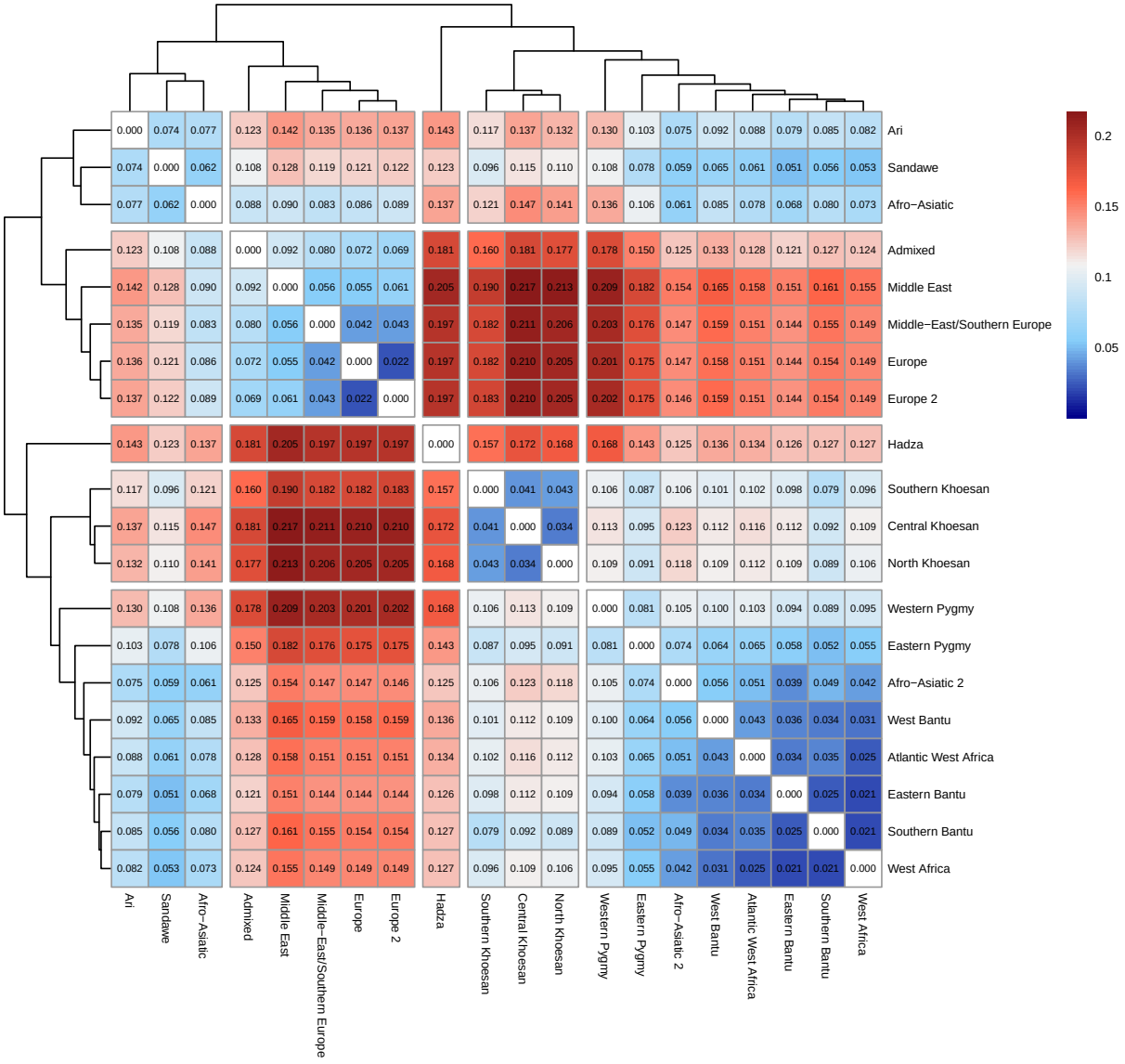
Supplementary Figure 1: **Geographic locations of analysed populations.** We analyzed 1856 individuals from 91 groups, genotyped with different Illumina platforms and the Affymetrix Axiom Genome-Wide Human Origins 1 array.



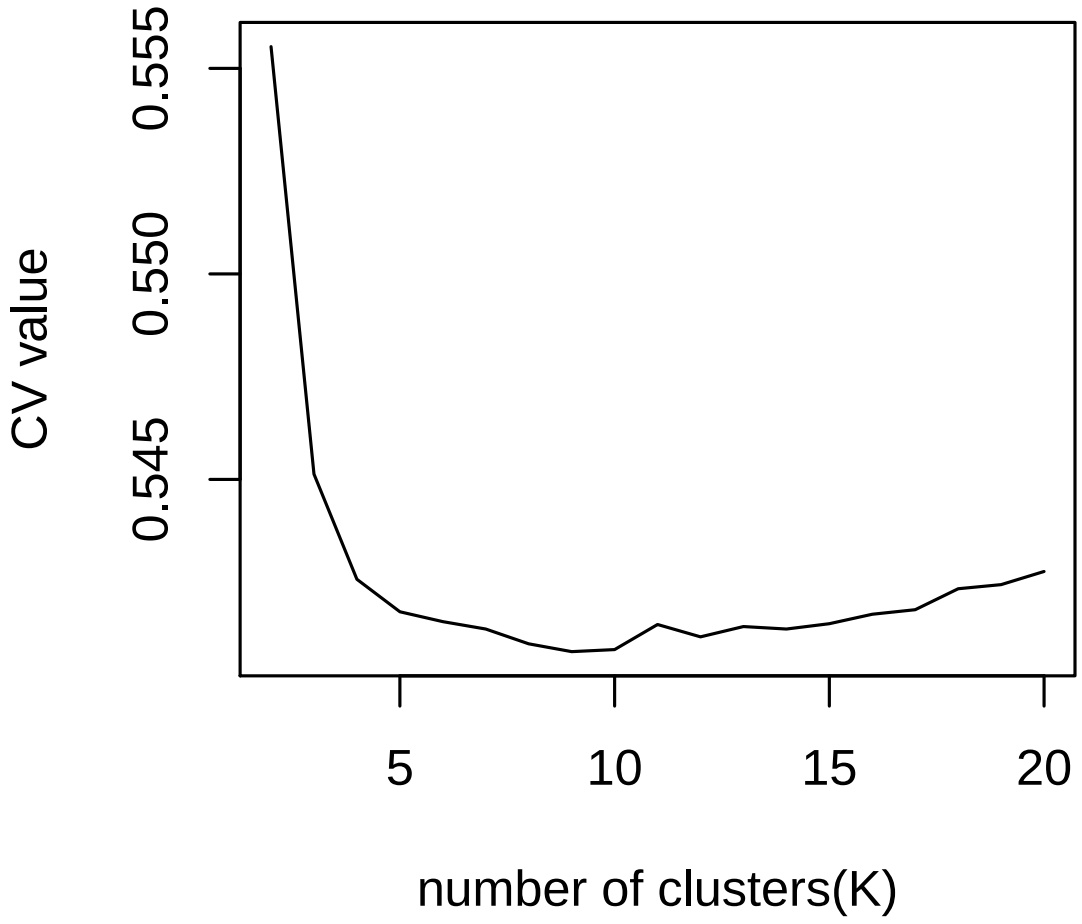
Supplementary Figure 2: **ADMIXTURE analysis of analysed populations for $K = 2..20$.** We performed ADMIXTURE analysis, with different random seeds for all the individuals and reported the population average.



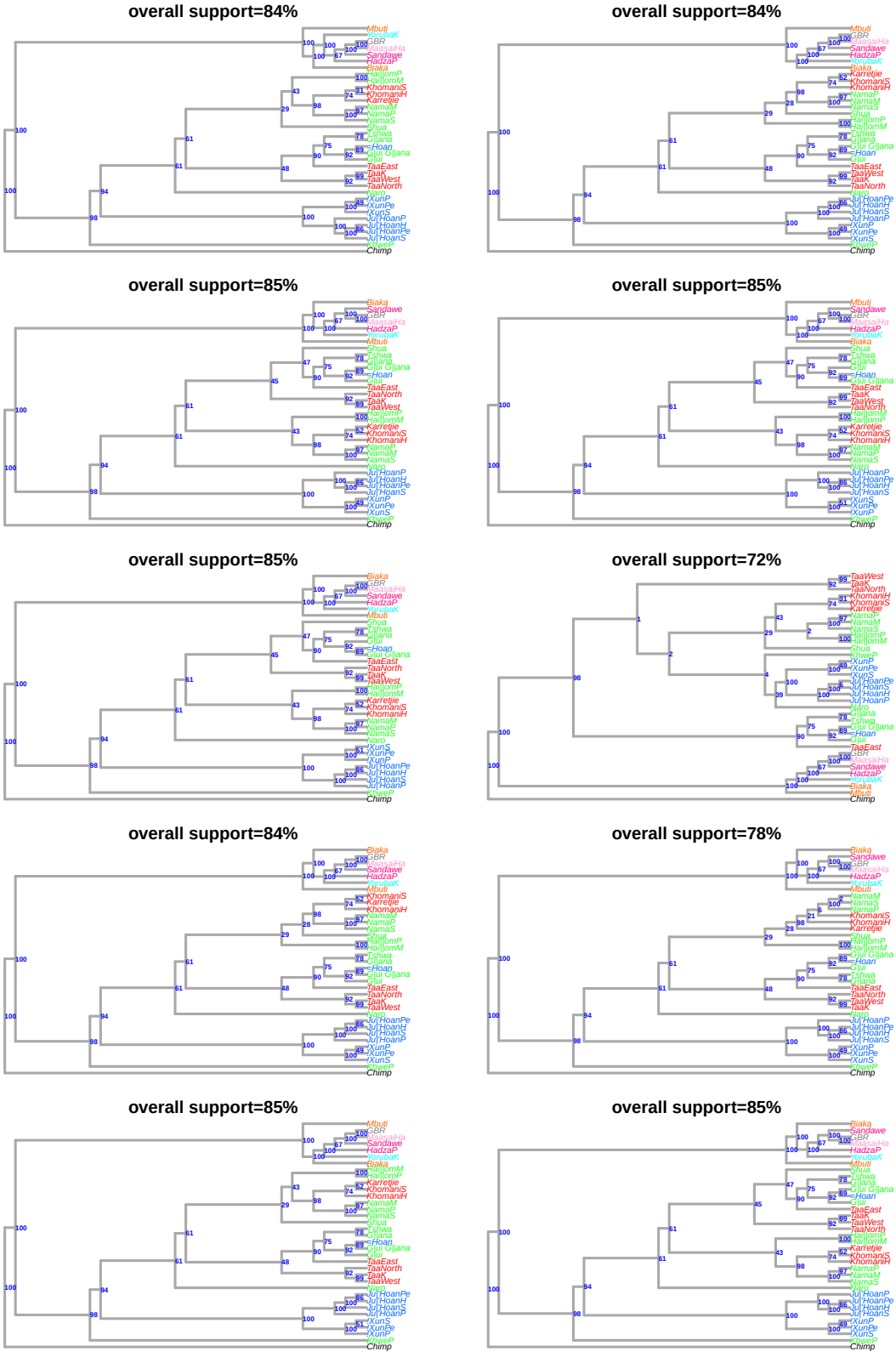
Supplementary Figure 3: **ADMIXTURE analysis of analysed populations for $K = 2..20$.** We performed ADMIXTURE analysis, with different random seeds for all the individuals and reported the individual clustering assignation.



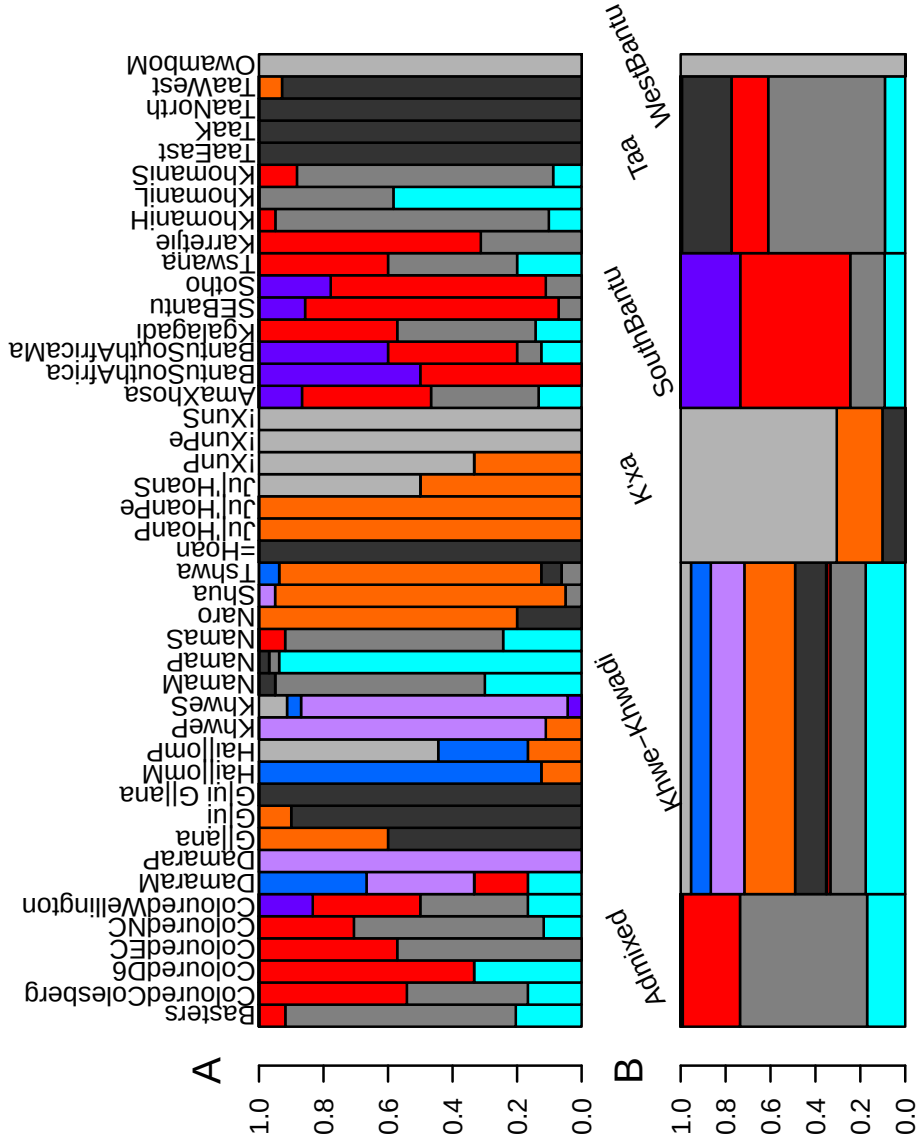
Supplementary Figure 4: **Heatmap and hierarchical trees of the genetic relationships between ancestral components**. We reported F_ST values between components ($K = 20$), and clustered them using a hierarchical approach as indicated in Methods section.



Supplementary Figure 5: **Cross validation error values for ADMIXTURE run for K=2..20**. The lowest values for CV error are between 9 and 10 clusters. We noted that analysis for larger number of K provided important insights into the genetic structure of analyzed populations and discussed these in the main text.



Supplementary Figure 6: **Maximum Likelihood trees of Khoesan populations.** We performed ten different runs, using different random seeds of TREEMIX, and assessed their support trough 100 bootstrap replications. We then reported one of the most supported trees in Fig 2B. Note that the most supported trees are identical in their topology.



Supplementary Figure 7: **Local ancestry MDS clustering analysis reveals Khoesan-related structure in admixed populations** Cluster Analysis of Multi-Dimensional Scaling coordinates. We grouped all the individuals in 9 clusters, using the ibs distance matrix between individuals, as inferred by mclust package (see methods) and visualized the results in barplot according to populations and language/ethnic affiliation. The results highlight the large heterogeneity in populations from the same affiliation and the existence of a slight but significant substructure between Bantu and Coloured populations.

Tables:

Table S1 – Populations used in this study. Information on number of individuals in the unlinked dataset, TREEMIX dataset, and dataset with 80% Khoesan, number of chromosomes in the Khoesan Ancestry dataset, coordinates, group and data source is reported.

Table S2 – Migration events incorporated in the TREEMIX analysis. The "Recipient" population is expected to have a percentage ("Amount") of ancestry from the "Source" population.

Table S3 – Three-population test (f3 test) results between "Target" and sources ("P1" and "P2") populations, including standard deviation (sd) and Z-score.

Table S4 – Geographic, Linguistic and subsistence affiliation used in the correlation analysis of MDS coordinates.