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East African pigs have a complex Indian, Far Eastern and Western ancestry

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Summary

In this study, we have characterized the mitochondrial diversity of 81 swine from Uganda. Median-joining network analysis of D-loop sequences from these individuals and others characterized in previous studies allowed us to determine that Ugandan pigs cluster with populations from the West (Europe/North Africa), Far East and India. In addition, partial sequencing of the Y-chromosome *UTY* locus in 18 Ugandan domestic pigs revealed the segregation of a single *HY1* lineage that has a cosmopolitan distribution. A Western and Far Eastern ancestry for East African pigs had been already reported, but this is the first study demonstrating an additional contribution from the Indian porcine gene pool. This result is consistent with the high frequency of zebuine alleles in cattle from East Africa. The geographic coordinates of East Africa, at the crossroads of many trading routes that, through the ages, linked Europe, Africa and Asia, might explain the rich and complex genetic heritage of livestock native to this area.

Keywords genetic diversity, indian ancestry, introgression, ugandan pigs

The history of pig domestication and breeding in Africa is mostly unknown because of a lack of sufficient zooarcheological and genetic data (Blench 2000; Amills *et al.* 2013). It has been generally assumed that pigs were not raised in East Africa until the arrival of the Portuguese sailors (Blench 2000). However, the analysis of Y-chromosome variation of pigs from Zimbabwe and Kenya revealed the presence of one haplotype that segregates at high frequencies in Asian pigs, whereas it is almost absent in European standard commercial breeds (Ramírez *et al.* 2009). This result points to the entry of Asian pigs into East Africa, maybe as a consequence of the ancient Indian Ocean trade between this region and China or because of a more recent introduction coming from the Portuguese colony of Macau (Ramírez *et al.* 2009). The aim of the current study was to characterize the genetic diversity of pigs from Uganda and make general inferences about the ancestry of East African swine.

Indigenous Ugandan pigs are black, and they are characterized by their hardiness, being bred under smallholder farming and scavenging conditions. Whole blood samples were obtained from 81 local pigs distributed in 13 different locations (Table S1). Total genomic DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen). A set of primers (forward 5′-CAACCAAAACAAGCATTCCA-3′, reverse 5′-GATTGTGGGCGTATGCTTAAA-3′) was used to amplify 1.2 kb (GenBank reference sequence AJ002189) corresponding to the mitochondrial control region. A second pair of primers (forward 5′-CAACCAAAACAAG CATTCCA-3′, reverse 5′-CCCGTAACCATTGACTGAAT-3′), yielding a 0.69-kb product, was used in the few cases when the aforementioned 1.2 kb-PCR did not work. Polymerase chain reactions were carried out in a 15-μl

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final reaction mixture containing PCR buffer, 2.5 mM of $MgCl_2$, 0.25 mM of dNTPs, 0.3 μM of each primer and 0.75 U of Taq Gold DNA polymerase (Applied Biosystems). The thermocycling profile consisted of one cycle at 95 °C for 10 min, followed by 35 cycles of 95 °C for 1 min, 62 °C for 1 min, 72 °C for 1 min and a final extension step at 72 °C for 7 min. Amplified products were purified with the ExoSAP-IT PCR Cleanup kit (Affymetrix) and sequenced in both directions with the same primers used in the amplification step. Sequencing reactions were prepared with the Big Dye Terminator Cycle Sequencing Kit v1.1 (Applied Biosystems) and electrophoresed in an ABI 3730 DNA Analyzer (Applied Biosystems). The resultant chromatograms were edited, and all sequences were submitted to GenBank (GenBank accession numbers KM597073–KM597153).

A final dataset comprising the 81 Ugandan pig mitochondrial sequences obtained by us (Table S1) plus 183 sequences retrieved from GenBank (Table S2) was subjected to genetic analyses. Because not all of these 264 sequences were exactly the same length, they were trimmed, and an alignment of 464 bp was used to build a median-joining network with NETWORK 4.6 (Bandelt *et al.* 1999). We employed the default parameters implemented in NETWORK 4.6, and the transversion/transition ratio was changed to 3:1. This analysis (Fig. 1, Fig. S1) evidenced that East African pigs have a complex ancestry, with roots in Asia and the West (Europe/North Africa). However, the most original finding of our analysis was that Kenyan, Ugandan and Indian pigs cluster together (Fig. 1, Fig. S1). This result suggests a direct introgression event, because modern domestic pigs in India and Bhutan carry unique and highly differentiated mitochondrial haplotypes that can be found only in Indian wild boars (Larson *et al.* 2011). This finding is supported by previous data obtained in East African cattle, where zebuine alleles reach very high frequencies (Hanotte *et al.* 2002; Decker *et al.* 2014), probably because of maritime introductions associated with the Indian Ocean trade. Similarly, an Indian origin has been postulated for chicken in Zimbabwe on the basis of mitochondrial data (Muchadeyi *et al.* 2008; Mtileni *et al.* 2011).

The majority of Ugandan pigs grouped with the Far Eastern ones, but some of them fell within the European cluster (Fig. 1, Fig. S1). An Iberian influence on East African pigs is conceivable because the Portuguese were the first Europeans to circumnavigate Africa and establish a string of trading posts and colonies (Kilwa, Sofala, Mombasa, etc.) on its eastern coast (Okello 2002). Besides, standard European breeds, such as Large White and Landrace, also are widespread in the African continent because of their excellent productive abilities, which often overcome those of local populations. Clustering of Ugandan and Kenyan pigs with those from the Far East was also evident (Fig. 1, Fig. S1). At present, we do not know the source of this introgression because East African pigs clustered indistinctly with specimens from China and South East Asia. The presence of Indian and Far Eastern haplotypes in East African pigs might have been facilitated by the existence of trading routes (e.g., *Carreira da Índia,* Spice Trade, etc.) used by the Portuguese ships that, during the 15th to 18th centuries, connected China (Macau), Malaysia (Malacca) and India (Goa and Calicut) with Kenya (*i.e.,* Malindi and Mombasa), Mozambique and other coastal regions of East Africa (Guinote 1999). Alternatively, this introduction may have taken place much before the Age of Discoveries, given

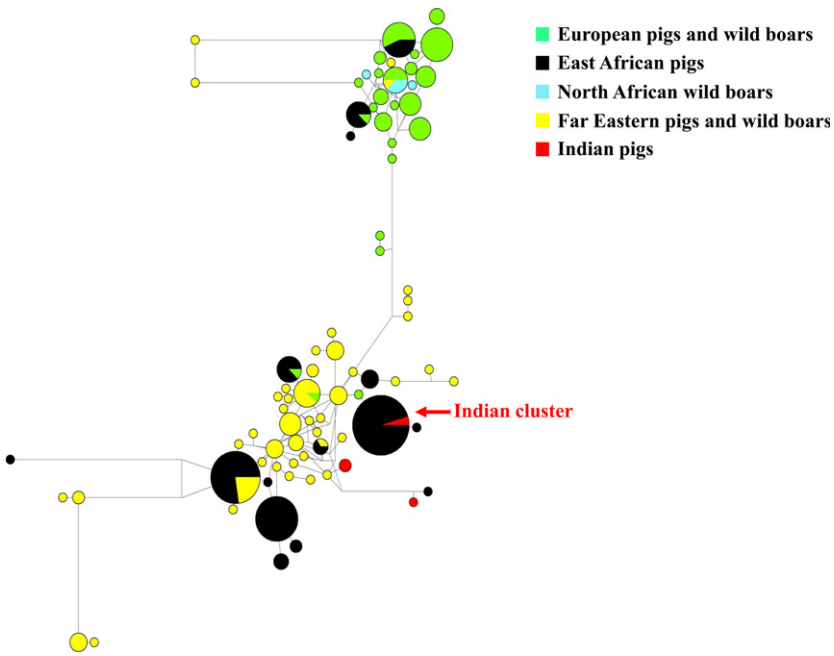

Figure 1 Median-joining network including sequences from European, Far Eastern, Indian, North African and East African pigs and wild boars (Tables S1 and S2). The clustering of Indian and East African pig samples is indicated.

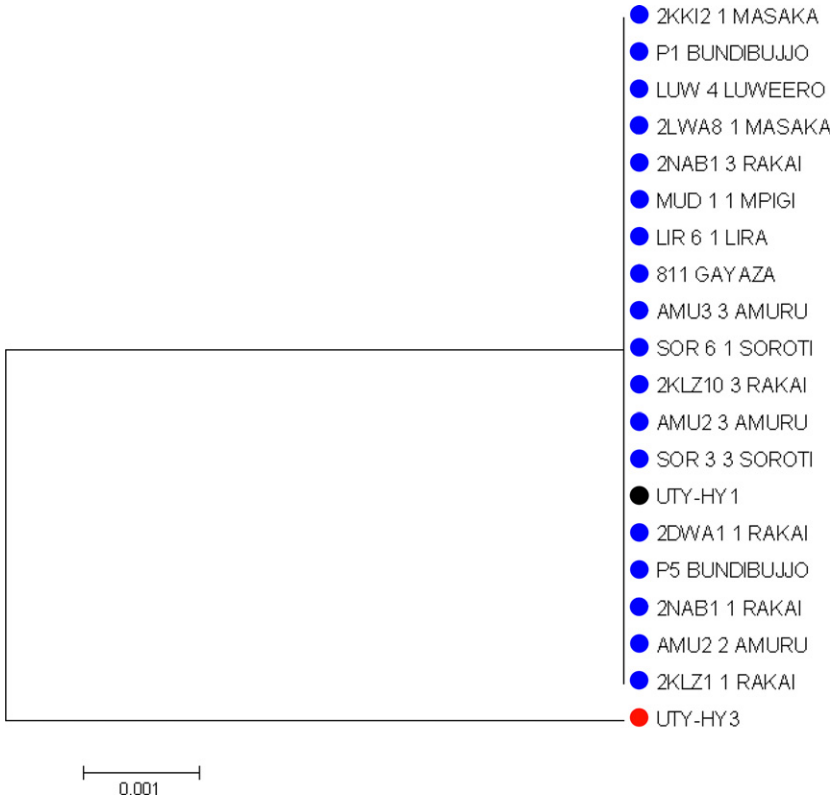

Figure 2 Neighbor-joining tree built with MEGA 5.05 (Tamura-Nei model, 1000 bootstrap replicates) of Y-chromosome *UTY* sequences from Ugandan pigs (blue) sampled at Masaka, Bundibujjo, Luweero, Rakai, Mpigi, Lira, Gayaza and Soroti. It can be seen that they all cluster with the *HY1* lineage (black), whereas the *HY3* (red) lineage appears as an outgroup.

that Indian Ocean trading routes have been used from immemorial times by the Arabs, Chinese, Persians, Romans, and many other civilizations (Kearney 2004).

Paternal genetic data also were obtained from Ugandan pigs by sequencing 0.37 kb of intron 1 and part of the flanking exons 1 and 2 of the *ubiquitously transcribed tetratricopeptide repeat containing, Y-linked* gene (*UTY*) with a single primer pair (forward 5′-AGCTGTTTTCGGTGAT GAGG-3′, reverse 5′-TGCCCAACAGAGTTTTAGTCC-3′). Amplification reactions were carried out in a 15-μl final volume containing PCR buffer, 2.5 mM of $MgCl_2$, 0.25 mM dNTPs, 0.3 μM of each primer and 0.75 of U Taq Gold DNA Polymerase (Applied Biosystems). The thermocycling profile consisted of one cycle at 95 °C for 10 min, followed by 35 cycles of 95 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min and a final extension step at 72 °C for 7 min. The PCR products were purified with the ExoSAP-IT PCR Cleanup kit (Affymetrix) and sequenced following the same protocols employed for the mitochondrial control region. The *UTY* amplicon contains two diagnostic SNPs that allow differentiation of the two main Y-chromosome lineages, that is *HY1* + *HY2* vs. *HY3* (Ramírez *et al.* 2009). After sequencing 18 Ugandan sires, we found only a single *HY1* haplotype (Fig. 2) that is widely spread both in Asia and Europe. Contrary to our expectations, we did not find any trace of the *HY3* haplotype, that could be considered as Asia specific and segregates in Kenyan (*HY3* frequency: 35%) and Zimbabwean Mukota (*HY3* frequency: 100%) pigs at significant frequencies (Ramírez *et al.* 2009). Probably, the impact of the Far Eastern introgression in African pigs has been especially important in Zimbabwe, where Mukota swine displaying a phenotype that resembles that of Chinese lard pigs have been reported (Ellert 1993). This genetic influence, in contrast, would have been less intense in other countries such as Uganda and Kenya.

In summary, this study demonstrates that pigs from East Africa have a very complex ancestry, with roots in the West, India and the Far East. This result is consistent with the enormous importance of East Africa as a point of intersection of intercontinental maritime routes linking these three geographic areas, a circumstance that has probably played a fundamental role in shaping and expanding African livestock diversity.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 A more detailed view of the geographic origin of the samples included in the median-joining network shown in Fig. 1.

Table S1 Ugandan samples analyzed in the current study and GenBank accession numbers of the corresponding mitochondrial control region sequences.

Table S2 European and Asian mitochondrial control region sequences.