INTROGRESSION OF EUROPEAN *Bos taurus* GENOME IN UGANDAN TAURINE AND ZEBUINE CATTLE BREEDS

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NEXTGEN PROJECT: MAIN AIMS

nextgen

SEVENTH FRAMEWORK PROGRAMME

http://nextgen.epfl.ch/

EU-funded project

P. Taberlet
LECA UJF-CNRS, Grenoble

Nextgen “*Next generation methods to preserve farm animal biodiversity by optimizing present and future breeding options*” is a 4-year EU-funded project.

- Identify genomic regions associated with adaptation and disease resistance

  Case studies: 1) adaptation in sheep and goats reared in contrasted environments of Morocco;
  2) relationship between genotypes and disease resistance/susceptibility in Ugandan cattle.

- Develop a novel freeze-drying approach for bio-banking cells and gametes at room temperature at very low cost.

- Evaluate the potential of sheep and goat wild ancestors as reservoirs of genetic diversity for the respective domestic species.

THE RATIONALE

Three main types of extant cattle: **the long-horned ankole** (crossbred between Asian *B. indicus* and African *B. taurus*)**; the short-horned zebu**, **the nganda** (crossbred between zebu and ankole),

Uganda is severely affected by tsetse fly and tick-born diseases, which involve theileriosis, anaplasmosis, babesiosis and cowdriosis.

Different level of resistance/tolerance to a number of diseases, since the available vector control methods are haphazard (Otim et al. 2004)

Since no major vaccination campaign against the diseases has ever been implemented in this country, cattle herders have traditionally selected local breeds with an emphasis on disease resistance and hardiness rather than on milk or beef production.

THE STRATEGY

Collection of biological samples from sick and healthy animals on the base of a spatial grid

Recording sample GPS coordinates

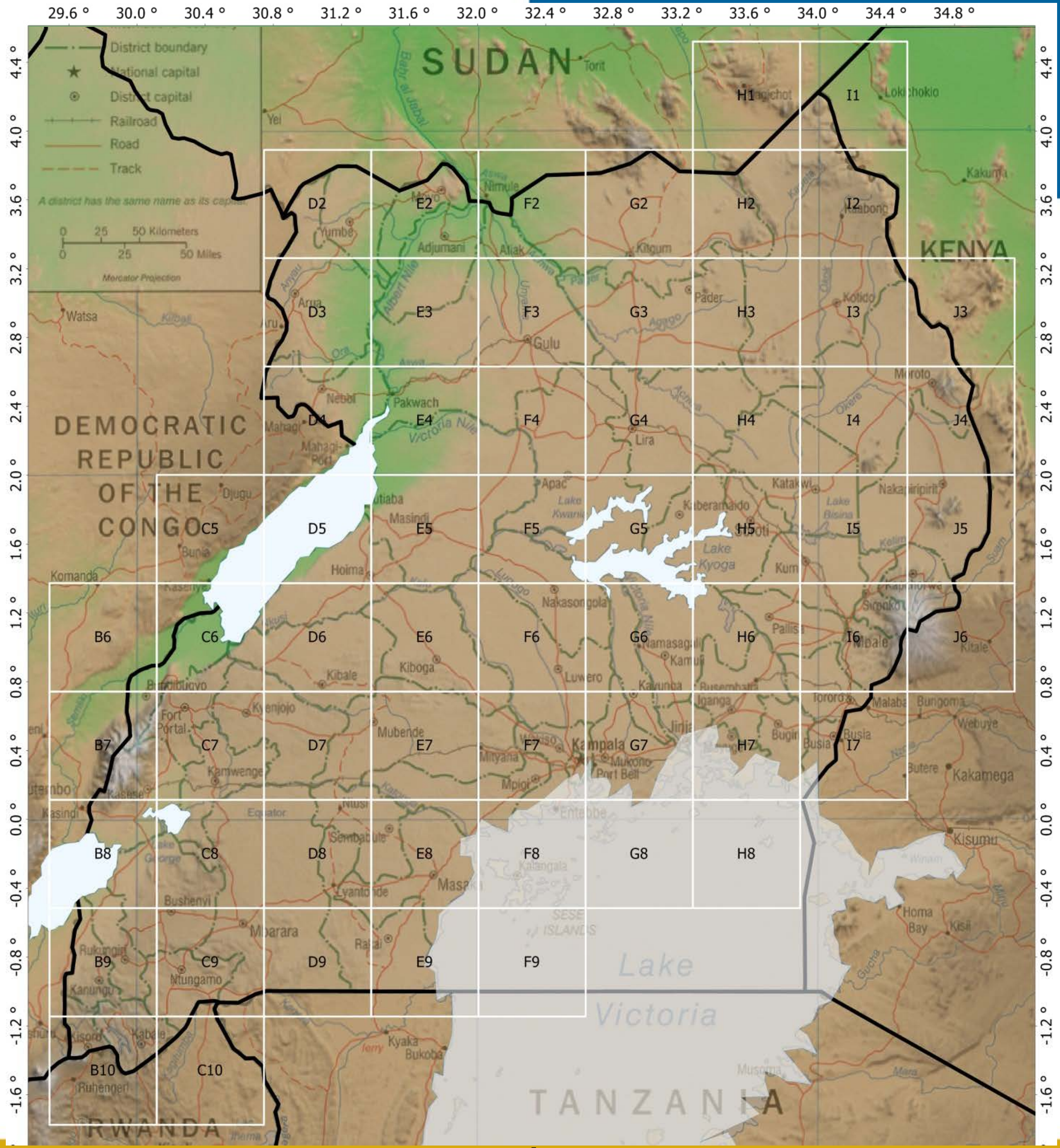
Assess animal health status via detection of disease specific antibodies

54k; 800k SNP data and whole genome sequencing

THE GOALS

- Investigate the genetic structure of the Uganda's cattle
- Detect possible introgression of foreign genome (European bos taurus ??) into Uganda's cattle genome
- Identify signature of selection comparing healthy and sick animals
- Search for spatial associations between the genomic footprints of selection and disease
- Pinpoint candidate genes and mutations in the genomic regions carrying selection signatures and spatially associated to disease tollerance/resistance.

NEXTGEN SAMPLING IN UGANDA



Side of cell 50 km

Sampled 20 animals per cells on average

THE BREEDS:
ANKOLE & RELATED BREEDS

Nkiga - NKI

Nsongora - NSO

Ntoro - NTO

Ankole - ANK

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The breeds: *B. indicus* and crosses

Small East African Zebu - SEZ

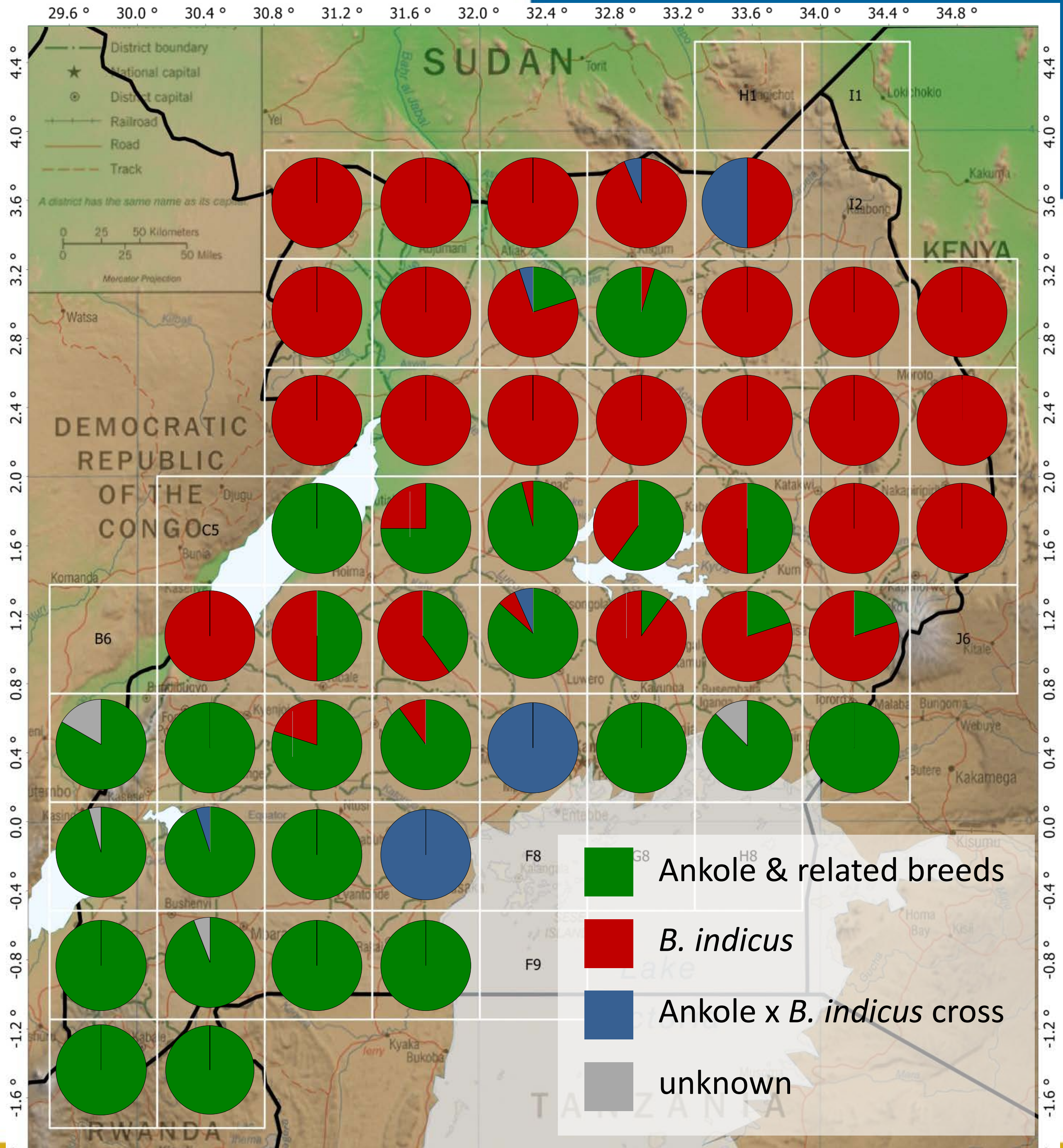
Shorthorn Zebu - SHZ

East African Shorthorn Zebu - EAZ

Nganda - NGA

Ankole x Zebu cross - AZC

NEXTGEN SAMPLING IN UGANDA



group	Label	Breed	N.
Ankole & rel. breeds	NKI	Nkiga	32
Ankole & rel. breeds	NSO	Nsongora	11
Ankole & rel. breeds	NTO	Ntoro	10
Ankole & rel. breeds	ANK	Ankole	338
Bos indicus	EAZ	East African Shorthorn Zebu	153
B. indicus	SEZ	Small East African Zebu	75
B. indicus	SHZ	Shorthorn Zebu	240
Ankole x *B. indicus*	NGA	Nganda	41
Ankole x *B. indicus*	AZC	Ankole Zebu Cross	11
Unknown			6
TOTAL			917

MATERIALS AND METHODS:

- **DNA extraction and SNP chip genotyping** have been carried out at LGS "Laboratorio di Genetica e Servizi" lab in Cremona (Italy) on **815** Ugandan cattle with Illumina *BovineSNP50 BeadChip* (~**54K markers**).

- **Datasets:** **54K SNPs**
  - **Ugandan cattle + Italian Holstein-Friesian (SELMOL + PROZOO projects) data**
  - **Uganda + other cattle projects (SELMOL + PROZOO + BOVINE HAPMAP projects) data**

- **Data analyses performed on all the datasets:**

  Raw data Quality Control and cleaning
  Multi-Dimensional Scaling plot
  Admixture software for population structure analysis

NEXTGEN + IT FRIESIAN, 54K:
THE DATASET & QC THRESHOLDS

Species	**Acronym**	**Origin**	**Subgroups**	**Sample size**
Bos indicus	EAZ	Uganda	East African Shorthorn Zebu	133
Bos indicus	SEZ	Uganda	Small East African Zebu	64
Bos indicus	SHZ	Uganda	Shorthorn Zebu	210
Cross	NGA	Uganda	Nganda	37
Cross	AZC	Uganda	Ankole Zebu cross	10
Ankole group	NKI	Uganda	Nkiga	27
Ankole group	NSO	Uganda	Nsongora	10
Ankole group	NTO	Uganda	Ntoro	10
Ankole group	ANK	Uganda	Ankole	287
Bos taurus	FRI		Holstein Friesian	400

Quality Control thresholds:
- SNP call rate = 0.95;
- individual call rate= 0.95;
- Minor Allele Frequency (MAF)= 0.01.

→

Cured dataset:
1189 individuals x 43494 markers

SOME EVIDENCES


UGBT-E 8190

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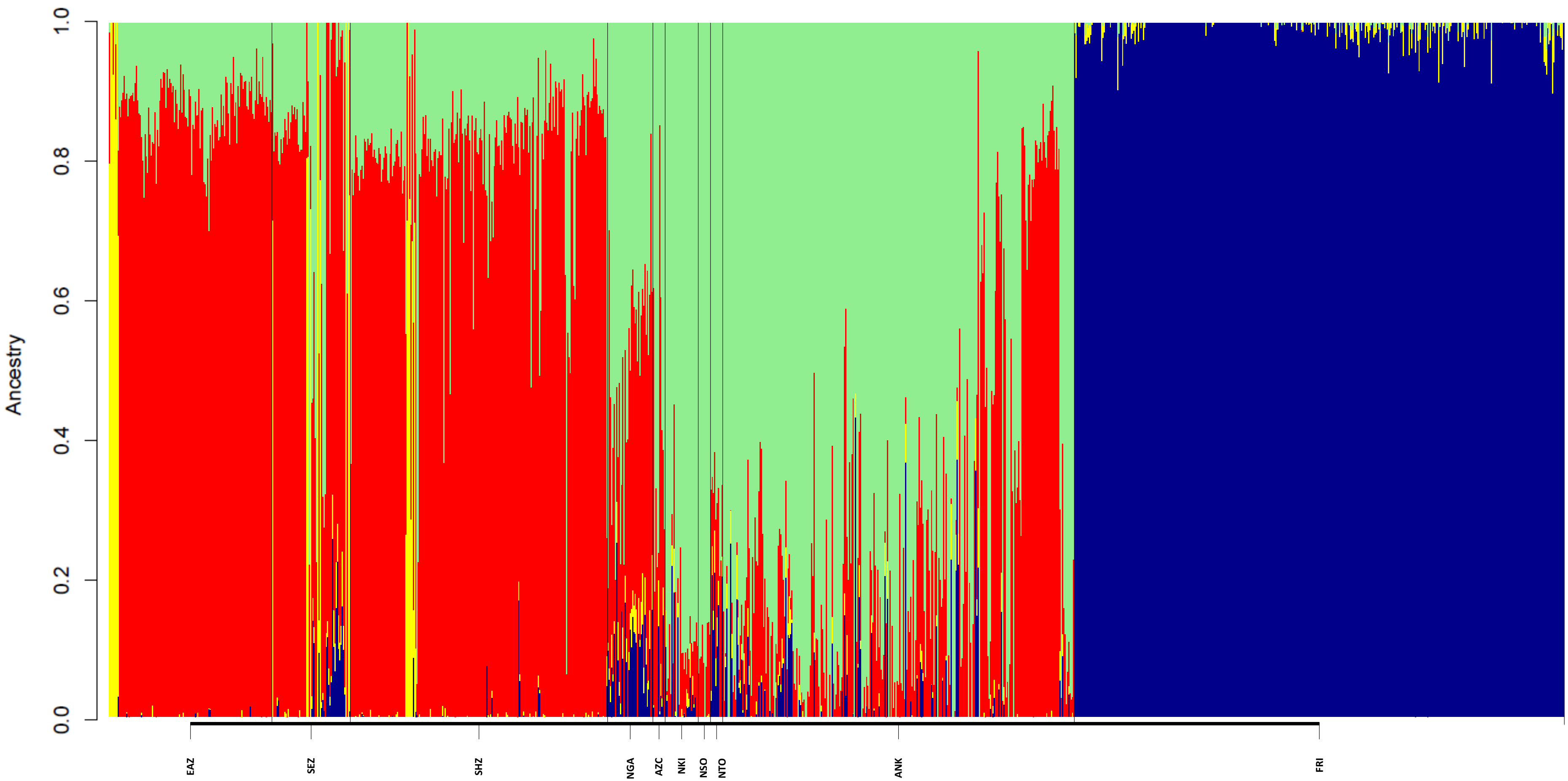
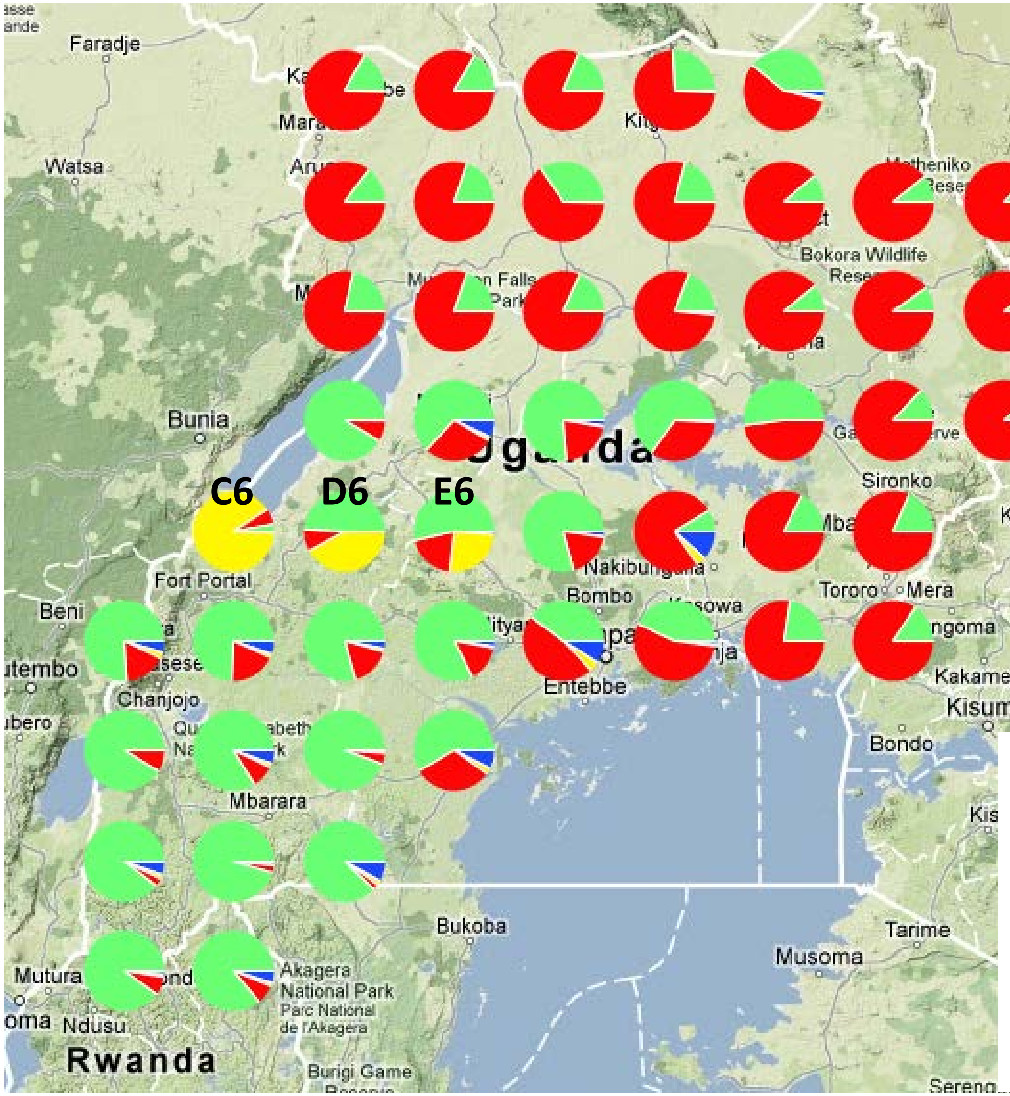
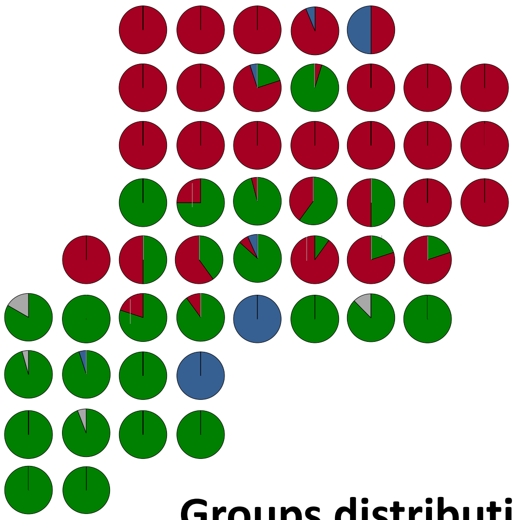
NEXTGEN+ IT FRIESIAN, 54K: ADMIXTURE ANALYSIS K = 4

Admixture software ver. 1.22 (Alexander *et al.*, 2009).

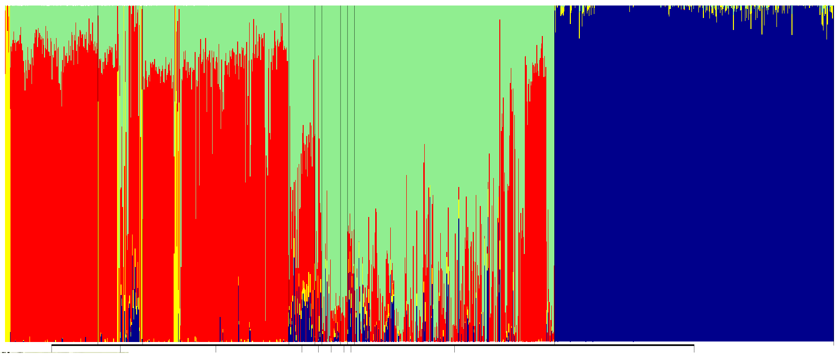


NEXTGEN+ IT FRIESIAN, 54K: ADMIXTURE PIECHARTS K = 4



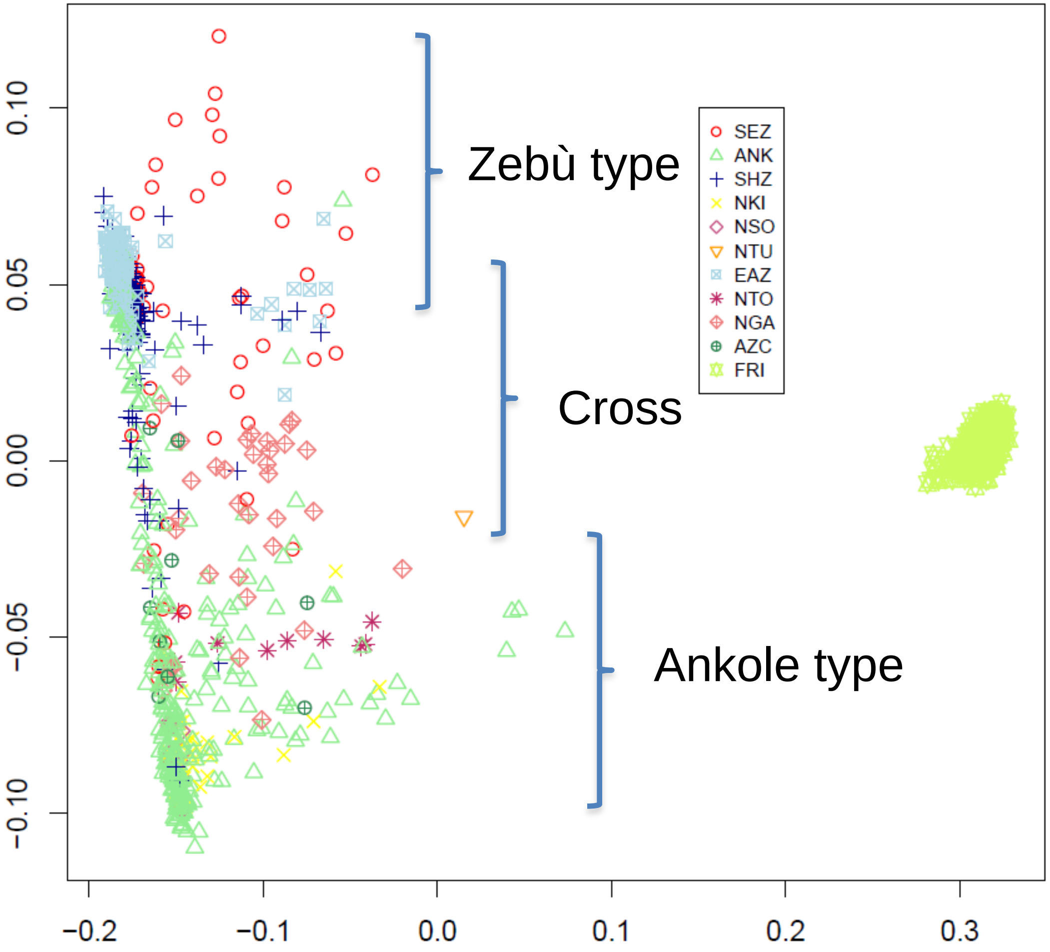


Groups distribution



NEXTGEN + IT FRIESIAN, 54K: MULTI DIMENSIONAL SCALING

MDS plots based on IBS distances = pairwise proportions of alleles not identical by state.



NEXTGEN + OTHER PROJECTS, 54K: THE EXTENDED DATASET & QC THRESHOLDS

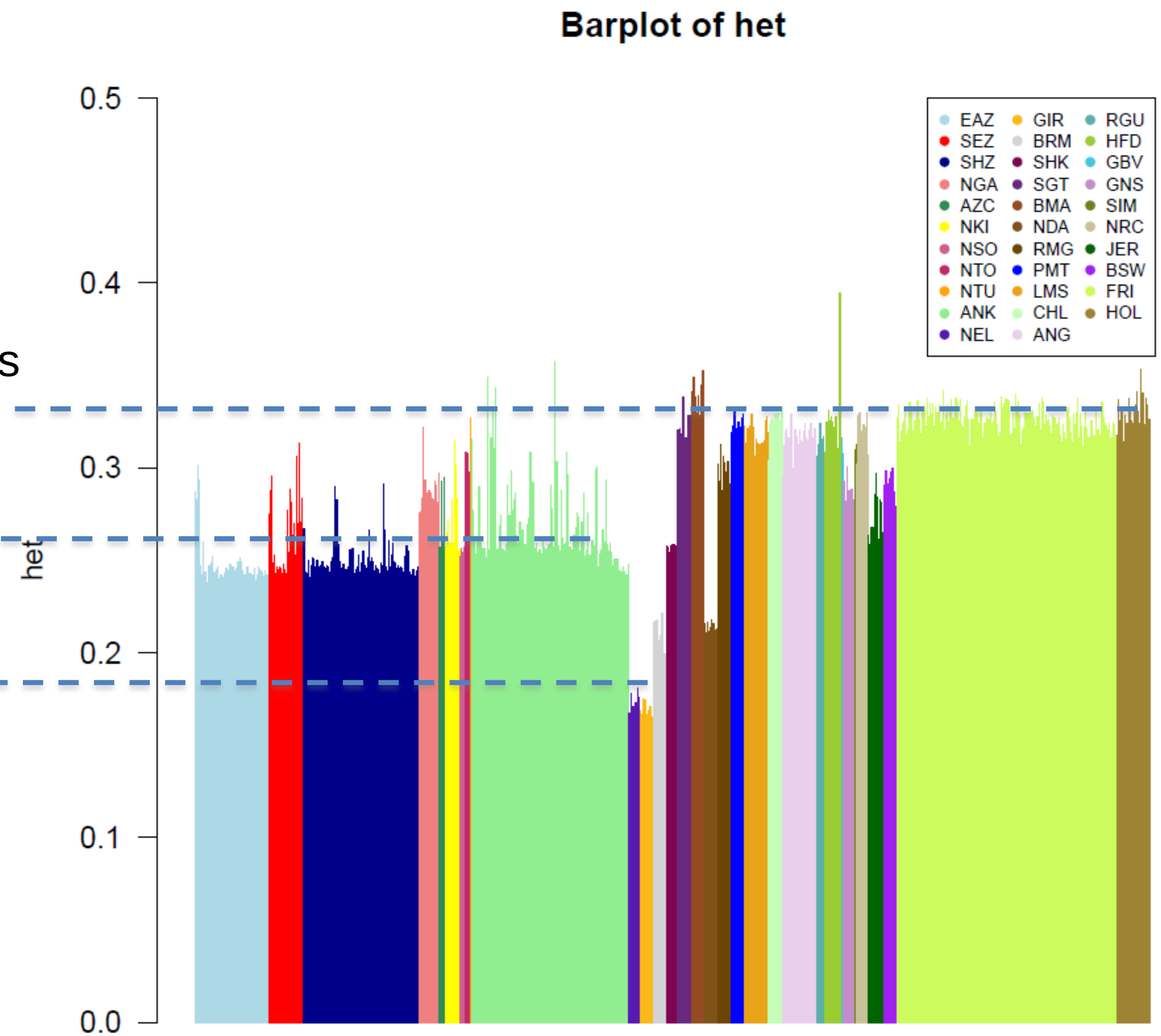
Ugandan cattle + 22 other breeds (17 taurine; 3 indicine; 2 crosses) = 31 breeds, 1765 individuals.

n. samples	label	breed	project	species
62	ANG	Angus	Hapmap	*B. taurus*
300	ANK	Ankole	Nextgen	*B. taurus*
11	AZC	Ankole Zebu Cross	Nextgen	*taurus x indicus*
24	BMA	Beefmaster	Hapmap	*taurus x indicus*
25	BRM	Brahman	Hapmap	*B. indicus*
24	BSW	Brown Swiss	Hapmap	*B. taurus*
26	CHL	Charolaise	Hapmap	*B. taurus*
135	EAZ	East African Shorthorn Zebu	Nextgen	*B. indicus*
400	FRI	Frisona Italiana	Selmol + Prozoo	*B. taurus*
3	GBV	Gelbvieh	Hapmap	*B. taurus*
24	GIR	Gir	Hapmap	*B. indicus*
21	GNS	Guernsey	Hapmap	*B. taurus*
31	HFD	Hereford	Hapmap	*B. taurus*
64	HOL	Holstein Friesian	Hapmap	*B. taurus*
28	JER	Jersey	Hapmap	*B. taurus*
44	LMS	Limousine	Hapmap	*B. taurus*
25	NDA	Ndama	Hapmap	*B. taurus*
24	NEL	Nelore	Hapmap	*B. indicus*
37	NGA	Nganda	Nextgen	*taurus x indicus*
28	NKI	Nkiga	Nextgen	*B. taurus*
21	NRC	Norwegian Red	Hapmap	*B. taurus*
10	NSO	Nsongora	Nextgen	*B. taurus*
10	NTO	Ntoro	Nextgen	*B. taurus*
24	PMT	Piemontese	Hapmap	*B. taurus*
15	RGU	Red Angus	Hapmap	*B. taurus*
24	RMG	Romagnola	Hapmap	*B. taurus*
65	SEZ	Small East African Zebu	Nextgen	*B. indicus*
24	SGT	Santa Gertrudis	Hapmap	*taurus x indicus*
20	SHK	Sheko	Hapmap	*B. taurus*
212	SHZ	Shorthorn Zebu	Nextgen	*B. indicus*
3	SIM	Simmental	Hapmap	*B. taurus*

Quality Control thresholds:
- SNP call rate = 0.95
- individual call rate= 0.95
- Minor Allele Frequency (MAF)= 0.01

Cured dataset:
1738 individuals x 41221 markers

NEXTGEN + OTHER PROJECTS, 54K: OBSERVED HETEROZYGOSITY



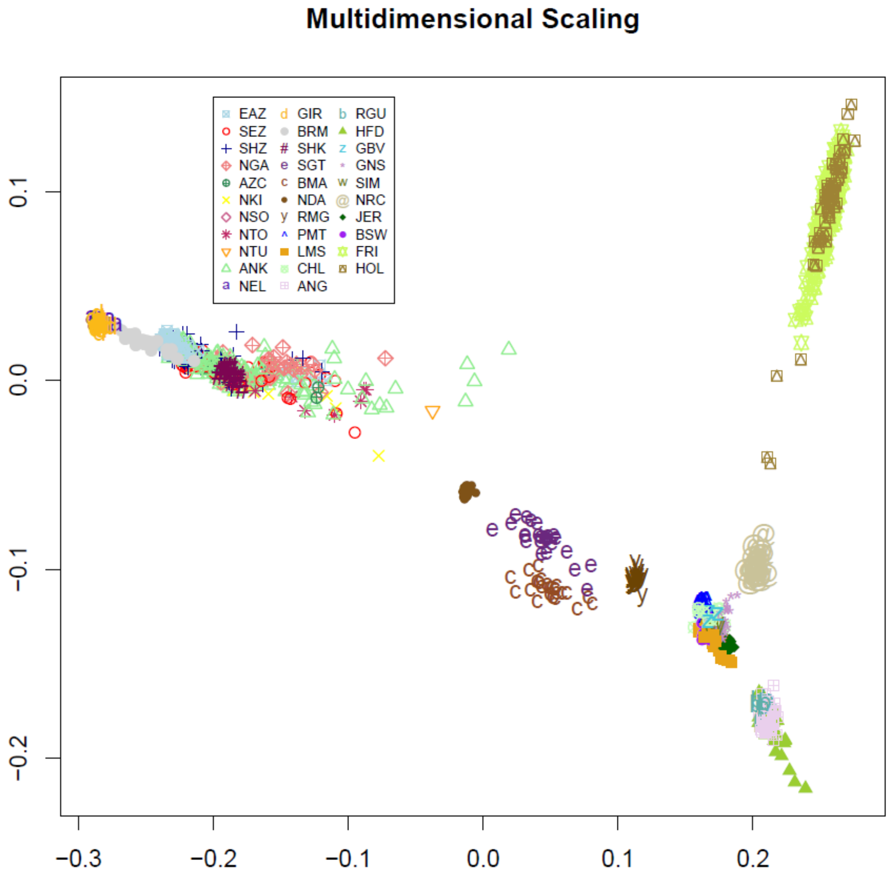
European bos taurus

African taurus and indicus

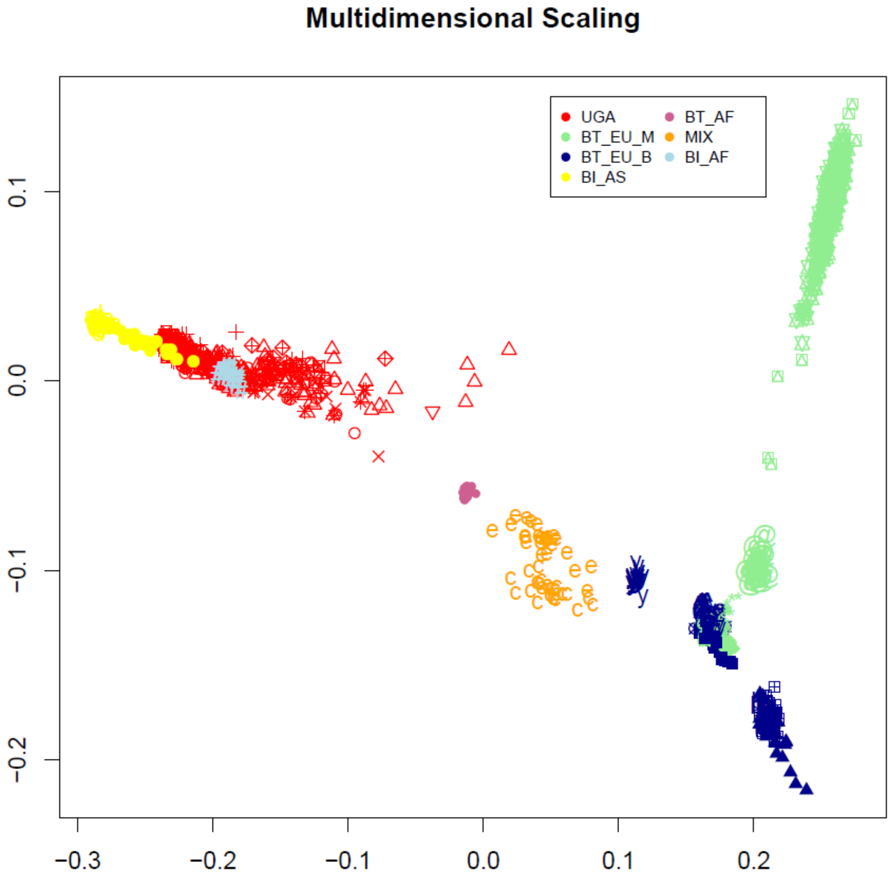
Indian indicus

NEXTGEN + OTHER PROJECTS, 54K: MULTI DIMENSIONAL SCALING

MDS plots based on IBS distances = pairwise proportions of alleles not identical by state.



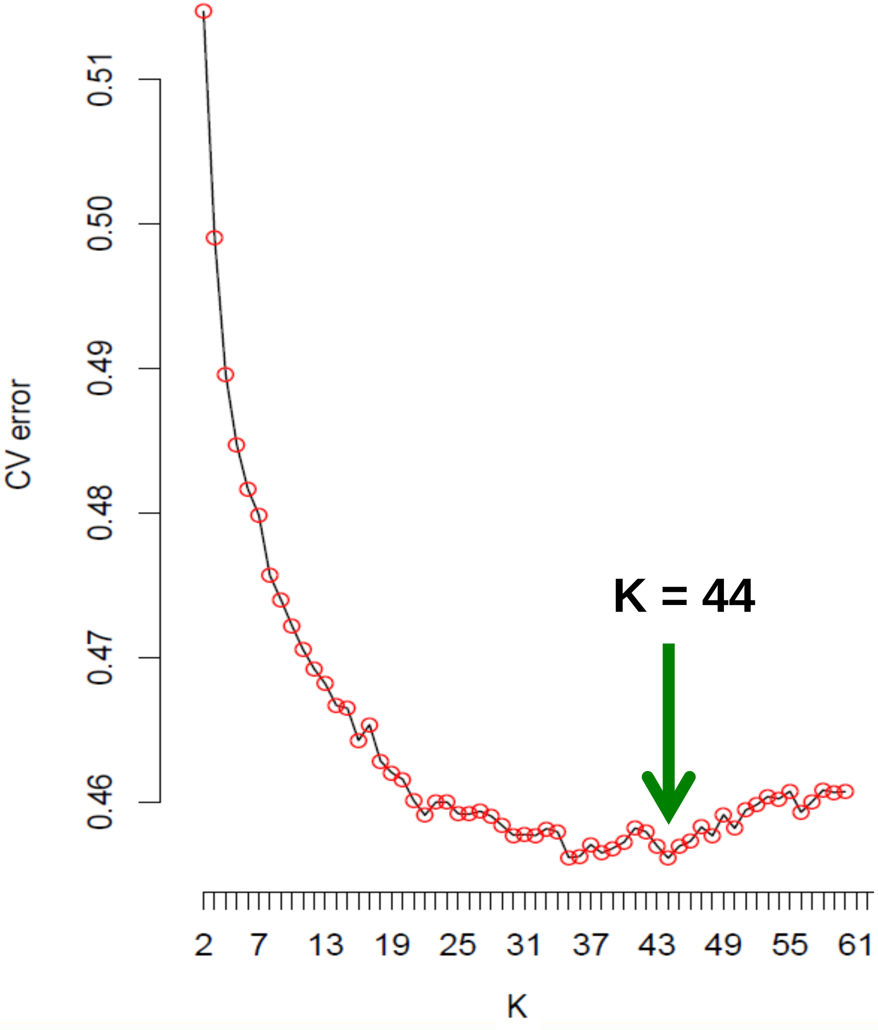
Single breeds



Groups / provenance / breed main purpose

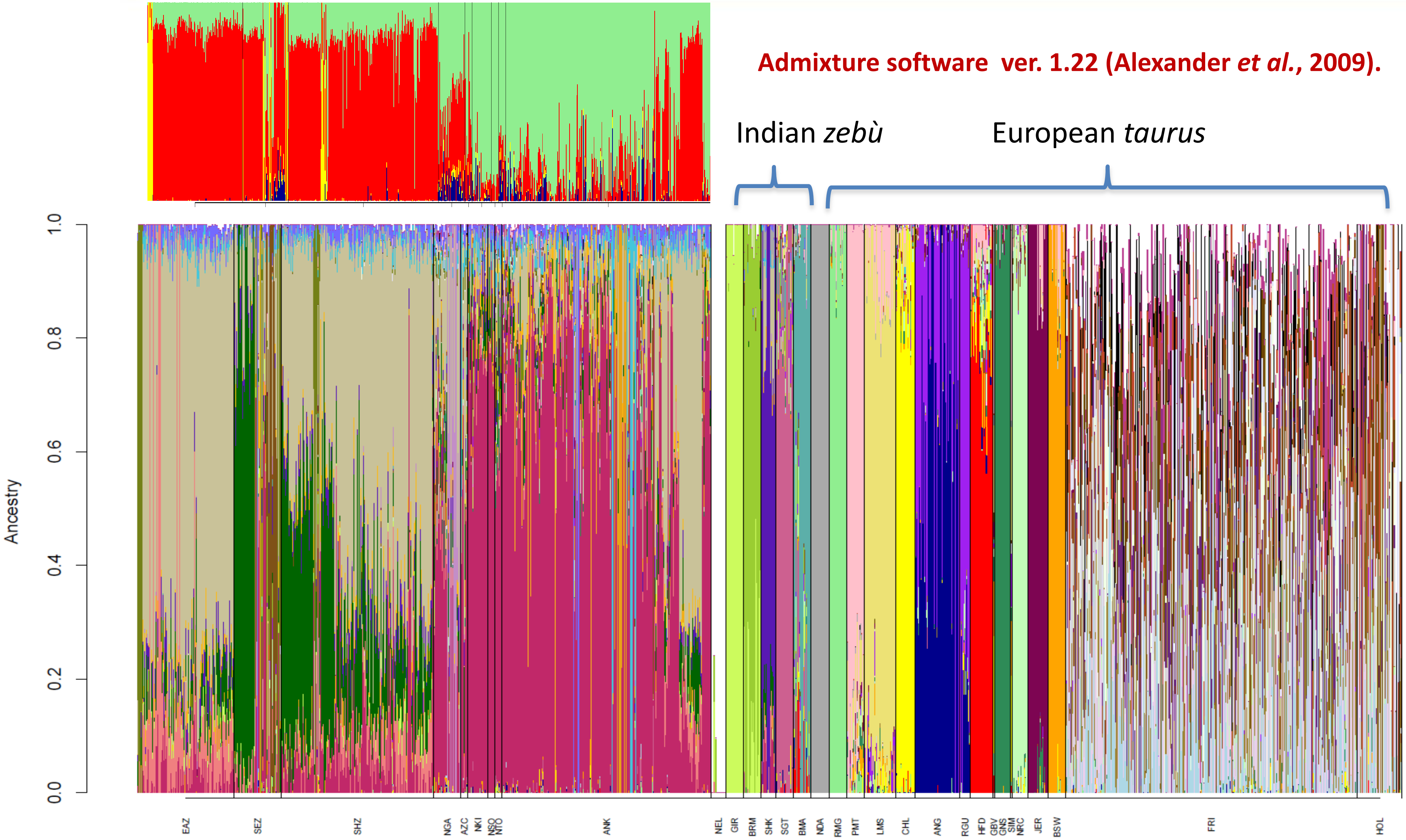
NEXTGEN + OTHER PROJECTS, 54K:
CROSS VALIDATION ERROR OF ADMIXTURE RESULTS

Admixture software ver. 1.22 (Alexander *et al.*, 2009): K from 2 to 60.



Alexander et al., 2009. Genome Research, 19: 1655 – 1664.

NEXTGEN + OTHER PROJECTS, 54K: ADMIXTURE ANALYSIS K = 44



Admixture software ver. 1.22 (Alexander *et al.*, 2009).

CONCLUSIONS:

- Up to now **lack of evidence of any introgression from the European cattle breeds**
- **54K SNP chip is affected by ascertainment bias** (varying degrees in EU taurines, Ankole group, Ugandan zebu, other zebu)
- The **main population structure** of Ugandan cattle **is consistent across datasets** (54K-U + IT Friesian, 54K-U + other projects) and comprises **three major genomic components** of probable **African indicine/taurine origin and a still unknown genomic component geographically well localized**

NEXT STEPS

1- Try to *in silico* correct the ascertainment bias (e.g. Guillot and Foll 2009; Nielsen R, Signorovitch J. 2003)

2- Complete the Elisa test and start comparing healthy and sick animals to pinpoint region (and genes) carrying signature of selection and search for significant spatial associations between the genomic footprints of selection and diseases

3- Pinpoint the origin of the mysterious genomic component

4- Share with you the results at the 65° EAAP meeting in Copenhagen

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SELMOL, PROZOO and Bovine HAPMAP projects.

THANK YOU for your attention!