

## Supplementary Online Material for

### Multiple paternal origins of domestic cattle revealed by Y-specific interspersed multilocus microsatellites

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#### This PDF file includes:

**Supplementary Table S1:** Description of sampling.

**Supplementary Table S3:** Consistency with the haplogroups previously identified by Götherström et al. (2005).

**Supplementary Figure S1:** Detailed representation of cattle Y-chromosome clades.

**Supplementary Figure S2:** Electropherograms showing paternal compatibility of the two Y-specific IMMs used.

#### References

**Supplementary Table S2**, containing all the genotypes and haplotypes obtained, is provided in a separated Excel file.

**Supplementary Table S1** Distribution of the 453 male individuals analysed per continent, country of origin and breed or zone of sampling. The number of samples per haplogroup (Y1, Y2 and Y3) and breed is also detailed. Additionally, each breed is assigned to a given geographical area: a) Atlantic Europe (103 samples); b) Continental Europe (248 samples); c) Mediterranean Africa (12 samples); d) Sub-Saharan Africa (32 samples); e) India (33 samples); and f) Rest of Asia (26 samples).

Further information on sampling:

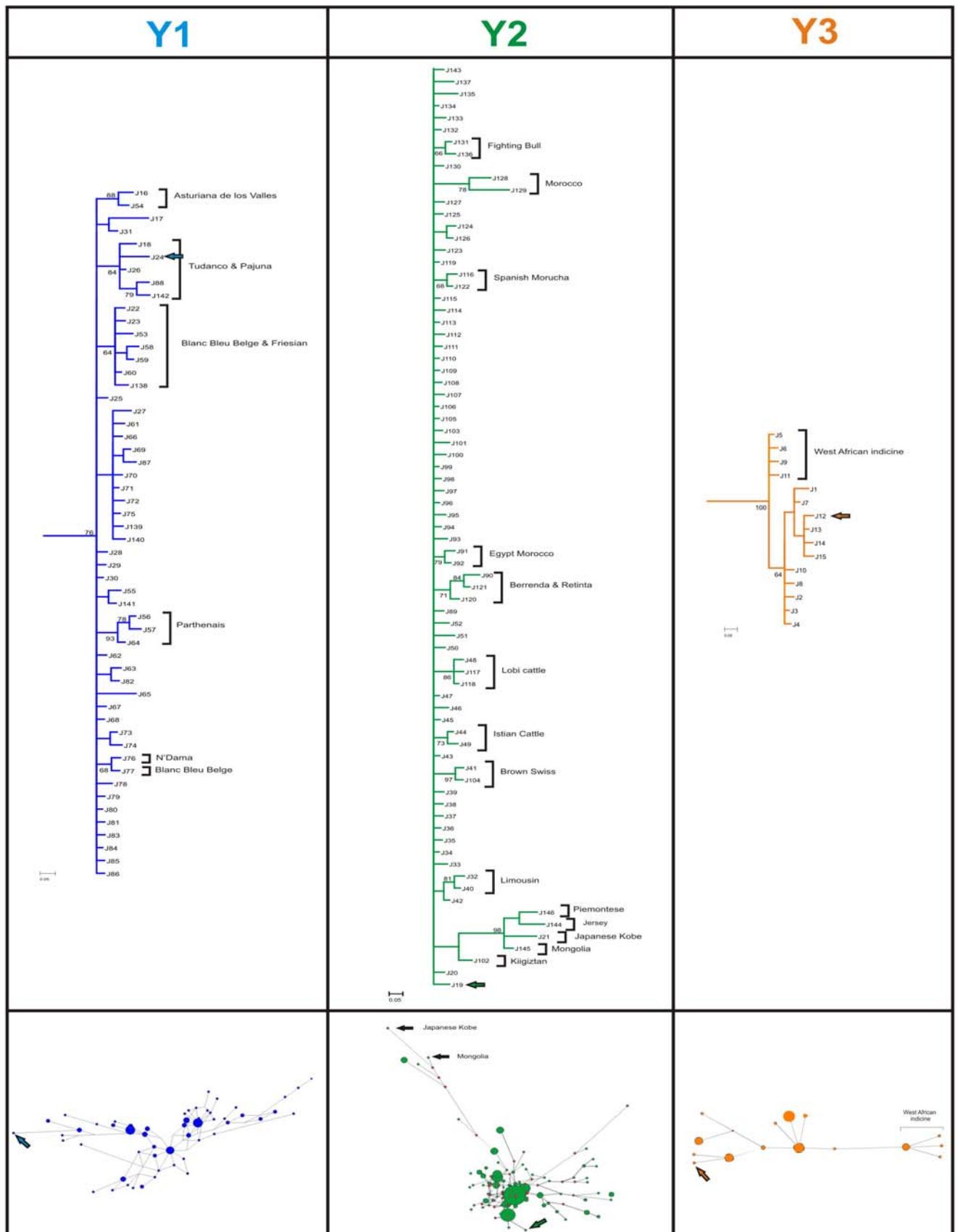
- a) Brahman samples were obtained from Argentinean populations. However, they are assigned to Asia.
- b) The samples obtained in Central India were basically sampled from the Maharashtra and Rajstan provinces of India
- c) The N'Dama samples were obtained from 2 ranches of the JVL Company located in the Kolo province of Congo. The history of these herds started in 1927 with the first acquisition of 10 local bovine heads (mixed Afrikander, Baoulé, and Devon cattle) and 50 heads of Barotsé cattle from North-East Angola. From 1930, 2 bulls and 40 heifers belonging to N'dama cattle breed were directly imported from Guinea (Fouta-Djalou) to sequentially replace the more than 200 extant heads due to very poor performance. From this year replacement was selected according to performance and N'Dama phenotype. In 1945 the herd numbered 12,000 heads and consists mainly of healthy and sturdy cattle with a strong predominance of the N'dama phenotype. At present the two JVL ranches keep more than 40,000 N'Dama heads.
- d) The Holstein Friesian Samples mainly consisted on sires born in Spain, France, Italy and The Netherlands. However, their pedigrees could be traced back to Canada and U.S.A. To avoid inconsistencies the Holstein samples were assigned to The Netherlands, in accordance with the ancestral origin of this cattle.
- e) The Jersey samples were basically obtained from Belgium. A few samples from the Danish Jersey population were also analyzed.

Continent	Country		Breed_area		Haplogroup frequency			Geographic Area
	Name	Frequency	Name	Frequency	Y1	Y2	Y3	
<b>AFRICA</b>	Burkina Faso	23	Lobi	23	0	8	15	Sub-Saharan Africa
	Congo	7	N'Dama	7	1	6	0	Sub-Saharan Africa
	Egypt	1	Egypt	1	0	1	0	Mediterranean Africa
	Morocco	11	BVOI	6	1	5	0	Mediterranean Africa
			BVTd	5	0	5	0	Mediterranean Africa
	Mozambique	2	Landim	2	2	0	0	Sub-Saharan Africa
<b>ASIA</b>	India	33	Brahman	7	0	0	7	India
			Central India	8	0	0	8	India
			NorthEast India	8	0	0	8	India
			Southern India	10	0	0	10	India
	Japan	1	Kobe	1	0	1	0	Rest of Asia
	Kazakstan	5	Kazakstan	5	2	2	1	Rest of Asia
	Kirgizstan	5	Kirgizstan	5	1	4	0	Rest of Asia

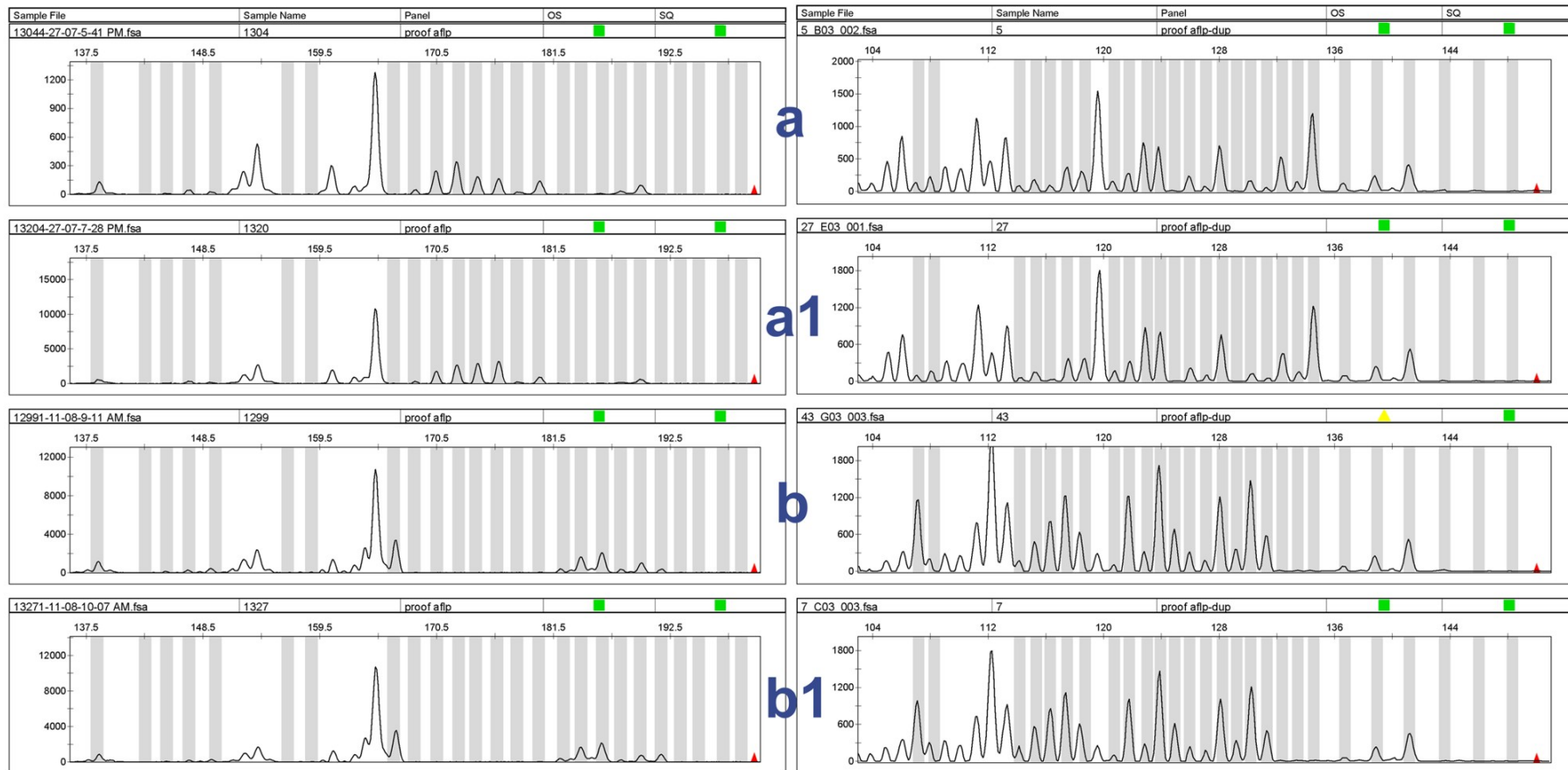
<b>EUROPE</b>	Mongolia	1	Mongolia	1	0	1	0	Rest of Asia
	Turkmenistan	6	Turkmenistan	6	2	2	2	Rest of Asia
	Yemen	8	Yemen	8	0	0	8	Rest of Asia
	Austria	51	Brown Swiss	15	0	15	0	Continental Europe
			Fleckvieh	6	0	6	0	Continental Europe
			Pinzgauer	9	0	9	0	Continental Europe
			Tyrolean Grey	21	0	21	0	Continental Europe
	Belgium	14	Blanc Bleu Belge	9	9	0	0	Atlantic Europe
			Jersey	5	0	5	0	Atlantic Europe
	Croatia	18	Istrian	5	0	5	0	Continental Europe
			Simmental	13	3	10	0	Continental Europe
	Denmark	6	Danish Red	6	5	1	0	Atlantic Europe
	France	64	Blonde d'Aquitaine	5	0	5	0	Continental Europe
			Charolais	15	0	15	0	Continental Europe
			Limousin	4	0	4	0	Continental Europe
			Normande	10	10	0	0	Continental Europe
			Parthenais	10	3	7	0	Continental Europe
			Salers	10	0	10	0	Continental Europe
			Tarentaise	10	0	10	0	Continental Europe
	Germany	10	European Friesian	10	10	0	0	Atlantic Europe
	The Netherdlands	9	Holstein	9	9	0	0	Atlantic Europe
	Ireland	3	Dexter	3	3	0	0	Atlantic Europe
	Italy	40	Chianina	18	0	18	0	Continental Europe
			Maremmana	17	0	17	0	Continental Europe
			Piemontese	5	0	5	0	Continental Europe
	Spain	121	Asturiana de la Montaña	10	9	1	0	Atlantic Europe
			Asturiana de los Valles	29	21	8	0	Atlantic Europe
			Berrenda	5	0	5	0	Continental Europe
			Betizu	9	0	9	0	Continental Europe
			Fighting Bull	12	1	11	0	Continental Europe
			Morucha	12	0	12	0	Continental Europe
			Pajuna	4	1	3	0	Continental Europe
			Parda Alpina	8	0	8	0	Continental Europe
			Pirenáica	8	0	8	0	Continental Europe
			Retinta	6	0	6	0	Continental Europe
			Rubia Gallega	11	0	11	0	Continental Europe
			Tudanco	7	7	0	0	Atlantic Europe
	UK	15	Angus	8	8	0	0	Atlantic Europe
			Hereford	7	6	1	0	Atlantic Europe

**Supplementary Table S3** Summary of the test of the consistency between the cattle Y-chromosome haplogroups identified in this study using IMM markers with those previously identified by Götherström et al. (2005) using Y-specific loci. One sample belonging to each of the 146 haplotypes identified using IMMs was genotyped for two out of the five sites reported by these authors to avoid redundant information: zfy9 and zfy10indel. Both markers were amplified using primer pairs as described by Ginja et al. (2008) in Table 1. zfy9 amplicons were purified with Exosap (Amersham) and sequenced using Big Dye Terminator v3.1 kit (Applied Biosystems, Foster City, CA) on an ABI310 DNA sequencer (Applied Biosystems) and analyzed with Analyses 5.2 software (Applied Biosystems). zfy10indel was genotyped on an ABI3130 DNA sequencer (Applied Biosystems) and analyzed with GeneMapper software (Applied Biosystems). A summary of the obtained results is given in the table below. All samples belonging to each Y-chromosome haplogroups identified using IMMs gave the same genotype which in turn, was fully consistent with those defined by Götherström et al. (2005) as haplogroups Y1, Y2 and Y3.

Haplogroup	locus	
	zfy9	zfy10indel
Y1	C	--
Y2	C	GT
Y3	T	GT



**SupplementaryFigure S1** Detailed representation of cattle Y-chromosome clades (Y1, in blue; Y2, in green; and Y3 in orange) identified using the program MrBayes 3.1 and Network 4.1.1. Computations are described in the legend of the Figure 1 of the manuscript. Posterior probabilities higher than 0.60 are given to illustrate the statistical confidence on branching order. The breeds on which the haplotypes identified were found are also identified, pointing out different local diversity events. Examples of the latter are the zebuine haplotypes of the West African Lobi cattle within the Y3 cluster, the Spanish Cantabrian, Belgian Blue and European Friesian samples within the Y1 cluster and the Brown Swiss, Istrian, Southern Spanish or Mediterranean African samples within cluster Y2. Arrows without legend identify ancestral haplotypes within haplogroups according to internal number of square differences ( $d^2$ ; see Materials and methods and legend of Table 2).



**Supplementary Figure S2** Electropherograms showing paternal compatibility of the two Y-specific IMMs used for phylogenetic analyses in cattle: UMN2405, on the left; and UMN2303, on the right. Fathers are indicated with a letter and sons are identified with the corresponding letter of the father and the number 1. Grey bars represent the loci assessed as polymorphic across the whole samples used. Following Pérez-Pardal et al. (2009), amplification on female DNA and paternal compatibility was tested on two pedigrees belonging to the Asturiana de los Valles beef cattle breed. They consisted on: a) 1 sire and 3 male offspring; and b) 1 sire and 1 male offspring. Additionally, a couple of sire cousins and a couple of sire sibs belonging to two well-known Holstein grandfather families were tested (Figure S3). In all families used, paternity was confirmed with the 11 genomic StockMarks Kit for Cattle Bovine Genotyping (Applied Biosystems Inc., Foster City, CA).

## References

- Ginja C, Telo da Gama L, Penedo MCT (2008). Y Chromosome haplotype analysis in Portuguese cattle breeds using SNPs and STRs. *J. Hered.* **100**: 148-157.
- Götherström A, Anderung C, Hellborg L, Elburg R, Smith C, Bradley DG *et al.* (2005). Cattle domestication in the Near East was followed by hybridization with aurochs bulls in Europe. *Proc. R. Soc. B* **272**: 2345–2350.
- Pérez-Pardal L, Royo LJ, Álvarez I, Ponce de León FA, Fernández I, Casais R *et al.* (2009). Female segregation patterns of the putative Y-chromosome specific microsatellite markers INRA124 and INRA126 do not support their use for cattle population studies. *Anim. Genet.* **40**: 560-564.