Supplementary Materials

Historical information

The analysis of the Y chromosome of the Bourbons also puts an end to rumors that the branch of Bourbon Orleans would be illegitimate. Indeed, at the height of the Revolution, Louis Philippe Joseph d'Orléans (1747-1793) - aka "Philippe Egalité" - had declared himself to be the result of an adulterous affair of his mother, Louise-Henriette de Bourbon-Conti, with a coachman named Lefranc. During the next generation, a certain Maria Stella, baronne de Sternberg, will claim to be the true heiress of Orleans, exchanged against the future king Louis-Philippe, who supposedly would be the child of an Italian jailer Lorenzo Chiappini. Both legends were false. These DNA results also serve to confirm that Louis XIV and his brother Philippe d'Orléans were indeed sons of the same father, most probably the King Louis XIII, and not of supposed and different lovers of Queen Anne of Austria - as Mazarin, Rochefort or Rivière [1].

Supplementary tables

Supplementary Table S1 Primer sequences and concentrations for the analysis of the main genotyped Y-SNPs for R1b in this study with for a) PCR amplification and b) Single-base extension.

a)

			PCR amplification				
Locus	**Haplogroup**	**Mutation**	**Primer sequences (5’-3’)**			**Conc. (µM)**	**Amplicon size (bp)**
M343	R1b	C->A	F	M343-F	CGTAGCCCGAGAGAAAACTG	0.300	90
			R	M343-R	CCCCACATATCTCCAGGTGT	0.300	
P310=S129	R1b1b2a	A->C	F	P310-F	GAAAAGTGTGCAACCAGCAC	0.300	109
			R	P310-R	CCATTGTCTCTGCAGCTCAC	0.300	
P297	R1b1b	G->C	F	P297-F	GGTGGTGGGAGAGAGAGAGA	0.300	124
			R	P297-R	TTGGAAAGGAACATAGCCAAA	0.300	
M335	R1b1c	T->A	F	M335-F	ACGGTGACGAATGTCTGAACGGCACAGTACTCACTTTAGGTTTGC	0.300	148
			R	M335-R	GGTCTCTGGACACCTGGCATCGTGTTCTAAATGCCCTTGAATTG	0.300	
M160	R1b1b2a2g2	A->C		Locus M73		0.300	98
P312	R1b1b2a2	C->A	F	P312-F	GACGGTGACGAATGTCTGAACGTTGGGCAACTTTGGAATACG	0.300	170
			R	P312-R	GGTCTCTGGACACCTGGCATCGGTGGAGTTGGGGCTAAAGTG	0.300	
M269	R1b1b2	T->C	F	M269-F	AAGGGGAATGATCAGGGTTT	0.300	103
			R	M269-R	GCCCGGCCACTATACTTCTT	0.300	
U106=S21	R1b1b2a1	C->T	F	U106-F	GCTCTGGTGCATAGGGATTC	0.300	83
			R	U106-R	GGGAAGGCAGGTATTCAGAG	0.300	
M73	R1b1b1	ins->2bp del (G->T)	F	M73-F	AAAACAATAGTTCCAAAAACTTCTGA	0.300	98
			R	M73-R	CCTTTGTGATTCCTCTGAACG	0.300	
M18	R1b1a	del->AA ins (T->A)	F	M18-F	AAACGGTGACGAATGTCTGAACGTCACCAGAGTTTGTGGTTGC	0.300	130
			R	M18-R	GGTCTCTGGACACCTGGCATCGTCACAAAAATAGTTTGGCCACTT	0.300	
P66	R1b1b2a2f	C->T	F	P66-F	GGACCATCACCTGGGTAAAGT	0.300	197
			R	P66-R	GATCCCAGGAAGGAGAATCA	0.300	
U198=S29=M467	R1b1b2a1a	G->A	F	U198-F	ATTTACCTGCACTCCCACCA	0.300	168
			R	U198-R	TGAGGCTCAGTTTCAAATGC	0.300	

b)

Single-base extension					
Locus	**Primer sequence (5’-3’)**	**Conc. (µM)**	**Length (nt)**	**Orien-tation**	**Alleles (dye)**
M343-SBE	CCCCACATATCTCCAGGTGT	0.150	20	R	C (blue), A (red)
P310-SBE	CTGCTTAGCTCCACCATTCA	0.250	20	R	A (red), C (blue)
P297-SBE2	TTAATGACTTTTTAGGGTCAGGAATCT	0.250	27	F	G (blue), C (yellow)
M335-SBE	TTCTAAATGCCCTTGAATTGTAAGAAA	0.250	27	R	T (green), A (red)
M160-SBE	ACAAGTTTAATACATACAACTTCAATTTTC	0.250	30	F	A (green), C (yellow)
P312-SBE2	TGTCTGAACGGGAGTTGGGGCTAAAGTGAAAG	0.250	32	R	C (blue), A (red)
M269-SBE	AATGTCTGAACGGGAATGATCAGGGTTTGGTTAAT	0.250	35	F	T (red), C (yellow)
U106-SBE	AATGTCTGAACGGGAATGATCAGGGTTTGGTTAAT	0.250	35	R	C (blue), T (green)
M73-SBE	TGACGAATGTCTGAACGTGATTCCTCTGAACGTCTAACCA	0.250	40	R	G (yellow), T (green)
M18-SBE	ACGGTGACGAATGTCTGAACGAAAATTCACTTAAAAAAACCCCGT	0.250	45	R	T (green), A (red)
P66-SBE	TTTTTTTTACGGTGACGAATGTCTGAACGAGATCCCAGGAAGGAGAATCA	0.250	50	R	G (blue), A (green)
U198-SBE	GACTGACTGACTGACTGACTGACTGACTGACTTCATCATTGCATGGGTATAAC	0.250	55	F	G (blue), A (green)

Supplementary Table S2 Mutation rate of all genotyped Y-STRs based on Ballantyne et al. [2] and Chandler [3]. The Y-STRs are given in descending order of the mutation rate. The Y-STRs wherefore different alleles were found between the three living donors of the House of Bourbon are given in bold.

Y-STR	Mutation rate
DYS724a,b	**3.50E-02**
DYS576	1.43E-02
DYS570	**1.24E-02**
DYS449	**1.22E-02**
DYS442	9.78E-03
DYS458	8.36E-03
DYS464a,b,c,d	7.27E-03
DYS460	6.22E-03
DYS389a	**5.51E-03**
DYS456	4.94E-03
DYS19	4.37E-03
DYS385b	4.14E-03
DYS607	4.11E-03
DYS635	3.85E-03
DYS439	3.84E-03
DYS389b	3.83E-03
DYS391	3.23E-03
GATA H4	3.22E-03
DYS459a,b	2.67E-03
DYS447	2.12E-03
DYS393	2.11E-03
DYS385a	2.08E-03
DYS437	1.53E-03
DYS390	1.52E-03
YCAIIa,b	1.23E-03
DYS392	9.70E-04
DYS438	9.56E-04
DYS454	4.75E-04
DYS455	4.26E-04
DYS388	4.25E-04
DYS426	3.98E-04
DYS448	3.94E-04

References

1 Delorme P: Anne d'Autriche. Paris, Pygmalion, 1999.

2 Ballantyne KN, Goedbloed M, Fang RX *et al*: Mutability of Y-chromosomal microsatellites: rates, characteristics, molecular bases, and forensic implications. *American Journal of Human Genetics* 2010; **87**: 341-353.

3 Chandler JF: Estimating per-locus mutation rates. *Journal of Genetic Genealogy* 2006; **2**: 27-33.