

# Haplogroup of the Y Chromosome of Napoléon the First

Gérard Lucotte (Corresponding author)

Institute of Molecular Anthropology

44 Monge street, Paris 75005, France

Tel: 33-698-829-261 E-mail: Lucotte@hotmail.com

Thierry Thomasset

Laboratory RX, USTL, Center of Transfert

66 de Landshut Avenue, Compiègne 50201, France

Peter Hrechdakian

Unifert Group S.A., 54 Louise Avenue, Immeuble Stéphanie

Bruxelles 1050, Belgium

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## Abstract

This paper describes the finding of the determination of the Y-haplogroup of French Emperor Napoléon I (Napoléon Bonaparte). DNA was extracted from two islands of follicular sheaths located at the basis of two of his beard hairs, conserved in the Vivant-Denon reliquary. The Y-haplogroup of Napoléon I, determined by the study of 10 NRY-SNPs (non-recombinant Y-single nucleotide polymorphisms), is E1b1b1c1\*. Charles Napoléon, the current collateral male descendant of Napoléon I, belongs to this same Y-haplogroup; his Y-STR profile was determined by using a set of 37 NRY-STRs (non-recombinant Y-microsatellites).

**Keywords:** Napoléon the First, Beard hairs, Ancient DNA, Y-chromosome haplogroup, Y-STR profile

## 1. Introduction

The genetic identification of old biological specimens is limited to the analysis of short, degraded DNA fragments, but the development and application of comprehensive DNA testing for identification of historical samples is of considerable interest. These sorts of studies, for those concerning French dynasties, include the analysis of the heart of Louis XVII, son of Louis XVI, king of France (Jehaes *et al.*, 2001), and the genetic analysis of the presumptive blood of Louis XVI (Lalueza-Fox *et al.*, 2010). I have recently (Lucotte, 2010) described the finding of a rare variant (16184C>T) in the sequence of the hypervariable segment (HVS1) of mitochondrial DNA (mtDNA) extracted from two preserved hairs conserved in the Vivant-Denon reliquary, authenticated as belonging to French Emperor Napoléon I (Napoléon Bonaparte ; 1769-1821). We are now reporting now the precise haplogroup of the Y chromosome of Napoléon I, based on DNA extracted from cell debris adherent to two of his beard hairs, conserved in the reliquary.

## 2. Materials and Methods

### 2.1 Preserved beard hairs

The Vivant-Denon reliquary (deposited in the Bertrand Museum of Châteauroux) contains in his right lateral compartment an authenticated lock of Napoléon's own hairs (two of them having been used to detect the rare variant of the mtDNA sequence). It also contains, in the lower part of the corresponding compartment, at least three beard hairs. Examined by electronic microscopy (SEM), these beard hairs differ from head hairs in their important diameters, their angular sections, and in the fineness of their scales (which corresponds to a more rapid growth).

## 2.2 SEM and energy dispersive X-ray

The samples were observed by scanning electron microscopy (SEM), without any preparation. The observations were conducted using a Philips XL30 instrument, equipped with a Bruker AXS energy dispersive X-ray (EDX). The system of analysis is PGT (Spirit Model, of Princeton Gamma Technology).

## 2.3 DNA extraction

DNA was extracted from the two calcium-phosphate rich islands observed at the basis two of the three beard hairs, using an efficient at-home, long and manual procedure, previously published (Gautreau *et al.*, 1983). Briefly, the two island samples were separately incubated overnight at 50°C in a lysis solution (0.5% SDS, 50 mM TRIS and 100 µg/µl of proteinase K in sterile H<sub>2</sub>O). Subsequently the DNAs were extracted by three successive phenol-chloroform extractions, and concentrated by using a Centricon-30 filter column (Millipore) up to a 10 µl volume. To control any potential DNA contamination, an extraction control was prepared at the start of the procedure.

## 2.4 Y-chromosome markers and the Y haplogroup

The amelogenin test was realized with the AmpFISTR Identifiler PCR amplification kit (AmpFISTR Yfiler™). Y microsatellites (Y-STRs) genotyping were determined as previously published for DYS19 (Roewer *et al.*, 1992), and for XCAIIa and YCAIIb (Mathias *et al.*, 1994). The pre –and post-PCR steps were carried out in physically separated laboratories, to avoid cross-contamination; the working conditions (concerning gloves, facemasks, pipette tips, microcentrifuge and PCR tubes) are those previously described (Lucotte, 2010).

Ten single nucleotide polymorphisms (Y-SNPs) were used to establish the Y-haplogroup; they were genotyped in the following hierarchical order: M125, M174, M35, M33, M123, M81 and M78 at first (to determinate the basal branches of the phylogenetic tree defining the major clades), and then M34, M84 and M290 (to determine the terminal differentiation).

The non-recombinant part of the Y-chromosome (NRY) haplogroup was deduced from genotyping by PCR of the NRY-SNPs, according to the most recently described (YCC 2008) marker phylogeny (Karafet *et al.*, 2008). I was the only one experimentator in this study, and my Y-haplogroup is R1b.

## 2.5 Examination of the current descendant of Napoléon's family

The current descendant (Ch. N.) of Napoléon buccal smear DNA was genotyped for the first 37 genetic markers of the Family Tree DNA (FTDNA) kit, in order to establish his Y-STR profile; these Y-STRs markers are, in order: DYS393, 390, 19, 391, 385a, 385b, 426, 388, 439, 389-1, 392, 389-2, 458, 459a, 459b, 455, 454, 447, 437, 448, 449, 464a, 464b, 464c, 464d, 460, GATAH4, YCAIIa, YCAIIb, 456, 607, 576, 570, CDYa, CDYb, 442 and 438. Ch. N. DNA was also genotyped for three Y-SNPs of the terminal differentiation (M34, M84 and M290).

## 3. Results

The examination of the three beard hairs shows that they are, in some parts, completely covered by small microparticles and micels of a potassic soap (the shaving soap), analyzed by energy dispersive X-ray. On the edge of the cutted sections of these hairs, there are some micro-fragments of iron of industrial type (without manganese and chromium), which correspond to micro-debris of the razor used to cut the beard.

There is little doubt that the shaving and cutting of these beard hairs were realized *post-mortem*, because at the basis of two of the three hairs we observe by SEM relatively voluminous extremities of blades of corneous and desiccated tissues (that must correspond to upper extremities of follicular sheath surrounding bulbs). The analysis of these pieces by X-ray shows that they are calcium-phosphate-rich (which correspond to corneous and desiccated structures); these islands of tissues contain some blood cells, well observable by SEM (Figure 1).

DNA was extracted from the two islands (one for each of the two beard hairs). An estimated DNA quantity of 32 ng and 21 ng of total DNA were obtained from each of these two islands (i1 and i2, respectively). All the subsequent analysis of these two DNAs were realized in parallel and gave exactly the same results for the genetic markers used.

The first Y-specific probe used was that of the amelogenin gene; that confirms that the DNAs tested correspond to a male individual (XY). The following three markers used were the Y-STRs DYS19, YCAIIa and YCAIIb; they gave allelic values of 13, 19 and 22, respectively (Table 1). Computed with the Whit Athey's haplogroup predictor program (Athey, 2006), these three values considered together estimate that this male individual corresponds (with a probability value of 76.5%) to one belonging to the Y haplogroup E1b1b.

Figure 2 summarizes the results we obtain concerning the Y-haplotype of this male individual belonging to the

E1b1b haplogroup; the ten Y-SNPs were genotyped by PCR: this individual is M215<sup>+</sup>, but M174<sup>-</sup>; being M33<sup>-</sup> and M35<sup>+</sup>, his DNA belongs to the E1b1b1 cluster; it belongs to the E1b1b1c sub-haplogroup, according to the last published system of Y-Chromosomal Haplogroup Tree Nomenclature.

More precisely this individual, being M34<sup>+</sup> and M84<sup>-</sup>, M290<sup>-</sup>, is classified as being E1b1b1c1\*(and not as E1b1b1c1a\*-M84, nor E1b1b1b-M290).

In order to verify this Napoléon I Y-haplogroup, we chose to study the NRY-DNA of the current male descendant of his family. Prince Charles Napoléon (Ch.N.), born in 1950, is the actual family head of the Napoléon I dynasty. Ch.N. is the elder son of Louis-Napoléon-Jérôme Bonaparte (1914-1997), the “Napoléon Prince”; Ch.N. is the 4<sup>th</sup> generation descendant of Jérôme Bonaparte (1784-1860), the “King of Westphalie”, the youngest brother of Napoléon I (Figure 3).

The Y-STR profile of Ch.N., based on 37 Y-STRs, is represented on Table 1. This profile is highly indicative of the E1b1b1 Y-haplogroup, Y-STRs allelic values at DYS19 (allele 13) and at DYS464a, DYS464b, DYS464c and at DYS464d (allele values of 14, 15, 16 and 17, respectively) being discriminant in the establishment of this Y-haplogroup. The allelic values for DYS19, DYSYCAIIa and DYSYCAIIb are the same for Ch.N. and for Napoléon I.

Ch.N. NRY-DNA is also M34<sup>+</sup>, M84<sup>-</sup> and M290<sup>-</sup> for the terminal markers of the haplogroup differentiation; so he belongs to the E1b1b1c1\* Y-haplotype, like Napoléon I.

#### 4. Discussion

We have shown, by Y-SNPs analysis on the basis of two of his beard hairs, that the Y-haplogroup of Napoléon I is E1b1b1c1\*. This result is confirmed by the study of the three Y-SNPs of the terminal haplogroup differentiation on Charles Napoléon, his current collateral male descendant. The use of 37 Y-STR markers permits us to establish the corresponding Y-STR profile.

The E1b1b1c1 (E-M34) haplogroup was found at small frequencies in North Africa and in Southern Europe (6.6 % in Sicily, for example) and has its highest concentration in Ethiopia and in the Near East (Cruciani *et al.*, 2004). According to the classification of the haplozone E3b Project by FTDNA, the more recent version being “Y-DNA haplogroup E and its subclades”, may 2008, the known haplogroup clusters of E1b1b1c1 are identified as E1b1b1c1\*-A (the “European” cluster, discovered among Germans and Spaniards), E1b1b1c1\*-B (the “Arabian” cluster, found among Arabs from Persian Gulf Countries), E1b1b1c1\*-C (the “British” cluster, found among the British and Irish), E1b1b1c1\*-D1 (the “Jewish” cluster, found among Askenazi) and E1b1b1c1\*-D2 (the “mixed” cluster, found both among Europeans and peoples of the Levant and Turkey).

The ages of these clusters were calculated (Aliiev *et al.*, 2010). The most plausible results can be expected when calculating the age of the E1b1b1c1\* cluster, whose samples were genotyped with numerous microsatellite markers. Its estimate age was 3850 ± 450 years. Parts of the descendants later migrated to Europe, which confirms the close age proximity of the E1b1b1c1\*-A cluster (equal to 3525 ± 650 years). Apparently the emergence of these two clusters were linked to the same period in the history of the Middle East. To determine the age of the common ancestor of all E1b1b1c1 members of the cluster, authors have compiled samples (with involvement of about 10 microsatellite markers) of Lebanese, Syrian, Palestinian and Turkish haplotypes. The modal haplotypes of all noted clusters and haplotypes are not related to any of the five previous clusters, and are designated as E1b1b1c1\*-miscellaneous. Age of the most recent common ancestor of all modern carriers of E1b1b1c1\* is 7000 ± 850 years. This sort of analysis suggests the presence of haplogroup E1b1b1c1 in the peoples of Western Asia Minor since V millennium B. C.

The masculine line of the Buonaparte family was from Tuscany, and the mother of Napoléon I (Laetizia Ramolino) was from Liguria. The 16184C>T mutation in that HVS1 sequence of mtDNA (or more exactly the mt-haplogroup containing this mutation) is probably characteristic of the maternal ancestors (Lucotte, 2010). The E1b1b1c1\* haplogroup of the Y chromosome must be characteristic of the paternal line. Napoléon I's paternal ancestors have been known since the 17<sup>th</sup> generation (Galantini, 2004). The most remote ancestor, Gianfardo, born and living in Sarzane (a little Italian town founded to the south of La Magra, a river that separated Liguria and Tuscany), between the end of the XII<sup>th</sup> and the beginning of the XIII<sup>th</sup> Century. Giovanni (11<sup>th</sup> generation), the only son of Cesare (married in 1440 to Apollonia Malaspina, Marquese of Verrucola, and Prior of Sarzane), was the first paternal ancestor of Napoléon I to leave Sarzane for Corsica. Giovanni was the Superintendent of the Saint-Martin palace of Fabrizio Colonna. Since Giovanni, nine generations of paternal ancestors had succeeded in Corsica (Francesco, Gabriele, Girolamo, Francesco, Sébastiano, Carlo, Giuseppe, Sébastiano and

Giuseppe) until Charles-Marie Buonaparte (born in 1746 and married to Laetizia in 1764), the father of Napoléon I.

Napoléon I himself knew his ancestry. In the St-Helen Memorial (Walter, 1956), he declared: “The mother of Pope Nicolas V originated from Sarzane; she was also a Buonaparte”. He confidentially said to Dr Francesco Antommarchi, his latest physician, about this male ancestry (Antommarchi, 1975): “My most remote ancestor, who inhabited Toscane, had the principles that I profess”. Probably Napoléon also knew his remote oriental patrilineal origins, because Francesco Buonaparte (the Giovanni son), who was a mercenary under the orders of the Genoa Republic in Ajaccio in 1490, was nicknamed “The Maur of Sarzane”. But, at this time (Tulard, 1999), the knowledge of his ancestry did not have the same importance as today.

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Table 1. Allelic values at 37 Y-STRs for Charles Napoléon NRY-DNA. Three Y-STRs (DYS19, DYSYCAIIa and DYSYCAIIb) only were tested for Napoléon I

Locus	Y-STRs	Allelic values	
		Napoléon I	Charles Napoléon
1	DYS393		14
2	DYS390		24
3	DYS19*	13	13
4	DYS391		10
5	DYS385a		16
6	DYS385b		16
7	DYS426		11
8	DYS388		12
9	DYS439		12
10	DYS389-1		14
11	DYS392		11
12	DYS389-2		31
13	DYS458		19
14	DYS459a		9
15	DYS 459b		9
16	DYS455		11
17	DYS454		7
18	DYS447		21
19	DYS437		14
20	DYS448		20
21	DYS449		28
22	DYS464a**		14
23	DYS464b**		15
24	DYS464c**		16
25	DYS464d**		17
26	DYS460		10
27	DYSGATAH4		11
28	DYSYCAIIa	19	19
29	DYSYCAIIb	22	22
30	DYS456		15
31	DYS607		12
32	DYS576		18
33	DYS570		19
34	DYSCDYa		35
35	DYSCDYb		36
36	DYS442		12
37	DYS438		10

\* Discriminant for E1b1b.

\*\* Highly discriminants for E1b1b.

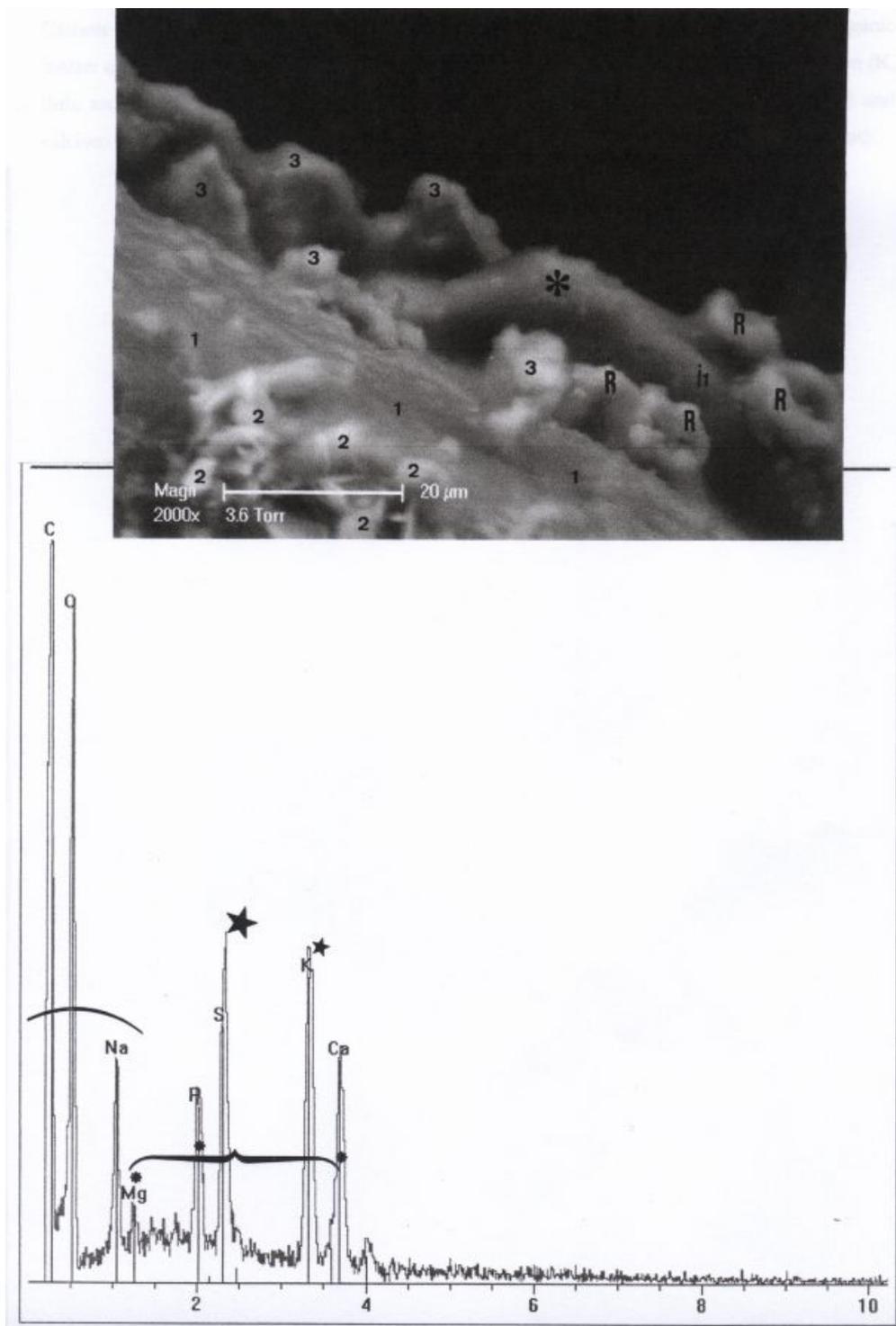


Figure 1. SEM microphotography and X-ray elementary microanalysis of some part of the first beard hair (in island) of Napoléon, in a region at the top of a follicular sheath

*Above:* SEM microphotography (x 2000). 1, corpus of the hair; 2, deposits of the shaving soap; 3, five detached parts of hair scales; star indicates the top region of the follicular sheath; in the corresponding island (i1), four red cells I are visible. *Below:* X-ray elementary microanalysis. Carbon I, oxygen (O), sodium (Na) and magnesium (Mg) peaks correspond to the organic matter of the hair, rich in sulfur (S); some parts of the sulfur peak (big star) and the potassium (K, little star) peak correspond to the potassium sulfate of the shaving soap; phosphorus (P) and calcium (Ca) peaks correspond to the calcium phosphate rich top region of the follicular sheath.

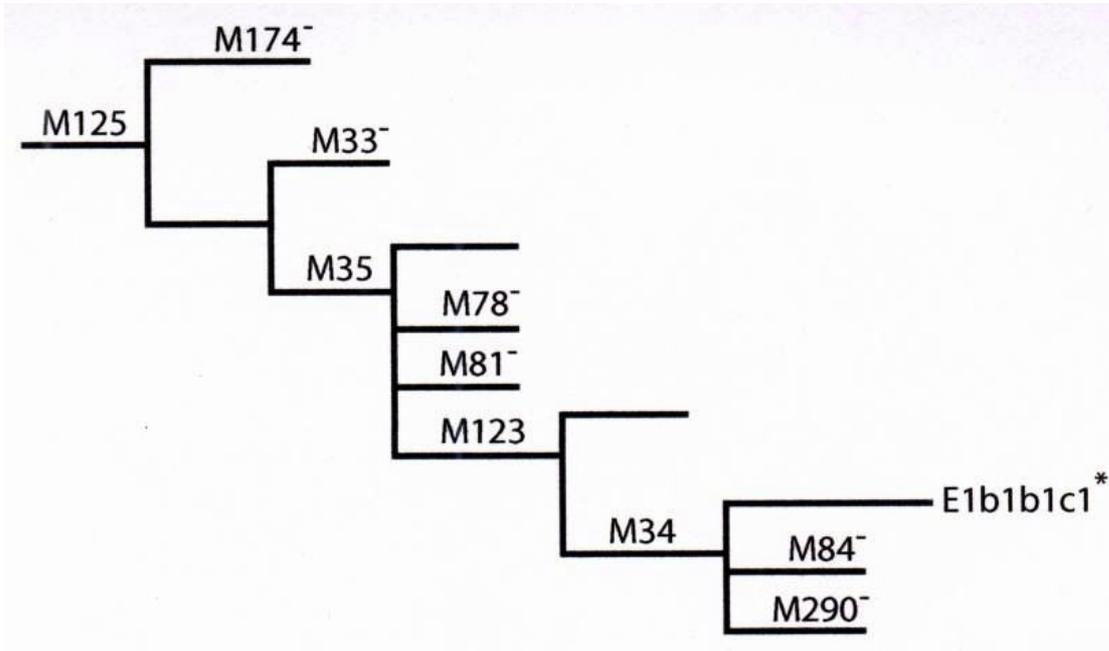


Figure 2. Phylogenetic tree of NRY SNPs

This figure summarizes the procedure used to determine the Y-haplogroup of Napoléon I. Ten different SNPs were used consecutively; the E1b1b1c1\* haplogroup is characterized according to YCC.

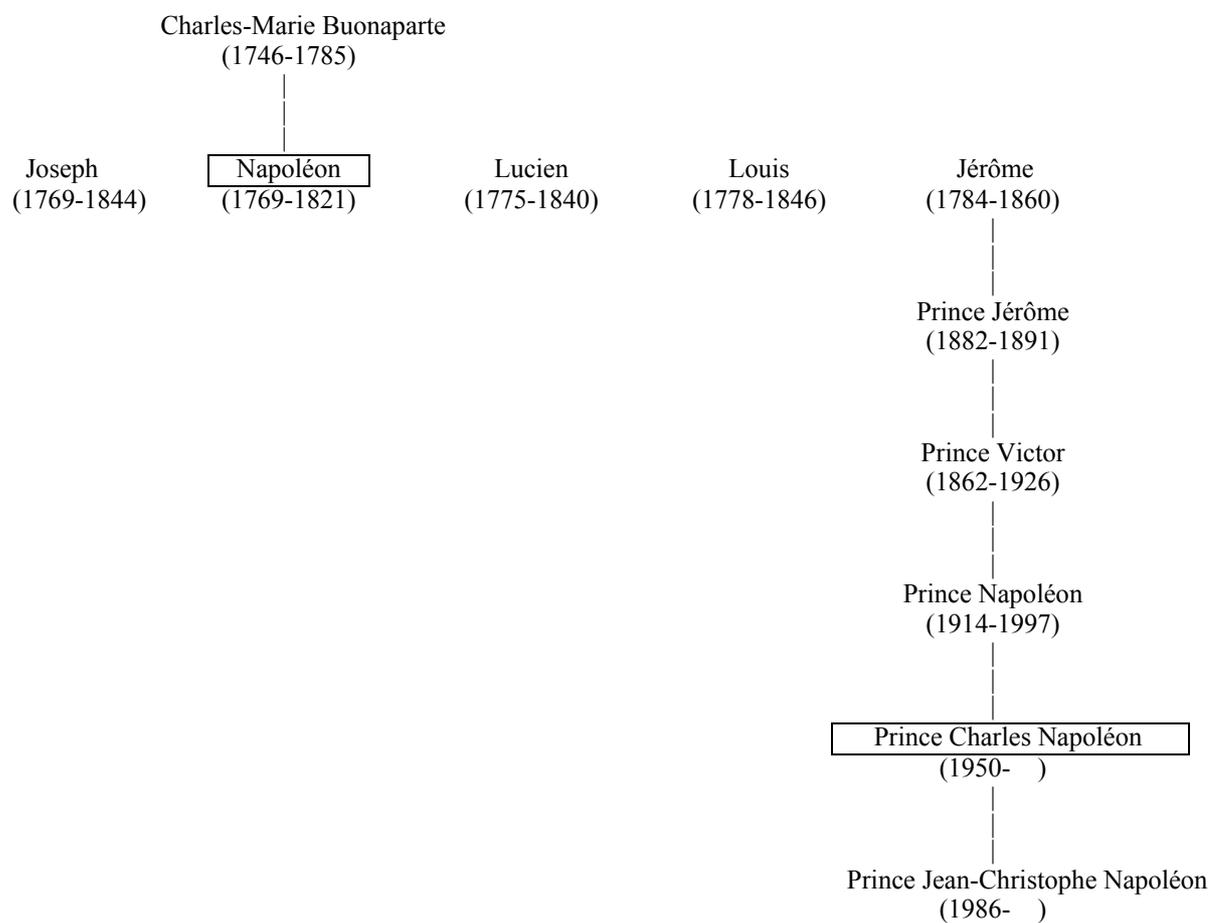


Figure 3. Napoléon's brotherhood and the masculine descent of Jérôme