

Brief Report

On the Origin of Cultivated Roses: DNA Authentication of the Bourbon Rose Founding Pedigree

Abdelmalek Alioua and Pascal Heitzler *

Institut de Biologie Moléculaire des Plantes, Centre National de la Recherche Scientifique (CNRS),
Université de Strasbourg, 67084 Strasbourg, France

* Correspondence: pascal.heitzler@ibmp-cnrs.unistra.fr

Abstract: Rose flowers have been cultivated for their fragrance and their garden value since ancient times. Very ancient cultivars became famous locally for their specific use, and competitive horticultural activities progressively established, leading, with time, to landraces with limited polymorphism. The most famous examples are the oil-bearing Damask roses from Iran and the Yueyue Fen garden strain from China. In 1817, a new rose, allegedly a hybrid from the two previous lineages, was discovered at Reunion. From this plant, as early as the 1820s, a new founder group, the Bourbon roses, was developed in France, which immediately stirred up deep passions among botanists and skilled enthusiasts. Today, more than 30,000 named cultivars have been raised either as garden and landscape plants for the cut rose market or as indoor pot plants. The market handles billions of euros a year, making the rose the most economically important crop worldwide. Following the inheritance of SSR DNA markers, we here propose a reconstitution of the very early lineage of Bourbon roses, clarifying one of the major steps, if not the major one, that links these very ancient heritage roses to modern roses.

Keywords: rose genetics; founder pedigree; Bourbon roses; SSR markers



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1. Introduction

During the antiquity, rose cultivation emerged and became important in few temperate countries of the world, where specific local strains were grown for their perfume and/or garden value. Over the last few centuries, the production of rose water, and then, rose oil, became a desired and precious activity that progressively emerged in more or less remote geographical regions. As the evolution of the production of rose oil develops to perfection, a tight conjunction is established with agronomically important criteria: the definition of a terroir with optimal minerality, the growing of a more diversified pool of elite strains, and finally, sophisticated and competitive know-how for flower extraction methods.

Fortunately, these old strains have been maintained through their ancestral traditional use, and today, clusters of close related clones and even landraces were recorded as a consequence of the hereby long-range horticultural activity [1–5]. The best-known are the Damasks, the Yueyue Fen, and the *moschata* roses. They have been important for their traditional use: for cosmetics and social wellness and/or for their very ancient garden value. They have also particularly influenced the history of the domestication of the roses, and increasing molecular data converge with their implication as the ancestors of modern roses [2,5–10].

Damask roses represent the most important roses for attar, the volatile oil distilled from the flowers [9,11]. The production of flowers and Rose water, most probably from *R. damascena*, is knowledge derived from the ancient Greeks and Romans. It seems that this tradition originated from Iran, the ethnobotanical and historic birthplace of *R. damascena*, where maximal DNA polymorphism diversity was found [1,3,12,13]. Today, rose oil production is of great commercial importance in Bulgaria and Turkey. The ‘*trigintipetala*’

strain, grown in the latter countries, shares 100% of the SSRs markers found in many Iranian accessions, indicating that this clone is derived from cuttings of only one Iranian elite Damask accession among others. Interestingly, the old Damask roses, known in Europe as garden roses, such as 'York and Lancaster' and 'Quatre Saisons', also exhibit the same DNA profile as *trigintipetala*, suggesting that they represent vegetative mutant derivatives (sports) selected over hundreds of years of vegetative diffusion [9]. Thus, 'Quatre Saisons' seems to be a reblooming sport of the non-recurrent original *R. damascena* that flowers twice in spring and again in autumn (hence the synonym of autumn Damask). This cultivar became quite iconic worldwide for this new promising trait.

The Yueyue Fen (pink flowered) and the Yueyue Hong (red flowered) Chinese germplasm represent another example of a very ancient cluster of accessions with close phylogenetically based relationships. They are supposed to derive from hybrids between the *R. chinensis/lucidissima* complex and *R. odorata* [2,5,8], and were grown in China for more than 3000 years [14]. Recurrent Chinese roses were created and developed in China, most probably during the Song dynasty (960–1279) a thousand years ago [14]. These continuous-blooming bushy mutants represent the quintessential innovation of the evolution of garden roses. So, when a few cultivars were sent to Europe at the end of the 18th century, they caused a surge of demand among rose lovers. From the 1820s, they were extensively used for breeding in order to improve the assortment of garden roses. Among the Chinese Yueyue Fen accessions, one seemed to emerge, most probably due to its particular rusticity. It was renamed 'Old Blush' and was proved to be of particular importance during the initial steps of rose domestication. For these reasons, the genome of 'Old Blush' was chosen for a high-quality assembled and sequenced genome [6,8]. The recessive mutation leading to continuous flowering is due to amorphic alleles of the *TFL1a* gene that encodes a floral repressor [15]. The critical point for varietal selection must have been to transmit then the *TFL1a* alleles to the other categories of roses [7,16]. The fever of hybridizing roses started in France, where most of the important groups of hybrids were created.

The emergence of Bourbon roses constitutes one of the most important steps during the evolution of horticultural roses, as they are the main source of Hybrid Perpetuals, that in turn led to the makeup of Hybrid Teas, and then, most modern roses. 'Rose Edouard' is the first raised Bourbon rose, and its origin is very unusual. The genesis and the history of Bourbon roses were reviewed in 1849 by Louis Chaix, followed and endorsed by notes of Antoine Jacques, published in *Jardins de France* (40:410–411) in the same year. Both 1849 texts were reprinted much later, in the *Journal des Roses* (pp. 124–139, 1899). However, on no account does this report on the origin of 'Rose Edouard' given by Chaix constitute a scientific document due to a lack of historical references. But we will briefly summarize here this most diffused version of the supposed chronology.

After the loss of Mauritius and its botanical garden, the 'Jardin des Pamplemousses', to the UK in 1810, the French Marines missioned a botanist to rebuild a new botanical garden at Reunion. André Thouin, the eminent botanist from the Museum d'histoire naturelle of Paris, sent one of his best students, Jean-Nicolas Bréon (1785–1864), to Reunion for this purpose. Bréon rebuilt the botanical garden on the site of the former abandoned 'jardin du Roi' at St-Denis. He also established the 'jardin d'acclimatation' on the surrounding heights of St-Denis, where he introduced hundreds of new plant species, food crops, fruit trees, medicinal herbs, ornamentals, and agricultural implements for the optimal self-sufficiency of the local farmers.

In 1817, the year of his arrival on the Island of Reunion, he discovered a new hybrid of roses, the Rose Edouard. The name 'Edouard' would come from Monsieur Edouard Périchon, then owner of the plot of land where Nicolas Bréon recognized in this 'Rose Edouard' a hybrid between a China rose and a Damask rose. In the past, it was the tradition to cultivate such roses to form perfumed deterrent hedges delineating the properties. Bréon considered that this hybrid rose would represent a new class of roses, promising from a horticultural point of view. He sent achenes and then sticks from this initial plant to Antoine Jacques: a famous rose specialist, botanist, and the chief gardener of the Duke of

Orléans, the future King of France, Louis-Philippe the first. In France, Jacques and few other French rose specialists raised a few descendants of Rose Edouard, the first generation of the Bourbon roses. This new group of rose hybrids depicts a complete breakthrough in the history of rose varietal selection. As Bourbon roses were the direct ancestors of Hybrid Perpetuals, they represented the first key step towards the generation of modern roses.

In 2017, for the bicentenary of the discovery of the first Bourbon rose by Bréon, a safeguard project was coordinated by the botanical garden of Reunion for prospecting and conserving the original Bourbon roses found on the island. It was then of critical importance to know whether the Rose Edouard, as actually grown in Reunion and worldwide, still represents the correct variety, and whether DNA-based experiments could confirm the founding pedigree of the Bourbon roses. Furthermore, as we will discuss below, the knowledge of the exact pedigree may help in following the inheritance of important traits, as well as the chemical genetics of volatiles and health-beneficial compounds that have been transmitted to the base of different horticultural groups of roses. Using an improved resolution for the detection of DNA fingerprints, we clarify many aspects of the founding pedigree of the Bourbon roses and confirm the central role of Bréon in broadly sharing the Reunion roses to one and all in the field of rose botany and cultivation.

2. Materials and Methods

The plant material used for this study is summarized in Table 1. Rose Edouard accessions were supplied by Odile Masquelier from her garden 'La Bonne Maison' near Lyon, as well as Daniel Lemonnier (the collection of Normandy Roses); Girija and Viru Viraraghavan (Western Ghats, India); the Botanical Garden of Reunion; Reunion members of the association des Jardins Créoles; and from our experimental rosarium (Colmar, N-E of France). The 'Rose Edouard' accession from Colmar came from the collection of Mme Loubert (Les Rosiers sur Loire).

Table 1. Origin of the rose cultivars used in this study.

Cultivar	Ploidy	Origin	Location
Old Blush	2N = 14	China, Song Dynasty (960–1279) Imported to Europe in 1752	Rosarium experimental de Colmar, France
Old Blush climbing	2N = 14	Unknown origin, occurred during the 19th Century Climbing sport of Old Blush	Rosarium experimental de Colmar, France
Slater's Crimson	3N = 21	China, Old Imported to Europe in 1792	Rosarium experimental de Colmar, France
Bengal Crimson	2N = 14	China, Old Grown in Reunion prior 1800	Rosarium experimental de Colmar, France
Kazanlik	4N = 28	Iran, non-recurrent Damask	Rosarium experimental de Colmar, France
Quatre Saisons	4N = 28	Very Old recurrent sport of Kazanlik, most probably	Rosarium experimental de Colmar, France
Rose Edouard (Colmar)	4N = 28	found by Bréon, Réunion, 1817	Rosarium experimental de Colmar, France
Rose Edouard (BM, India)	4N = 28	India	Rose collection of Viru and Girija Viraraghavan, India The same clone is growing at La Bonne Maison, near Lyon (the rose collection of Odile Masquelier)
Rose Edouard (BR1, Réunion)	4N = 28	Réunion	Rose collection of Bruno Ricquebourg, Réunion
Rose Edouard (JBM, Réunion)	4N = 28	Réunion	Jardin Botanique Mascarin, Réunion

Table 1. Cont.

Cultivar	Ploidy	Origin	Location
Rose Edouard (COL1, Réunion)	4N = 28	Réunion	Found in Colimaçon, near Saint-Leu, Réunion
Bourbon Jacques	4N = 28	seeds from Bréon, 1817, raised by A. Jacques, France	Rosarium experimental de Colmar, France
Reine des Ile-Bourbon (syn. Bourbon Queen)	4N = 28	Mauget 1834, France	Rosarium experimental de Colmar, France
Champneys' Pink Cluster	2N = 14	Champneys 1802, Charleston, United States	Rosarium experimental de Colmar, France
moschata	2N = 14	moschata, the original clone from Leonie Bell, United States	Rosarium experimental de Colmar, France
Stanwell Perpetual	4N = 28	Lee, pre-1821, Stanwell, UK	Rosarium experimental de Colmar, France

DNA extraction was performed using the CTAB and magnetic beads methods. Young leaves were collected and stored at -80°C until extraction. The leaf material was ground in 750 μL of a grinding buffer with metal beads for 2 min on a Tissue Lyser (Quiagen, S.A.S., Courtaboeuf, France) at 30 Hz. The samples were incubated for 30 min at 65°C and extracted with 700 μL of chloroform/isoamyl alcohol (24/1, *v/v*, Carlo Erba Reagents, Cornaredo, Italy). After a centrifugation step (5 min, 10,000 rpm, room temperature), the aqueous phase was collected and added to 550 μL of isopropanol (Carlo Erba Reagents, Cornaredo, Italy). Precipitated DNA was pelleted via centrifugation (5 min at 10,000 rpm, room temperature), and the pellets were washed with 70% ethanol (Sigma Aldrich, L'Isle-d'Abeau Chesnes, France) and dried at room temperature before resuspension in 100 μL of DNase/RNase-free water. The DNA samples were then incubated with Rnase A/T1 at 37°C for 30 min, and the isolated genomic DNA was purified with 1 volume of Ampure XP magnetic beads (Beckman, Coulter, Villepinte, France) according to the manufacturer's protocol. gDNA QC was performed on a Nanodrop (Thermo Fisher Scientific, Illkirch, France) and a Qubit Fluorometer (Thermo Fisher Scientific, Illkirch, France) according to the manufacturer's protocol. The gDNA samples were stored at -20°C .

Nucleic lysis buffer: Tris base 0.2 M, EDTA 0.05 M, NaCl 2 M, CTAB 2%. DNA extraction buffer: Sorbitol 0.35 M, Tris base 0.1 M, EDTA 0.005 M. Grinding buffer: 25 mL of DNA extraction buffer, 25 mL of nuclei lysis buffer, 10 mL of sarkosyl 15%.

For SSR amplification for parentage analysis, we used the following 12 SSR markers: RMS070 (LG1), RMS015 (LG1), RMS065 (LG2), RMS132 (LG2), Rh58 (LG3), Rh50 (LG3), Rw55E12 (LG4), RhAB38 (LG5), Rw52D4 (LG5), Rw22A3 (LG6), CL2980 (LG6), and H10D03 (LG7). The references are [17] for BFACT047; [18] for the Rw, H and CL series; [19] for the Rh and RhAB series; and <http://www.wipo.int/pctdb/en/wo.jsp?wo=2003097869&IA=WO2003097869&DISPLAY=STATUS> (accessed on 11 December 2003) for the RMS series. The forward primers were labeled with a fluorescent chemical (FAM). PCR was conducted with 50 ng of genomic DNA, a 1 μL mix of FAM fluorescently labeled forward/non-fluorescently labeled reverse primer, 5 μL of a 5 \times GoTaq Reaction Buffer (Promega Corporation, Charbonnières-les-Bains, France), 2 μL of 2.5 mM dNTP, 1.5 μL of 25 mM MgCl_2 , 0.1 μL of GoTaq DNA polymerase 5U/ μL (Promega Corporation, Charbonnières-les-Bains, France), and DNase/RNase-free water for a final volume of 25 μL . The PCR reactions were performed in a GeneAmp[®] 9700 thermal cycler (Applied Biosystems, Illkirch, France) using the following program: 95°C for 5 min; 2 cycles (95°C for 30 s, 60°C for 30 s, 72°C for 30 s); 2 cycles (95°C for 30 s, 58°C for 30 s, 72°C for 30 s); 2 cycles (95°C for 30 s, 56°C for 30 s, 72°C for 30 s); 2 cycles (95°C for 30 s, 54°C for 30 s, 72°C for 30 s); 2 cycles (95°C for 30 s, 52°C for 30 s, 72°C for 30 s); 15 cycles (95°C for 30 s, 54°C for 30 s, 72°C for 30 s); 72°C for 6 min; and then held at 4°C . One μL of the PCR product was added to 9 μL of Hi-Di Formamide (Thermo Fisher Scientific, Illkirch, France)

and a Genscan 400HD-ROX dye Size Standard (Thermo Fisher Scientific, Illkirch, France) master mix, followed by denaturation at 95 °C for 5 min. The mixture was suggested for capillary electrophoresis on an ABI3130xl Genetic Analyzer and POP7 polymer (Applied Biosystems, Illkirch, France). The FSA files generated were analyzed with the GeneMarker software, Version 3.0.1 (BioGene, Huntingdon, UK) for calling the allele sizes. In this study, the color code used in the tables reflects the putative origin of common alleles, whereas black values indicate non-related alleles. Due to transient shortage of resources, we had to replace the HD400 dye mix, explaining the occasional length variation (in general one unit shift) given for the same allele.

3. Results

3.1. Definition of the DNA Profile of 'Rose Edouard'

The 'Rose Edouard', with its characteristic cup-shaped flowers, is semi-double and very fragrant, a trait inherited from the ancient Damask roses. In the same line, the upright shrub has few agglomerated terminal flowers, the supple stems are covered with prickles of a heterogenous size, and the infundibuliform hips are also typical of the Damasks. The larger triangular prickles and the strongly recurrent ability to flower are typical traits of China roses. We concentrate our investigations on clones exhibiting the standard deep-pink fragrant flowers, best matching the description of the 'Rose Edouard'. A similar or even identical 'Edward' rose has been described in India for many decades.

We then compare the DNA profile of 'Rose Edouard' accessions provided from Reunion, Metropolitan France, and India. For this, we developed an acceptable resolution for the detection of DNA fingerprints. We PCR-amplified fluorochrome-coupled simple sequence repeats (SSRs), which are now largely used for parentage analysis in plants. They were separated via capillary electrophoresis, and the size of the different alleles was revealed based on their sharp curve profiles. Altogether, the tested SSRs represent a specific identity card for any accession. In principle, for each marker, a maximum of two peaks can be detected in a diploid variety (two septets, 14 chromosomes), three peaks for a triploid (21 chromosomes), and four peaks for a tetraploid (28 chromosomes). For the robustness of the data, we almost always tested two independent SSR markers per chromosome ($x = 7$). Thus, the 12 SSR markers used defined the predicted inheritance of a maximum of 24 different alleles for diploid offsprings or 48 alleles for tetraploids. The profiles are consistent with a tetraploid rose, a feature that is expected for 'Rose Edouard'. The profiles matched pretty well for all markers and for all chromosomes (Table 2), though some results remained incomplete for few accessions, depending on the quality of the genomic DNA. These results indicate that the typical 'Rose Edouard' that is now widely spread around the world corresponds to one clone with its own DNA profile.

Table 2. SSR haplotypes and DNA profile comparisons among the different accessions of 'Rose Edouard'.

Locus (LG)	Edouard Colmar	Edouard BM/India	Edouard BR1	Edouard JBM	Edouard COL1
RMS070 (LG1)	150	150	150	150	
	168	168	168	170	164
	170	170	170	174	170
	174	174	174		
RMS065 (LG2)	105	105	105		
	109	109	109	105	-
	117	117	117		
	124	124	124		
Rh50 (LG3)	275	275	275	275	
	299	299	299	301	
	302	302	302	331.5	-
	331.5	331.5	331.5		

Table 2. Cont.

Locus (LG)	Edouard Colmar	Edouard BM/India	Edouard BR1	Edouard JBM	Edouard COL1
Rw55E12 (LG4)	164.5 173 179–181	164.5 173 179–181	164.5 173 179–181	173 179–181	164.5
Rw52D4 (LG5)	209 212/212	209 212/212	209 212/212	210 213	210
RhAB38 (LG5)	103.5 134 140 160	103.5 134 140 160	103.5 134 140 160	104.5 140 161	134
CL2980 (LG6)	211.5 222.5 236.5	211 222.5 236.5	211 222.5 236.5	212 224.5 239	215 224 228.5 239
H10D03 (LG7)	222 225.5 232.5	222.5 225.5 232.5	222.5 225.5 232.5	224.5 227.5 235	224.5 227.5 235 241.5

The color code is specified below Table 3.

However, among the different primitive Bourbon roses we found at Reunion, we noticed some differences in the intensity of the pink color of the flowers, in the serration of the leaves, and in the density of the thorns (stems). It is quite possible that after decades of confined cultivation in Reunion, the actual insular clones developed a more or less consanguineous population, most likely derived from self-pollination or from backcrosses with the original parental plants. The prospection of more ‘Edouard’ derivatives and the analysis of subsequent DNA-based comparisons deserve further investigation. Another research program is planned for this purpose. Nevertheless, we loaded the DNA of one such accession (COL1) found on the west coast of the island. It reveals minor differences in its DNA profile beside the typical ‘Edouard’ rose (Table 2). This very preliminary result supports our above hypothesis.

3.2. Identification of the Damask Parent of ‘Rose Edouard’

We next compare the identified profile of the common ‘Edouard’ rose with that of her alleged parents. According the earlier descriptions, ‘Quatre Saisons’ was a privileged candidate for the Damask parent of ‘Rose Edouard’. In principle, for a parent–offspring relationship, the profile of the curves must show an identity of at least half of the alleles for every considered marker. ‘Quatre Saisons’ is tetraploid, and its DNA could amplify three or four different alleles for each SSR marker. Most probably, three alleles may represent partial homozygote situations, where a marker is amplified from two similar or identical alleles. For any marker, whether similar or different, two alleles of ‘Quatre Saisons’ have been transmitted to ‘Rose Edouard’ (Table 3). This strongly suggests that ‘Quatre Saisons’ is the Damask parent of ‘Rose Edouard’.

Table 3. SSR haplotypes and DNA profile comparisons for the genealogy of Rose Edouard.

Locus (LG)	Old Blush	Quatre Saisons	Edouard	Bourbon Jacques	Bourbon Queen
RMS070 (LG1)	150 170	168 174 186	150 168 170 174	150 170 174	152 170 174 186

Table 3. Cont.

Locus (LG)	Old Blush	Quatre Saisons	Edouard	Bourbon Jacques	Bourbon Queen
RMS015 (LG1)	153.5	130	130		153.3
	171.5	145	153.5	166	166
		166	166	171.5	171.5
RMS065 (LG2)	109	96.5	105	96.5	112.5
	117	105	109	109	114.5
		114.5	117	124	117
RMS132 (LG2)	181	124	124		124
	192	167	167	167	173
		173	173	176	177
Rh58 (LG3)	247	177	181	181	191
	287.5	196.5	191	197	196
		226	226		
Rh50 (LG3)	302	231	231	252.5	226
	331.5	252.5	287.5	287.5	231
		264			
Rw55E12 (LG4)	164.5	275	275	302	275
	179–181	299	299	331.5	299
		333.5	302	333.5	302
Rw52D4 (LG5)	209	348.5	331.5		331.5
	212	113.5	164.5	173	173
		156	173	179–181	179–181
RhAB38 (LG5)	134	173	179–181		179–181
	140	203	209	209	203
		212	212/212	212	209
CL2980 (LG6)	222.5	103.5	103.5	103.5	103.5
		142	134	117	134
		148.5	140	140	140
H10D03 (LG7)	222	160	160		158
	232.5	211	211.5	223	211.5
		214.5	222.5	237	222.5
H10D03 (LG7)	222	236.5	236.5		222.5
	232.5	208	222	208.5	225.5
		225.5	225.5	222.5	233
	239	232.5	232.5		239

Color code: the red values specify the alleles originated from 'Old Blush', while the blue ones are from 'Quatre Saisons'.

Previous studies report that several old once-blooming damasks grown in Europe, like 'Kazanlik' (syn. *Trigintipetala*), share the same DNA profile as 'Quatre Saisons'. Rusanov et al. suggest that, among the different landraces of the Damask roses from Iran, the ethnobotanical center of origin, one clone is distinguished for its robustness and its ability to produce an attar of particularly good quality [9]. This clone has been widely grown as the activity of oil production has developed and been exported to other countries. The same clone was used in the Bulgarian valley of Kazanlak and then in the Turkish region of Isparta. Since the original Damasks from Iran are non-reblooming roses, it is tempting to predict that 'Quatre Saisons' occurs somewhere as a (vegetative) sport from the former. 'Quatre Saisons' has slightly more compact flower stems, and blooms at least twice (in spring and in fall). It is possible that this mutation reveals the expression of a hypomorphic, not-yet-identified allele of TFL1a that is present in those plants and responsible for its

partial reblooming phenotypes [16]. Alternatively, a lucky TFL1a combination between ‘Old Blush’ and ‘Kazanlik’ may have produced ‘Rose Edouard’.

3.3. Identification of the Chinese Parent of ‘Rose Edouard’

The other parent of ‘Rose Edouard’ is assumed to be a China rose. Among several possible candidates that have been cultivated on Reunion Island for at least 200 years, we tested ‘Bengal Crimson’, ‘Slater’s Crimson’, and ‘Old Blush’, roses that are already found in the creole gardens of Reunion today. Their DNA profiles were compared to that of ‘Rose Edouard’. The first two seem to have profiles that are not compatible with that of the ‘Rose Edouard’ (Table 4), especially for Rw55E12, RhAB38, or H10D03. Both ‘Bengal Crimson’ and ‘Slater’s Crimson’ can therefore be excluded as parents of ‘Rose Edouard’. In contrast, the alleles of ‘Rose Edouard’ that do not come from the first parent, ‘Quatre Saisons’, all correspond to those of ‘Old Blush’. As the latter seems to be a good candidate, it is nevertheless diploid. The crossing between a diploid rose and a tetraploid rose should make ‘Rose Edouard’, a triploid rose. In fact, ‘Rose Edouard’ is tetraploid, but this would not necessarily represent an inconsistency. In crosses with different ploidy levels, occasional non-disjunctions are always possible in roses [20]. Furthermore, polyploid offsprings of diploid roses like ‘Old Blush’ are fairly common events when the pollen is provided from a triploid or a tetraploid father (ibid). Following our experimental data for every single locus, ‘Old Blush’ should not simply have given half of its chromosomes to ‘Rose Edouard’, but all (Table 3). This assumption is consistent with the nature of the patterns observed for ‘Rose Edouard’. ‘Old Blush’, the best known Yueyue Fen rose, is the other substantiated parent of ‘Rose Edouard’.

Due to its long history of cultivation, ‘Old Blush’ has produced and accumulated a range of sports or somatic mutants [21]. We confirmed that the climbing form has the same DNA profile as the original bush form (Table 4). This is a further argument that the parent of ‘Rose Edouard’ is the original ‘Old Blush’ clone.

Table 4. SSR haplotypes and DNA profile comparisons: Slater’s Crimson and Bengal Crimson are not the parents of ‘Rose Edouard’.

Locus (LG)	Old Blush Climbing	Old Blush	Edouard	Slater’s Crimson	Bengal Crimson
RMS070 (LG1)	150 170	150 170	150 168 170 174	150 156.5	150 152.5
RMS065 (LG2)	109 117	109 117	105 109 117 124	110.5 116.5	98 108.5
Rh50 (LG3)	302 331.5	302 331.5	275 299 302 331.5	302	331.5
Rw55E12 (LG4)	164.5 179–181	165 179–181	164.5 173 179–181	169	177.5 183.5
Rw52D4 (LG5)	209 212	209 212	209 212/212	209	213 219
RhAB38 (LG5)	134 140	134 140	103.5 134 140 160	137 179.5 183.5	-

Table 4. Cont.

Locus (LG)	Old Blush Climbing	Old Blush	Edouard	Slater's Crimson	Bengal Crimson
CL2980 (LG6)	222.5	222.5	211.5 222.5 236.5	223 232	212 222.5
H10D03 (LG7)	222 233	222 232.5	222 225.5 232.5	222	222 211.5 217

Color code: the red values specify the alleles originated from 'Old Blush', while the blue ones are from 'Quatre Saisons'.

3.4. Authentication of 'Rose Edouard' Parents

Both the Damask roses and the Yueyue Fen roses are very ancient hybrid tribes. In their ethnobotanical birthplaces, respectively, Iran and China, a given tribe entails multiple, genetically related, morphologically similar but molecularly distinct cultivars and landraces [1,3,5]. The original clones, 'Quatre Saisons' and 'Old Blush', differentiate from such tribes because they were more reliably performant as garden plants with excellent rusticity and optimal plasticity for adaptation to poor soils, such that these elites have probably existed for more than a thousand years. 'Old Blush' and 'Quatre Saisons' became popular worldwide and were grown in different continents, where they became the parents of other famous roses, putatively half-sisters of 'Rose Edouard'. At this point, we wondered whether to check the status of such half-sister plants. The aim was to establish whether the 'Old Blush' and the 'Quatre Saisons' plants that gave rise to 'Rose Edouard' at Reunion matched with the clones that produced acknowledged descendants elsewhere. We focused on Champneys' Pink Cluster and Stanwell Perpetual (Table 5), two contemporary possible half-sisters of 'Rose Edouard', through 'Old Blush' and 'Quatre Saisons', respectively.

Table 5. SSR haplotypes and DNA profile comparisons of two contemporary half-sisters of Rose Edouard.

Locus (LG)	Stanwell Perpetual	Quatre Saisons	Edouard	Old Blush	Champney's Pink Cluster	Moschata
RMS070 (LG1)	168 176	168 174 186	150 168 170 174	150 170	170 174	174
RMS015 (LG1)	145 156 166 172	130 145 166	130 153.5 166 171.5	153.5 171.5		165.5
RMS065 (LG2)	96.5 107.5 114.5 122	96.5 105 114.5 124	105 109 117 124	109 117	96.5 117	96
RMS132 (LG2)	171 173 177 181	167 173 177 196.5	167 173 181 191	181 192		192 197
Rh58 (LG3)	226 231 238 243	226 231 252.5 264	226 231 287.5	247 287.5		252

Table 5. Cont.

Locus (LG)	Stanwell Perpetual	Quatre Saisons	Edouard	Old Blush	Champneys' Pink Cluster	Moschata
Rh50 (LG3)	275	275	275			
	299	299	299	302	302	315
	317	333.5	302	331.5	333.5	332.5
	333.5	348.5	331.5			
Rw55E12 (LG4)	113.5	113.5	164.5	164.5	164.5	177
	156	156/173	173	179–181		
	184.5		179–181			
Rw52D4 (LG5)	203	203	209	209	209	212
	212	212	212/212	212		
	218					
RhAB38 (LG5)	103.5	103.5	103.5			
	130	142	134	134	134	162
	160	148.5	140	140	140	
	178.5	160	160		162	
CL2980 (LG6)	214.5	211	211.5		222.5	236.5
	236.5	214.5	222.5	222.5	236.5	
H10D03 (LG7)	208	208	222	222	208	197
	218	225.5	225.5	232.5	222	207
	239	239	232.5			

Color code: the red values specify the alleles originated from 'Old Blush', while the blue ones are from 'Quatre Saisons'.

'Champneys' Pink Cluster', is the diploid founding plant of the 'Noisette' group of roses. In the early 19th century, this group innovatively combined a tall bush or semi-climbing growing habit with a reliant recurrent flowering trait. As the first 'Bourbon' rose, the first 'Noisette Rose' was raised far from the French Metropole. 'Champneys' Pink Cluster' was raised by a market gardener and horticulturist, John Champneys, around 1802 in Charleston (South Carolina, USA) from 'Old Blush' × *R. moschata*. This plant and one important seedling, 'Noisette carnée' were largely diffused in France by the brothers Philippe et Louis Noisette, two nurserymen, based, respectively, in Charleston and Paris. Our partial data show that 'Champneys' Pink Cluster' inherited half of the 'Old Blush' alleles (Table 5). Previous work of Dr Morvillo's team [10], who used the RADP method, strongly suggests that 'Champneys' Pink Cluster' is a direct descendant of 'Old Blush'. The other set of the SSR alleles is also compatible with those from the *R. moschata* clone (Table 4), which is grown both in the US and in Europe (Table 1).

R. moschata accounts for another very ancient horticultural tribe, with Western Himalayan origins. A phylogenetic analysis places different *R. moschata* accessions within the *R. brunonii* genetic pool [4]. *R. moschata* most probably represents a partial reblooming horticultural version of *R. brunonii*, one of the most vigorous tree climbers among wild roses. *R. moschata*, which grows as large hedges, may express a hypomorphic allele of TFL1a [16]. In amorphic or hypomorphic TFL1a mutants, the gibberellins remain low, limiting the growth of the new shoots [15,22]. One clone of *R. moschata* was grown in Europe in the 16th century for its extreme and gorgeous fragrance. As it was not fully hardy, the plant disappeared during the 19th century. It was discovered again in Southern USA by Léonie Bell in the 1950s and then reintroduced to Europe by Graham Stuart Thomas in 1963. It is this very precise clone that we sampled from our experimental rosarium. Our partial results confirmed the fact that our material matches with the true *R. moschata* clone, as grown by Champneys (Table 5).

'Stanwell Perpetual', a popular and extremely fragrant rose, is a tetraploid hybrid that appeared spontaneously in a garden near London in the 1820s. It is supposed to derive from a cross between an unknown *R. spinosissima* and the Damask 'Quatre Saisons'.

We confirmed here that the Damask rose ‘Quatre Saisons’ is indeed the direct parent of ‘Stanwell Perpetual’ (Table 5).

Here, we propose that three contemporary roses raised in the early 1800s, ‘Rose Edouard’, ‘Champneys’ Pink Cluster’, and ‘Stanwell Perpetual’, are true half-sisters. We can deduce from this statement that the parents of ‘Rose Edouard’ were iconic enough to be grown and hybridized in such different places like Reunion Island, the United Kingdom, and South Carolina, US. These parallel relevant events strongly support the idea that ‘Old Blush’ and ‘Quatre Saisons’, sampled in our collection, represent the historical and authentic varieties. These arguments consistently reinforce the robustness of the pedigree proposed in our study (Figure 1).

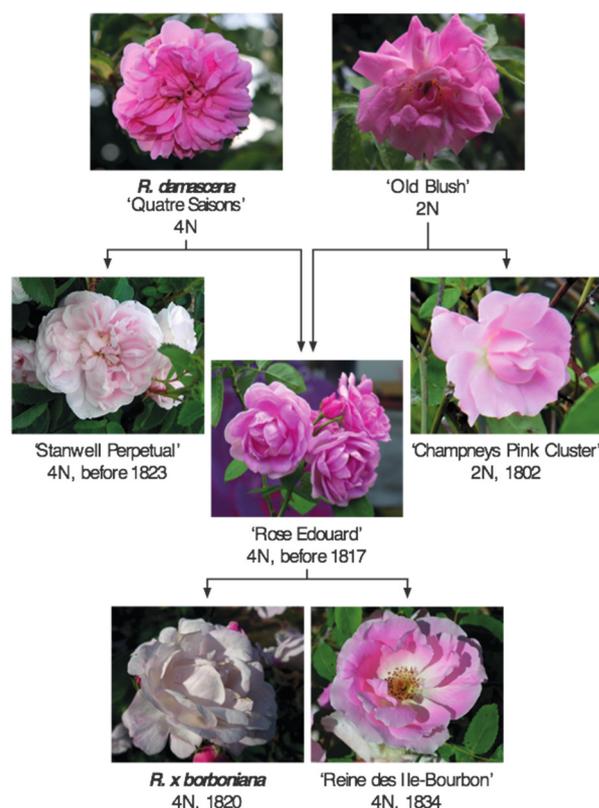


Figure 1. The Bourbon founding pedigree.

3.5. Identification of First-Generation Descendants of ‘Rose Edouard’

Due to its exotic origin, ‘Rose Edouard’ is one of the first named rose seedlings to appear in the southern hemisphere. The discovery of the first Bourbon rose has not only generated a local and anecdotal horticultural novelty, but the ancestor of a whole new group of roses, the Bourbon roses, themselves at the origin of the Perpetual Hybrids and by extension, many modern roses grown worldwide. The European history of the emerging diversity of Bourbon roses remains blurred today, but it was clear that this new class of roses would dramatically change the horticultural evolution of roses bred from then on. We know that Jean-Nicolas Bréon sent achenes from ‘Rose Edouard’ to Antoine Jacques, the famous rhodologist (1782–1866) and head gardener for Louis-Philippe, King of France. Two such earliest French Bourbon seedlings, which would have been good candidates for the mentioned Bréon-to-Jacques seedling transaction, were tested through SSR markers.

The first is a Bréon seedling from 1820, specifically from hips originating from Reunion. The corresponding rose still grows in internationally renowned rose gardens such as L’Hay and Sangerhausen, either as *R. x borboniana* (not to be confused: *R. x borboniana* is also the Indian name of ‘Rose Edouard’) or ‘Bourbon Jacques’ (the purple-colored rose, now extinct, drawn by Pierre-Joseph Redouté under the name ‘Bourbon Jacques’ in 1824, may

constitute a sister seedling). The rosebush, freely suckering, is very vigorous and sends its canes reaching upwards of two meters. The flowers are markedly a paler pink than those of 'Rose Edouard'. The second is 'Reine des Ile-Bourbon' or 'Bourbon Queen', released by Mauget in 1834. Since it is a later variety, this plant is assumed to derive from a seedling of 'Rose Edouard', established in France in 1823–1824. Alternatively, this rose might have been another seedling from Bréon. 'Reine des Ile-Bourbon' is itself the parent of several other Bourbon roses developed in France and Europe.

The genetic fingerprints of the two candidate roses described above, which are tetraploids, were compared with those of 'Rose Edouard'. The DNA tests show, in both cases, an identity of 50% of the markers for each locus considered, which means at least two out of four alleles (Table 3). Among these shared markers, there is often an 'Old Blush' allele and a 'Four Seasons' allele from the grandparents. The other markers are from an unknown parent. Our results provide evidence that 'Rose Jacques' and 'Reine des Ile-Bourbon' are direct descendants of 'Rose Edouard', and thus constitute the first generation of Bourbon roses (Figure 1).

4. Discussion

Nicolas Bréon noticed in 1817 that the 'Edouard' rose combines the qualities of an ancient China rose with the Damask 'Quatre Saisons'. He distributed seeds and cuttings of this hybrid rose in France, and so he was involved in the origin of the development of the major line of Bourbon roses. Our DNA results corroborate this timeline as well as also Bréon's work. We brought the technique of DNA comparisons to an optimal resolution, to better follow every marker. Our results offer five major arguments that together strongly support the authenticity of Bréon's 'Rose Edouard'. First, the clones of 'Rose Edouard' from Reunion, India, and Metropolitan France correspond to a single rose. Second, 'Old Blush', the worldwide best-known Yueyue Fen China rose, is a confirmed parent of 'Rose Edouard'. Third, the Damask 'Quatre Saisons' is the other recorded parent of 'Rose Edouard'. Fourth, the ancient popular roses, contemporary to the discovery of the first Bourbon, 'Champney's Pink Cluster' and 'Stanwell Perpetual', are proven half-sisters of 'Rose Edouard'. The genetic profiles of their common parents, 'Old Blush' and 'Quatre Saisons', match those of today's cultivated clones and simultaneously authenticate them in our collection. Fifth, two historic first-generation Bourbon roses are the direct descendants of this same 'Rose Edouard'. Altogether, these results complete each other and favor the idea that the first Bourbon Rose is the Rose Edouard, as grown today. We summarize the founding pedigree of the Bourbon roses in Figure 1. Our results agree perfectly with Bréon's botanical data. Finally, this story emphasizes the overriding role of the most fragrant roses as the starting point for the varietal selection.

Some confusion surrounds the chronology and historical aspects of the genesis of the first Bourbon roses. We will probably never know all the details of that timeline. For example, was the 'Rose Edouard' already well established and multiplied at Reunion in 1817? Did it already exist long before in India? Or did it appear shortly before Bréon's arrival in Reunion, on the property of Edouard Périchon? Our DNA tests will not be able to definitively decide these questions.

It is plausible that if 'Rose Edouard' had first appeared in India long before 1817, then Bréon would not have been able to give as much detail about the supposed ancestry of this rose. The hypothesis of the birth of the first Bourbon rose in Reunion thus seems more likely. Old documents on the history of the French colonies reported that Périchon family, who knew the 'Rose Edouard' before Bréon, were an influential family of Reunion who conducted business in Mauritius and India. Today, 'Rose Edouard' is common in the latter countries, and the influence of the Périchon dynasty on this diffusion remains possible. According to our contacts with Indian rose specialists, we are not aware of any ancient report that describes this rose in India long prior to Bréon's experience.

Our analyses also show that the tetraploid character of the 'Rose Edouard' is intimately linked to an event of non-disjunction of the 'Old Blush' genome. This genetic accident

seems innocuous, but it was more than providential, since, if the 'Rose Edouard' had been triploid, it would certainly never have had the fertility for the crucial and phenomenal offsprings that we know. The authentication of the parents we give here for the 'Rose Edouard' also explains the recessive transmission of its remontancy. In addition, the genome of 'Old Blush' has been sequenced [6,8], with 'Old Blush' being one of the pillars in the lineage of current roses and a pioneer source for the continuous flowering trait. Our study considerably reinforces these statements and shows that 'Old Blush', as the direct parent of 'Rose Edouard', is more than ever relevant in the transmission of important qualities in modern roses.

Finally, in India and Pakistan, the cultivation of 'Rose Edouard' has been widely replacing the Damask rose for perfumery and cosmetics, because it is better suited to mild climates without cold winters. On Reunion, 'Rose Edouard' was part of the local pharmacopoeia in the mountain areas where, historically, people lived in total self-sufficiency. It is primarily still used to combat eye septicemia. Our study will also help future investigations on the biochemical genetics of scent signatures and health-beneficial molecules, as revealed from the crude extracts of the plants.

The cultural and economic stakes of this history are deep. At the time of the bicentennial discovery of 'Rose Edouard' by Jean-Nicolas Bréon, a project to safeguard and enhance the horticultural and historical heritage of Reunion was thus established, in partnership with the associations 'Jardins Créoles', ARMEFLHOR, and the Union des Horticulteurs et Pépiniéristes de la Réunion (UHPR). One of the objectives of the Bourbon rose project is to support the emergence of a professional horticultural sector, specialized in roses and based on quality know-how with ecological and sustainable practices. Enthusiastic nurseries are being mobilized despite the difficult island economic context. Our study should help the project, bringing legitimacy to the heritage character of 'Rose Edouard' at Reunion. The founding pedigree that we propose here will serve as a solid basis to explore the other Bourbon roses found on the island in order to establish a kind of filiation between them. It will also be a robust foundation for any future investigations into phylogenetic relationships by progressing towards more recent roses.

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