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Molecular Typing and History of the Provins Roses Horticultural Group

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Summary

A molecular typing analysis by Randomly Amplified Polymorphic DNA (RAPD) was performed on the large collection of Gallica roses from the conservatory of the 'Roseraie de la Cour de Commer' (France; 281 cultivars). A Principal Components Analysis (PCA) of the PCR data produced a map of distances between varieties. The identification of varieties by date of creation and breeder reveals the occurrence of two allele flows in opposite directions during the domestication history (XIXth–XXth centuries) of this horticultural group. The first flow corresponds to an increased molecular polymorphism of PCR fragments resulting from a higher heterozygosity. This increased polymorphism is due to extensive crosses between Gallica varieties and selection of cultivars with original morphologies and hybrid vigour. The second flow corresponds to achievement of inter-specific crosses of Gallica (4n) varieties by *Rosa chinensis* (2n) and parent cultivars; these crosses result in introgression of chinenses characters (red coloration of young follicles, recurrence) together with some decrease of the molecular polymorphism probably due to some reduction of polyploidy and/or heterozygosity. The description in the horticultural literature of the pollination practices suggests that the many Gallica varieties corresponding to the first allele flow were created by productive breeders using simple cross intraspecific pollinations between Gallica cultivars. The few varieties corresponding to the second flow were created by careful breeders who practised strict interspecific pollinations between Gallicas and Chinenses roses.

Zusammenfassung

Molekulare Klassifizierung und Geschichte der Gruppe der Provins-Gartenrosen Eine molekulare Klassifizierung mittels 'Randomly Amplified Polymorphic DNA' (RAPD) wurde mit der sehr umfangreichen Sammlung von Gallica-Rosen des Konservatoriums 'Roseraie de la Cour de Commer' durchgeführt (Frankreich; 281 Varietäten). Eine 'Principal Components Analysis' (PCA) der PCR Daten ergab ein Diagramm mit Abständen zwischen den Varietäten. Zusammen mit der Beschreibung neuer Varietäten durch die Züchter und den genauen Daten der Kreation belegen diese Daten zwei Allelflüsse in entgegengesetzter Richtung während der Domestikation (19. und 20. Jahrhundert) dieser Gartenbaugruppe. Der erste Fluss entspricht einer erhöhten Rate von molekularen Polymorphismen der PCR Fragmente, die wahrscheinlich auf eine höhere Heterozygosität zurückzuführen ist, die ihrerseits vermutlich auf sehr zahlreichen Kreuzungen zwischen Gallica-Varietäten und der Selektion von Kulturvarietäten mit ursprünglicher Morphologie und Hybridstärke beruht. Der zweite Fluss entspricht interspezifischen Kreuzungen von Gallica- (4n) -Varietäten mit *Rosa chinensis* (2n) und ihren Vorfahren; diese Kreuzungen führen zu einer Introgression von Chinenses-Charakteren (rote Färbung der jungen Follikel, Wiederaustreiben) zusammen mit etwas Abnahme der molekularen Polymorphismen, vermutlich wegen einer Abnahme der Ploidie und/oder der Heterozygosität. Die Beschreibung der Bestäubungspraktiken in der Gartenbauliteratur führt zu der Schlussfolgerung, dass die zahlreichen Gallica-Varietäten des ersten Allelflusses durch sehr produktive Züchter geschaffen wurde, die einfache intraspezifische Kreuzungen zwischen Gallica-Kulturvarietäten ausführten. Die sehr viel geringere Anzahl von Varietäten des zweiten Allelflusses zeugt von umsichtigen Züchtern, die sehr gezielte interspezifische Kreuzungen zwischen Gallica- und Chinenses-Rosen ausübten.

Key words. interspecific pollination – introgression – genealogy – principal component analysis – UPGMA dendrogram – history – rose breeders

Introduction

The Modern Rose was created by the rose breeders of the XIXth century through many numerous cycles of interspecific crosses repeated between founders of Chinese origin (*R. chinensis* and its hybrids) and of European (or peri-mediterranean) origin (*R. gallica* and its hybrids: *R. damascena* and *R. centifolia*) (WYLIE 1954; MAIA and

VENARD 1976). The Chinese roses were introduced from Far East at the end of the XVIIIth and at the beginning of the XIXth centuries. They provided recurrence unknown in European roses. The latter conferred for their part the characters of hardiness and pest resistance.

The 'horticultural group' of Gallica roses was developed at the beginning the XIXth century. While Dutch breeders had already obtained a certain number of new varieties during the XVIIIth century, the first large rose collections of Gallicas were created in France under Napoleon 1st by Dupont, Godefroy, Vilmorin and the Empress Josephine herself. This stimulated a fashion of intense creation among botanists, collectors and breeders: in a period of thirty years (1820–1850) two generations of French rose breeders (Descemet, Vibert) produced more than two thousands of new varieties (JOYAUX 1998).

During the second half the XIXth century the breeders gradually turned away from the Gallicas. Vibert who had been the most important breeder of 'Provins Roses' since 1815 abandoned himself these varieties well before he retired from business. The fashion was now for Perpetual Hybrids that presented the extraordinary superiority to bloom until winter. In between 1848 and 1900 several thousands of Perpetual Hybrids and Hybrid Teas were put on the market (DICKERSON 1992).

It is well established that as soon as from the beginning of the XIXth century Gallica breeders attempted but without much success, to achieve the introgression into their roses of recurrence originating from *R. chinensis* (JOYAUX 1998). It is only in second half of the XXth century with the creation, for example, of the 'English Roses', that became associated into a same variety the recurrence of Hybrid Teas together with the floral morphology and the fragrance of old Gallica roses (AUSTIN 1992; BEALES 1992).

We analysed here by molecular typing (RAPD) the whole national collection of Gallica roses (281 cultivars) collected from different rose gardens in Europe (Mottisfont, UK; Sangerhausen and Wilhelmshöhe, Germany; Tête d'Or, Malmaison, l'Haÿ-les-Roses and Gap-Charance, France) and conserved at the 'Roseraie de la Cour de Commer' (Commer, Mayenne, France). Seventeen wild type *R. gallica* cultivars belonging to populations from the region of Lyon (France) and from the French Alps (Conservatoire Botanique National Alpin (CBNA) of Gap-Charance, Hautes-Alpes, France) were used as undomesticated reference. Our data show that only some rare breeders of the XIXth century succeeded to obtain punctually the introgression of Chineses alleles into the genome of Gallicas, while the 'English Roses' bred after 1960 show the systematic existence of such hybridisations. The horticultural literature suggests that the pollination process used by many French rose breeders of the XIXth century explains their failure to succeed in interspecific crossings.

Materials and Methods

Cultivars analysed

281 cultivars from the 'Roseraie de la Cour de Commer' (Commer, Mayenne, France) were analysed (see list in Table 1). Among them 17 pairs of cultivars had been collected from the various rose gardens under the same name and were supposed to have the same origin (pair#1 (=p#1): n°41 and 110 of Table 1; p#2: n°74+114; p#3: n°149+150; p#4: n°1+180; p#5: n°176+247; p#8: n°63+235; p#9: n°16+170; p#10: n° 136+155; p#11: n° 173+236; p#12: n° 31+108; p#13: n° 36+109; p#14: n° 7+65; p#15: n°47+53). They should be identical clones of the same original cultivars. They have been used here to evaluate the validity of our methodology, the genetic stability of the rose varieties and the quality of their identification. They were located as pairs on the ACP map of Fig. 2. Eight cultivars of wild type *R. gallica* harvested in the region of Lyon, and nine cultivars from the southern French Alps obtained from the CBNA have been used as reference of non-domesticated cultivars. *R. chinensis* cv. 'Old Blush' and three interspecific hybrid species of the Gallicaneae section (*R. alba*, *R. centifolia*, *R. damascena*) have been used as external references to the horticultural group. Among all these cultivars, *R. gallica* as well as the domestic Gallicas are known to be tetraploid; *R. alba* (6n) had been proposed to be a hybrid of *R. canina* (5n) x *R. gallica*; *R. damascena* (4n) would be a hybrid of *R. gallica* x *R. moschata* (2n) or *R. phoenicia* (2n) and *R. fedschenkoana* (4n); *R. centifolia* (4n) would be a complex hybrid of *R. gallica*, *R. damascena* and *R. alba* (HURST 1941; WYLIE 1954; IWATA et al. 2000).

DNA extractions

Fresh rose leaves were washed in water with non-ionic detergent, rinsed with distilled water and stored under 96 % ethanol in a cold room. 25 mg batches of alcohol dehydrated, air dried leaves were dry ground with two steel balls (2 mm diameter) in a grinding mill (MM200, Retsch, Hahn, Germany), then resuspended in 1 ml of 1 % Cetyl Trimethyl Ammonium Bromide - 0.7 M NaCl - 0.1 M Tris - 20 mM EDTA, pH 7.5 (DOYLE and DOYLE 1990) and incubated for 10 min at 65 °C with occasional inversions. The lysates were deproteinised by shaking with chloroform (1 volume) and centrifugation. The aqueous supernatants were precipitated with isopropanol (1 volume), rinsed with ethanol, air dried, resuspended in 2 ml of 10 mM Tris - 1 mM, pH 7.5, and stored at –20 °C.

PCR Amplifications

RAPD amplifications were performed with Klen Taq polymerase (Ab Peptides, St Louis MO, USA) according to recommendations of the manufacturer, using a MJ Research PTC100 thermocycler. Reaction mixes of 40 µl contained 10 µl of DNA stock solutions diluted 100 times. The primers were the 'P495', 'P496', 'P497', 'P498', 'P499', and 'P503' 20-mers designed for rose molecular typing by DEBENER et al. (1996). The PCR conditions were the following: initial DNA denaturation 3 min - 94 °C, followed by 35 cycles of 60 sec - 94 °C DNA denaturation / 60 sec - 55 °C primers annealing / 90 sec - 72 °C TAQ elongation. A last elongation step of 3 min - 72 °C followed the amplification cycles. The PCR products were then analysed by electrophoresis on 3 % acrylamide - 1 % agarose gels and visualised under UV light in the presence of Ethidium bromide.

Table 1. List of the Gallica varieties from the 'Roseraie de la Cour de Commer' analysed by RAPD, with the identity of their breeders and their date of creation. The numbering corresponds to the order in which samples were harvested and processed. Pairs of homonyms carry two or three numbers and have been identified as p#1, p#2… etc according to their position on the PCA map in Fig. 3. The identities of cultivars n°12 ('l'Enchanteresse'), 115 ('Estelle') and 117 ('Séguier') were doubtful and annotated by question marks.

N°	Variety	Breeder	Date
1	Désirée Parmentier	Parmentier	1841
2, 180, 117(?) p#4	Séguier	Robert	1853
3	Cramoisi des Alpes	?	pre 1829
4	Frédéric II	Verdier	1847
5	Le Grand Sultan	Descemet	pre 1815
6	Alector Cramoisi	?	pre 1811
7, 65 p#14	Nouvelle Pivoine	?	pre 1825
8, 35 p#7	Alexandre Laquement	?	pre 1899
10	Camaieu	Gendron	1826
11	Arlequin	?	pre 1841
13	Panachée superbe	Laffay	1841
14	Triomphe de Flore	Rouen	pre 1829
15	Park Wilhelmshöhe	?	1987
16, 170 p#9	Mécène	Vibert	1845
17	Ambroise Paré	Vibert	1846
18	Perle des Panachées	Vibert	1845
19	Fleur de Pelletier	Pelletier	pre 1824
20	Provins Renonculé	?	pre 1811
21	La Belle Sultane	Pays-Bas	pre 1811
22	Duchesse d'Angoulême	Vibert	1821
23	Alain Blanchard	Vibert	1839
24	Cosimo Ridolphi	Vibert	1842
25	Gloire de France	Bizard	1828
26	Ypsilanti	Vibert	1821
27	*R.g. pumila*		1773
28	Juliette	Miellez	pre 1828
29,33,106,107,126	Rosa gallica		
30	Esther	Vibert	1845
31,108 p#12	Agar	Vibert	1843
32	Alice Vena	?	XIX
34	Kean	?	pre 1843
36, 109 p#13	Belle Hélène	Descemet	pre 1815
37	Hippolyte	?	pre 1842
38	Adèle Prévost	?	pre 1836
39	Louis Philippe	?	pre 1836
40	*R.gallica grandiflora*		
41, 110 p#1	Duc de Fitzjames	?	pre 1837
42, 111, 181 p#17	Tricolore de Flandre	?	1846
43	Thérésa Scarman	Scarman	1996
44	Daphné	Vibert	1819
46	Pourpre charmant	?	pre 1811
53, 47 p#15	Anacréon	Vibert	1836
48	Valence Dubois	Fontaine	1880
49	Van Artevelde	Parmentier	pre 1847
50	Cora	Lecomte	1827
51	*R.g.* x *R.multiflora*		
52	César Beccaria	Robert	1855
54	Fanny Essler	Vibert	1835
55	Lucile Duplessis	Vibert	1836
56	Victor Parmentier	Parmentier	pre 1847
57	Général Donadieu	?	pre 1842
58	Rosier des Parfumeurs	?	18eme
59	Abailard	Vibert	1845
60	Belle Pourpre Violette	Descemet	pre 1811
61	Minerve	Descemet	pre 1812
62	Marie Tudor	?	pre 1846
63, 235 p#8	Oeillet Flamand	Vibert	1845
64	Nouveau intelligible	?	pre 1811
66	Charles Quint	Robert	1856
67	Belle Galathée	Descemet	pre 1815
68	Nouveau Monde (?)	Descemet	pre 1811
69	Bérénice	Vibert	1818
70	Pourpre ardoisé	?	pre 1813
71	Dumortier	Parmentier	pre 1843
72	Nouveau Vulcain	?	pre 1843
73	Duc de Bordeaux	Vibert	1820
74, 114 p#2	Perle de Weissenstein	Schwartz-kopf	1773
75	Cynthie	Descemet	pre 1815
76	La Revenante	Miellez	pre 1825
77	Comte Foy	Lecomte	1827
80	Paquita	?	pre 1841
81	Catinat	Vibert	1838
82	Adèle Courtois	?	pre 1842
83	Haddington	?	1837
84, 115 (?)	Estelle	?	pre 1810
85	Héloise	Descemet	pre 1815
86	Grande et Belle	Pays-Bas	pre 1811
87	Gazelle	?	pre 1843
88	Eulalie Lebrun	Vibert	1844
89	Octavie	?	?
90	Sultane favorite	Pays-Bas	XVIII
91	Panachée double	?	pre 1836
92	Lustre d'Eglise	Pays-Bas	pre 1790
93	Manette	Lecoffé	pre 1824
94	Orpheline de Juillet	?	pre 1836
95	La Maculée	DuPont	pre 1815
96	Rose Foucheaux	?	pre 1846
97	Alfieri	Vibert	1833
98	Malesherbes	Vibert	1834
99	Passe Velours: faux	Descemet	pre 1815
101	Fanny Pavetot	?	1820
102	Adèle	Descemet	pre 1815
103	Beau Narcisse	Miellez	pre 1828
104	Napoléon	Pays-Bas	pre 1814
112, 45 (?)	Dometil Beccard	?	pre 1883
116,164,100? p#16	Jenny Duval	?	pre 1842
118	Adèle Heu	Vibert	1816
119	Agatha	?	1800
120	Agathe Fatime	Descemet	pre 1815
121	Agathe Incarnata	?	1811

N°	Variety	Breeder	Date
122	Agathe Rose	Hardy	1823
123	Aimable Amie	Pays Bas	1805
124	Aimable Rouge	Vibert	1819
125	Antonine d'Ormois	Vibert	1835
127	Assemblage de Beautés	Delaage	1823
128	Avenant	?	pre 1837
129	Bacchante	?	pre 1811
130	Baron de Gossart	?	1802
131	Beckett's Single	Beckett House	1995
132	Belle Biblis	Descemet	pre 1815
133, 134 p#6	Belle de Crécy	Roeser	pre 1836
135	Belle des Jardins	Guillot	1872
136, 155 p#10	Belle de Yèbles	Desprez	pre 1835
137	Belle Doria	Parmentier	pre 1847
138	Belle Herminie	Vibert	1821-38
139	Belle Isis	Parmentier	1845
140	Belle Rosine	Vibert	1829
141	Belle sans Flatterie	Pays Bas	pre 1806
142	Belle Villageoise	Vibert	1839
143	Belle Virginie	?	pre 1826
144	Bijou des Amateurs	Jacquemet	pre 1830
145	Bizarre Triomphant	Pays-Bas	pre 1790
146	Boula de Nanteuil	Roeser	1834
147	Bouquet de Vénus	?	pre 1814
148	Brennus	Laffay	1830
150, 149 p#3	Capitaine Williams	?	pre 1843
151	Alcime	Vibert	1845
152	Amélie de Mansfield	?	pre 1842
153	Ariane	Vibert	1818
154	Bellard	?	pre 1842
156	Bouquet Charmant	Pays-Bas	pre 1811
157	Comte Boula de Nanteuil	Roeser	1834
158, 12(?)	L'Enchanteresse	François	pre 1824
159	Fanny Bias	Descemet	pre 1811
160	Ferdinand de Buck	?	pre 1842
161	Gil Blas	?	pre 1843
162	Grand Cramoisi	Vibert ?	1832
163	Hector	Parmentier	pre 1847
165	Julie d'Etanges	Vibert	1834
166	La Neige	Robert	1852
167	Louis-Philippe	?	pre 1836
168	Madame d'Hébray	Pradel	1857
169	Marie-Antoinette	Vibert	1829
171	Mercedès	Vibert	1847
172	Moïse	Parmentier	1828
173, 236 p#11	Oeillet Parfait	Foulard	1841
174	Omphale	Vibert	1839
175	Pepita	?	?
176, 247 p#5	Président de Sèze	Mme Hébert	1828
177	Président Dutailly	Dubreuil	1888
178	Reine d'Espagne	?	?
179	Royal Marbré	?	pre 1837
182	Marie-Louise	?	pre 1811
183	Cardinal de Richelieu	Parmentier	pre 1847
185	Château de Namur	Quétier	pre 1842
186	Chaucer	Austin	1970
187	Chianti	Austin	1967
188	Chloris	Descemet	1800
189	Cocarde pâle	?	pre 1813
190	Constance Spry	Austin	1961
191	Cramoisi Picoté	Vibert	1834
192	d'Aguesseau	Vibert	1836
193	Darius	Laffay	1827
194	Dis-moi Qui Je Suis	?	?
195	Dona Sol	Vibert	1842
196	Double Brique	?	pre 1842
197	Duc de Guiche	?	pre 1810
198	Duchesse de Berry	Vibert	1820
199	Duchesse de Buccleugh	Vibert	1837
200	Duchesse de Montebello	Laffay	1824
201	Elise Rovella	?	pre 1842
202	Eucharis	Descemet	pre 1815
203	Fornarina	Vibert	1841
204	Georges Vibert	Robert	1853
205	Georges Vibert,mutant	Robert	1853
206	Gonzalve	Vibert	1835
207	Grande Renoncule	?	pre 1842
208	Henriette	?	pre 1811
209	Henri Foucquier	?	pre 1842
210	Impératrice Joséphine	Descemet	pre 1815
211	James Mason	Beales	1982
212	Jeanne Hachette	Miellez	1828-42
213	La Bien-Aimée	?	pre 1814
214	La Gloire des Jardins	Descemet	pre 1815
215	La Nationale	?	pre 1836
216	LaplusbelledesPonctuées	?	pre 1929
217	Léa	Vétillard	pre 1828
218	Le Rosier Evêque	?	pre 1790
219	Les Saisons d'Italie	?	19ème
220	L'Invincible	Miellez	pre 1819
221	Lord Scarman	Scarman	1996
222	Louis van Tyll	Belgique	pre 1846
223	Lycoris	Vibert	1835
224	Manteau	?	?
225	Marcel Bourguoin	Corboeuf	1899
226	Marjolin	Roeser	pre 1838
227	Montalembert	Moreau	1852-61
228	Montigny	?	pre 1842
229	Narcisse de Salvandy	Parmentier	pre 1847
230	Néron	Laffay	1841
231	Nestor	Vibert	1834
232	Nouveau Rouge	Descemet	pre 1811
233	Nouvelle Transparente	Miellez	1835
234	Oeillet Double	Prévost	1829

N°	Variety	Breeder	Date
237	Ohl	?	pre 1843
238	Ombrée Parfaite	Vibert	1823
239	Ombre Superbe	Pays-Bas	pre 1811
240	Ornement de la nature	Pays-Bas	pre 1814
241	Paeonienrose	?	pre 1845
242	Palais de Laeken	Laeken	1824
243	Passe Princesse	Godefroy	?
244	Petite Orléanaise	?	pre 1843
245	Phénice	Vibert	1843
246	Pompon Panaché	?	1856
248	Prince Frédéric	Parmentier	pre 1846
249	Princesse de Nassau	Miellez	1830-40?
250	Provins ancien	Cochet	pre 1853
251	Provins Marbré	?	pre 1819
252	Pucelle de Lille	Miellez	pre 1829
253	Reine de Perse	?	pre 1825
254	Robert le Diable	?	pre 1837
255	Roi des Pourpres	Descemet	pre 1815
256	*R.francofurtana*		1774
257	*Rosa Complicata*		1864
258	*Rg conditorum*		1889
259	*R.g. officinalis*		
260	*R.g. velutinaeflora*		XIX
261	*R.g. versicolor*		mutant
262	*Rosa macrantha*		
263	*Rosa mundi* self-col.	UK	18ème
265	*RosaRichardii*(sancta)		
265	Rose de Boufarik		
267	Rose de la Maître-Ecole	Coquereau	1831
268	Rose de Schelfhout	Parmentier	pre 1847
269	Rose des Maures	?	1947
270	Sanchette	Vibert	1837
271	Scharlachglut	Kordes	1952
272	Soleil Brillant	Pays-Bas	pre 1790
273	Souvenir de Malmedy	Scarman	1996
274	Sterckmans	?	1840
275	Surpasse Tout	Pays-Bas	pre 1823
276	Temple d'Appolon	?	pre 1814
277	Thalie la Gentille	Descemet	pre 1811
279	Triomphe de Laffay	?	?
280	Turenne	Vibert	1846
281	Tuscany	?	1837
282	Tuscany Superb	Rivers	pre 1837
283	Velours Pourpre	Pays-Bas	pre 1811
284	Ville de Londres	Vibert	1839
285	Ville de Toulouse	Brassac	1876

Data analyses

The polymorphic fragments were scored for each cultivar. They were processed in two complementary ways:

1) By dendrogram construction, calculating in a first step pairwise distances between the DNA samples using the various metrics (Euclidean, Khi2, Jaccard) included in the 'Clusters' module of the 'ADE-4' software developed by THIOULOUSE et al. (1996). In a second step UPGMA dendrograms (Unweighted Pair-Group Method of Arithmetic Averages, SOKAL and SNEATH 1963) were built up with the 'Dendrogram' module of 'ADE-4'.
2) By Principal Component Analysis (PCA) using the PCA module of 'ADE-4'. PCA defines axes for the projection of the complex set of data of presence / absence of PCR bands, maximising the weight of the data on these axes and providing thus a map of positions of the varieties, identified each by the label of its name, number or breeder.

Results

Characteristics of PCR fragments distribution

A total of 147 polymorphic PCR fragments were obtained among the 302 varieties of Gallica roses using six long RAPD primers. The distribution of the various PCR fragments present in the population (value=1) is essentially continuous, meaning that these six primers produce six sets of independent PCR markers. They prime different regions of the genome, exploring the totality of the DNA without preferential annealing at some specific sites only.

UPGMA dendrograms of rose cultivars

The genetic distances between rose cultivars were represented by UPGMA dendrograms constructed from matrices of distances calculated using the Euclidean, Khi2 or JACCARD (1901) metrics available in ADE-4. All three UPGMA dendrograms had similar structures, differing only by the lengths of their branches. Very different dendrogram structures were however obtained with other methods available in ADE-4 to build trees from distance matrices (divisive algorithm, Ward's method, complete link, single link, UPGMA). We worked here with the most commonly used UPGMA method (REYNDERS-ALOISI et al. 1995a, b, 2000) that already proved reliable in our hands for rose classification (MARTIN et al. 2000). The UPGMA dendrogram of Fig. 1 was built using the JACCARD (1901) metrics; it shows the large genetic variability of the whole rose collection. Even the sub-populations of the wild type roses from the areas of Lyon and Gap display long branches characteristic of large genetic distances between individual cultivars; this observation is in good agreement with previous analyses of similar populations (REYNDERS-ALOISI et al. 1995a, b, 2000). In spite of their large variability these wild type cultivars are fairly well clustered on the dendrogram; the founder botanical Gallicas already known in the Middle-Ages are positioned further apart (*R. g. pumila* n°27 in Table 1, *R. g grandiflora* n°40, *R. francofurtana* n°256, *Rg conditorum* n°258, *R. g. officinalis* n°259, *R. g. velutinaeflora* n°260, *R. g. versicolor* n°261, *R. macrantha* n°262, *R. mundi*

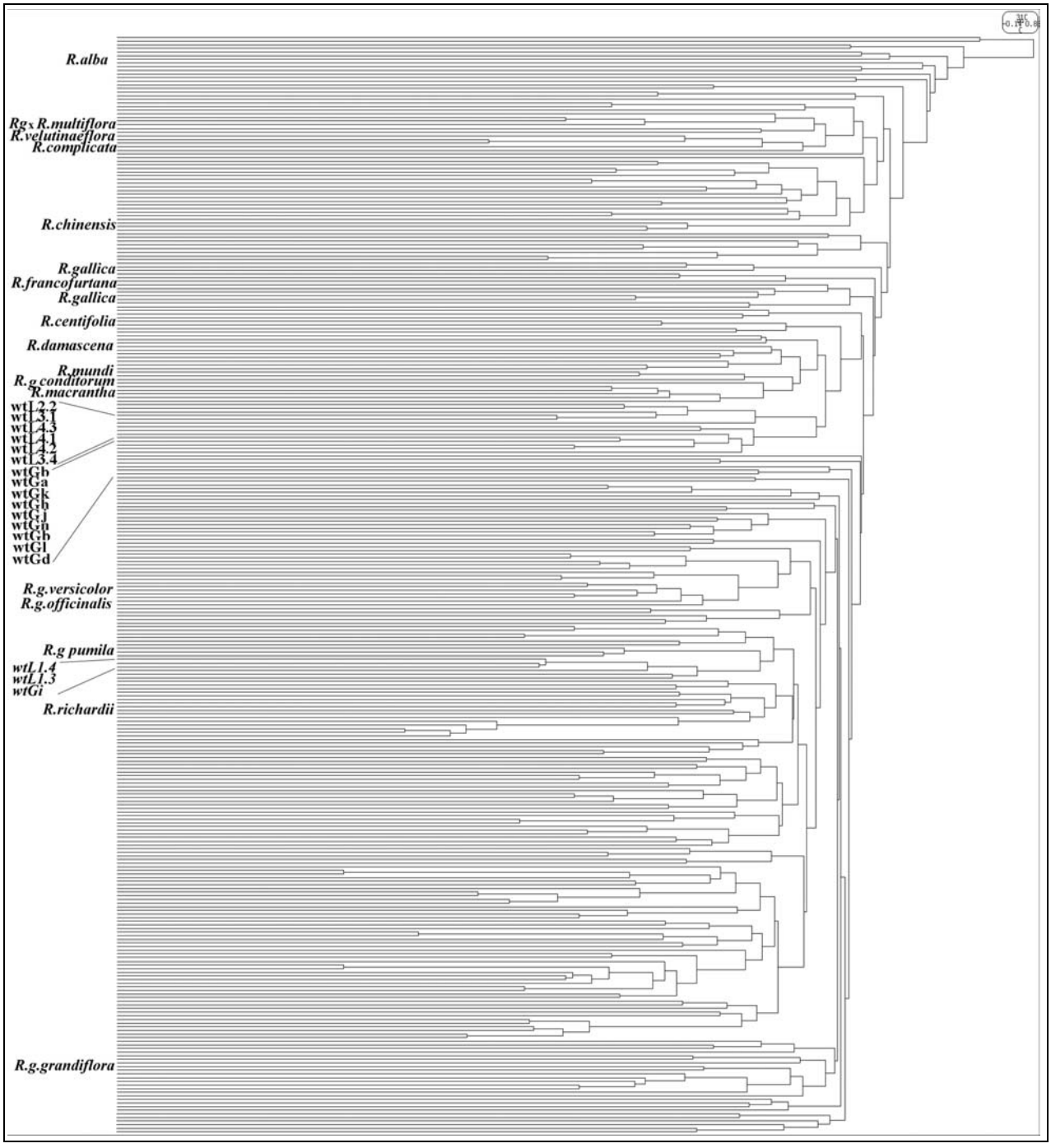


Fig. 1. UPGMA dendrogram constructed from the JACCARD (1901) distance matrix calculated from PCR data. The labels for the wild type cultivars ('wtG' and 'wtL'), for the founder botanical Gallicas and for four reference species (*R. alba*, *R. centifolia*, *R. chinensis* and *R. damascena*) have been added to illustrate the genetic distances and the polymorphisms between these varieties and the domesticated Gallicas.

n°263, *R. richardii* n°265), demonstrating significant modifications of their genome. The identification of the various cultivars by their labels of name, date of creation or breeder enables to visualise changes of genetic distances during the process of creation of the Gallica group. Reading of these changes along the dendrogram is however impractical, due to complexity of the pattern (302 individual branches) and to the possibility of rotation of these branches around their knots. We preferred therefore the presentation of the molecular data on genetic distance maps as made by Principal Components Analysis.

Principal Components Analysis of PCR data

PCA map of rose cultivars. The Principal Component Analysis (PCA) process projects the data resulting

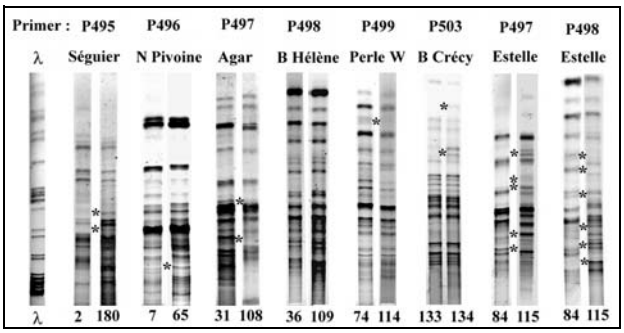


Fig. 2. Experimental PCR errors: the electrophoretic patterns of homonym pairs of varieties (listed in Materials and Methods) processed independently vary by 3 to 5 bands among 100 to 120 (about 5 %). The migration was from bottom to top. Upper line: identification of PCR primers. Bottom line: cultivar n° as listed in Table 1. The molecular size marker (extreme left lane) is Lambda DNA digested with Pst I + Hind III. *point to bands of variable intensities. 'Estelle' (4 right lanes) shows many variations, confirming that n°115 had been misidentified (Table 1).

from the analysis of PCR fragments on multiple axes defined so as to maximise the weight of the data on these axes. The two first axes carry the most significant information while the next ones bear the background of experimental error. The significant data will thus be represented at best on the plane formed by the two first PCA axes.

Evaluation of experimental error. The methodological error affecting the data represented on the PCA map was illustrated by examination of the 17 pairs (or triplet) of the supposed synonymous varieties mentioned in Materials and Methods. The corresponding electrophoretic patterns show a very strong similarity between the PCR profiles obtained for independent amplifications on these varieties (Fig. 2). In each of the 17 pairs of homonym cultivars, 3 to 5 PCR bands were found to show variable levels among the 100 to 120 obtained for each variety. This demonstrates the reasonable reliability of the procedure used (less than 5 % variation in the number of PCR bands), as well as the quality of the identification of the varieties in the collection. The cultivars of homonym pairs are projected close to each other on the PCA map, defining experimentally the precision of the position of each variety as a circle of about one map unit (Fig. 3).

PCA maps of wild-type vs. domesticated Gallicas. The map of genetic distances between Gallicas on the PCA plane provides a continuum of 302 points. However the existence of a strong structure in the population was demonstrated by analysis of variance (ANOVA). The 17 wild type (wt) *R. gallica* cultivars from Lyon and Gap and the 281 domestic cultivars are distributed

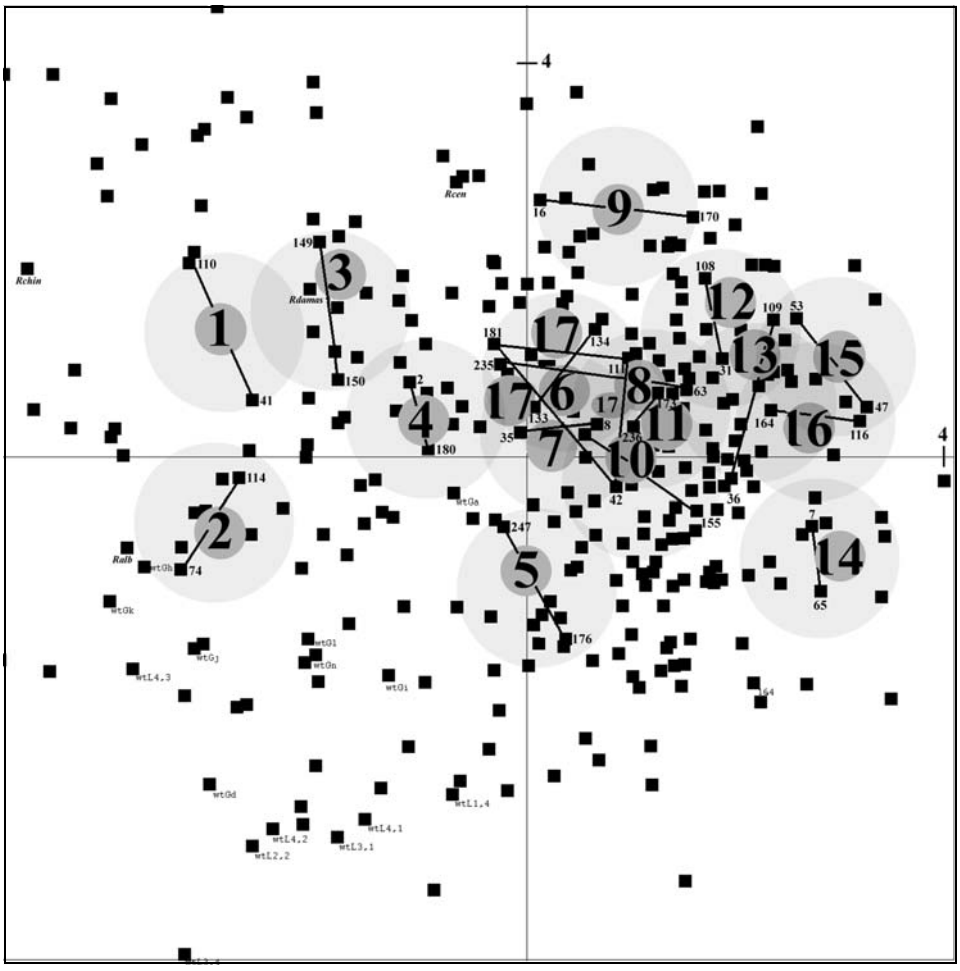


Fig. 3. PCA map of homonym varieties (4 distance units are indicated on X and Y axes). The 17 pairs of homonym varieties are identified by grey circles numbered 1 to 17 and the cultivar n° of Table 1. The experimental errors affecting the position of the cultivars are estimated by circles of one map unit as shown for the synonym pairs.

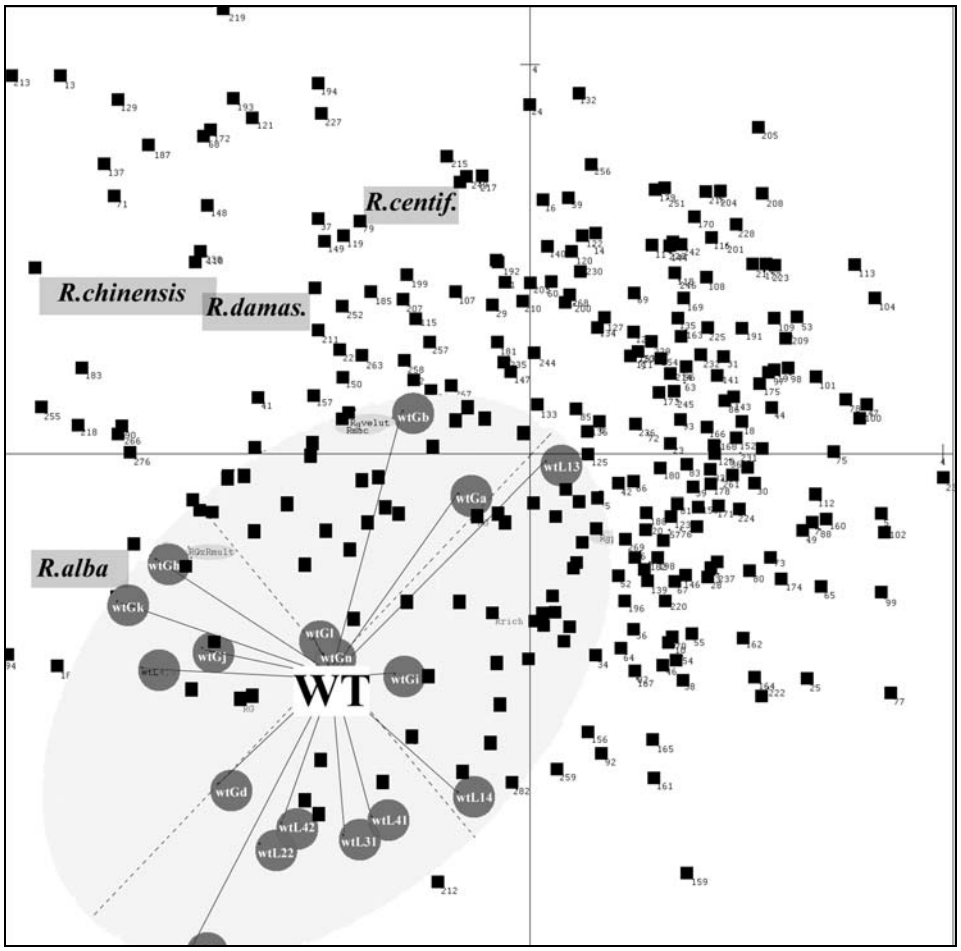


Fig. 4. Structure of the Gallica population (302 cultivars). The wild type *R. gallica* from Lyon and Gap are identified as grey circles respectively as 'wtL' 'wtG'. A light grey ellipse delimits their sub-population. The four reference species (*R. alba*, *R. centifolia*, *R. chinensis* and *R. damascena*) are identified by grey rectangles. The domesticated varieties are represented by black squares.

quite differently on the map. The former are essentially located in the lower left quarter of the map, while the latter are positioned in the three other quarters. The ANOVA demonstrates that the null hypothesis of an identical distribution of the two sub-populations has only a probability of 8,3 x 10^{-13} (Fig. 4). The various sub-populations of Gallicas that can be defined on the basis of their breeder identity or date of creation will therefore be significantly different. *R. alba*, *R. centifolia* and *R. damascena* are located close to the wild type *R. gallica* on the PCA map, in good agreement with the interspecific hybrid state of these species. On the other hand *R. chinensis* is logically located far away (in extreme left limit of the map) since totally unrelated with European roses.

Map position changes during the domestication of Gallicas

The identification of the various Gallica cultivars on the PCA map depicts the positional changes related to the domestication of this horticultural group. Four sub-groups of roses appear on the background of the French history JOYAUX (1998):

1) The Gallicas created before 1815 that had been collected under the First Empire of Napoleon Ist. This sub-population includes namely the founder botanical Gallicas already known in the Middle - Ages as well as the varieties bred by the Dutch breeders of XVII–XVIIIth centuries (data not illustrated). It spreads from the lower left part towards the upper right part of the map;
2) The roses bred from 1815 to 1850 during the time of the great Gallica fashion (French periods of the 'Restauration' and of the 'Monarchie de Juillet') (Fig. 5) are mainly positioned in the upper right part of the map;
3) Those created during second half of the XIXth century while the Perpetual Hybrids became more fashioned. They are located in the extreme upper right part of the map;
4) At last the 'English Roses' of the XXth century created by hybridisation between recurrent Hybrid Teas and old Gallica roses (Fig. 5). They are all located on the upper left part of the map, near to *R. chinensis*.

We have also analysed the position on the PCA map of the creations of the most important breeders (Descemet, Vibert, Parmentier and Laffay) so as to characterise their working methods and the nature of the cultivars they bred. In addition we have taken into account the more or less pronounced crimson edge of young follicles on cultivars of the 'Roseraie de la Cour de Commer'. This character is probably dominant and brought by the Chinenses (while recurrence is recessive). It is considered as an indication of interspecific hybridisation.

For each of these four sub-groups the numbers of varieties located in the different parts of the PCA map have been estimated and summarised in Table 2 below.

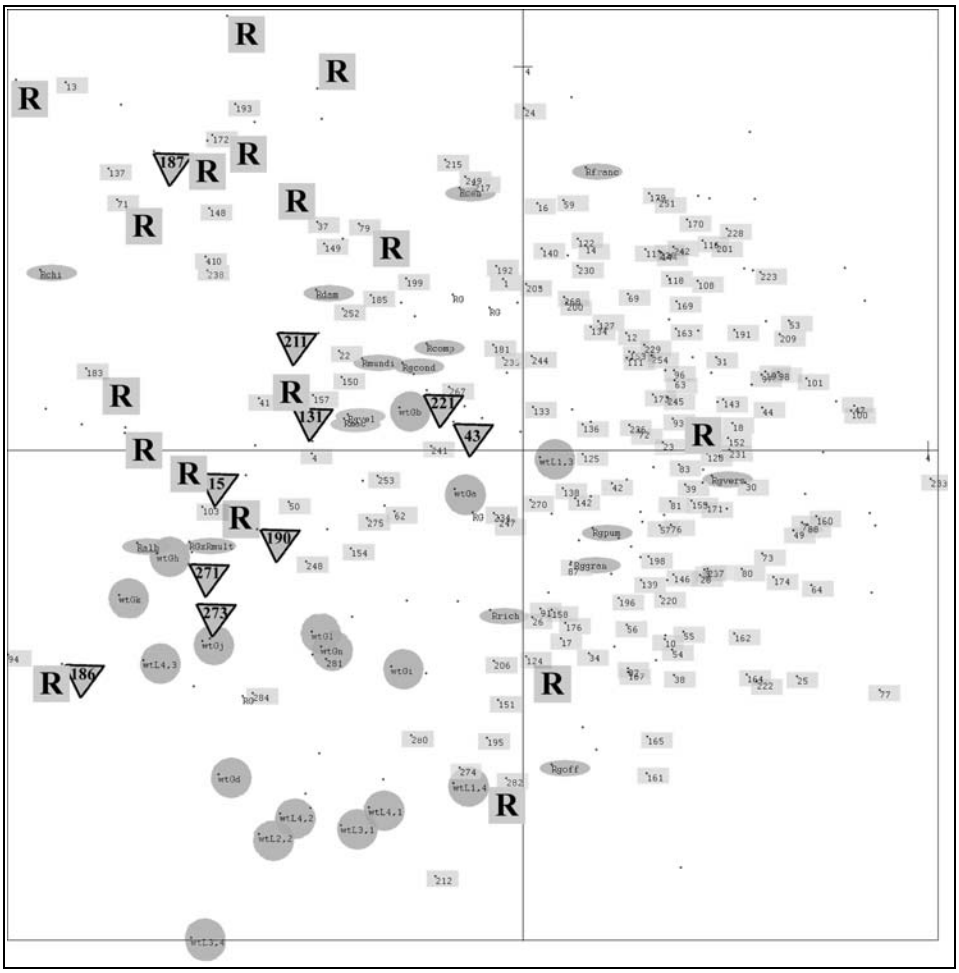


Fig. 5. Gallicas bred from 1815 to 1850 ('French Restauration' and 'Monarchie de Juillet') and after 1960 ('English Roses'). The 159 varieties of the collection bred from 1815 to 1850 are identified by grey rectangles; 70 % of them are positioned on the right half of the PCA map (as reported in Table 2). The 'English Roses' of the sample (triangles) are all (100 %) on the left half of the map, and of the 17 cultivars showing strong red coloration of young follicles (a chinenses character identified by 'R' signs) 88 % are on the left half of the map.

Discussion

Our results do not allow to define precise limits between the different horticultural groups of european roses (Gallicas, Centifolias and Damascenas) and thus to individualise clearly the Gallicas themselves. Intersections between the domains of these horticultural groups are quite logical since, as mentioned in Materials and Methods, they all originate from interspecific crosses that have involved in common the same species and alleles. They belong to the same botanical section of the Gallicanae (HURST 1941; WYLIE 1954; BEALES 1992; IWATA et al. 2000). On the contrary European and far-eastern roses are well differentiated by RAPD molecular typing since the latter belong to the botanical section of the Chinenses. Our analysis demonstrates the existence of two allele flows of opposite directions in the course of the domestication history of the Gallicas. The first flow resulted for from crosses exclusively between European roses, while the second one involved hybridisations with cultivars of the Chinenses section.

The first flow has for origin, on the PCA map, the lower left quarter on which are placed the wild type *R. gallica* found in the last sites existing in the French nature. This flow is directed towards the right part of the map. The first founder botanical Gallicas to be cultured (*R. g. officinalis* and others mentioned above) differentiate significantly from wild type cultivars on our map (Fig. 5). Their morphological characters have evolved in parallel: they show doubling of the flowers (10 to 20 petals or more) for many varieties (JOYAUX 1998) and acquisition of a more erected habit than that of wild type cultivars in their natural biotope. The same displacement on the PCA map continues all along the breeding of the Gallicas in the course of the XIXth century. Cultivars defined by their extreme positions of the map show globally, compared to those of the centre of gravity of the cultured Gallicas, an increase of their floral complexity. They are more systematically double and full, with a disposition of the petals in cups and in quarters. It appears also that the breeders have selected preferentially strong fragrances and dark red colours (purple, crimson, carmine), which are characters naturally, found in the Gallica group.

In our molecular typing analysis these varieties provide the higher numbers of PCR fragments. Our interpretation of this fact is that the genetic reshuffling imposed by repeated crosses among the horticultural group followed by selection of varieties with marked morphological characters have yielded cultivars highly heterozygous with good hybrid vigour. These highly heterozygous individuals display logically a large polymorphism of RAPD fragments after PCR amplification.

They represent the major part of the conservatory of 'La Cour de Commer' simply because they result from the work of the most productive rose breeders of the first half of the XIXth century (Descemet, Vibert). The horticultural literature (JOYAUX 1998) describes the

Table 2. PCA map positions of the Gallicas bred during the history of rose domestication. The positions on the left or right parts of the PCA map were recorded for roses bred at different periods, by various breeders or displaying red coloration of the young follicles. The percentages and total number of cultivars reported in columns 2, 3 and 4 illustrate the two opposite allele flows that occurred during the history of the Gallica group.

	Left Half	Right Half	Total nb of plants
Periods			
Wild type	94 %	6 %	17
Before 1815	53 %	47 %	75
1815 to 1850	30 %	70 %	159
1850 to 1899	27 %	73 %	22
English Roses	100 %	0 %	10
Breeders			
Vibert	11 %	89 %	46
Descemet	20 %	80 %	20
Parmentier	57 %	43 %	14
Laffay	60 %	40 %	5
Colour of follicles			
Strong	88 %	12 %	17
Moderate	71 %	29 %	7
Weak	38 %	62 %	16

nb = number

protocols of pollination used in that time and gives an explanation of the two opposite allele flows in the course of the Gallica domestication. Descemet and Vibert used simple open spontaneous pollinations between multiple varieties planted side by side to let operate the crosses. Thus in 1820 the nursery of Vibert in Chennevières-sur-Marne counted several tens of thousands of plants organised in 'classes' of very similar morphologies. The purpose of this 'scientific' organisation was 'to better recognise and memorise similar Provins roses'. At most, labourers attempted to improve pollination by dragging ropes through the rosebushes, simulating some wind pollination effect (but sexual reproduction in rose proceeds essentially through insect pollination). The most beautiful varieties were selected from the seedlings thus obtained. The process was empirical and gave plethora of results: among the some 2000 varieties bred between 1815 and 1850, 350 figured in the selling catalogues of Vibert. This simplistic pollination technique had evidently little chances to promote interspecific crossings with Chinenses roses since it has been described that pollen competition systematically favours intraspecific pollen grains at the expense of the interspecific ones (HEIZMANN et al. 2000). Among the 600 creations of Vibert 51 are conserved in the 'Roseraie de la Cour de Commer' and 46 of them are projected on the right part of the PCA map.

The second allele flow is opposite to the former and directed towards roses related to *R. chinensis* 'Old Blush'. This flow appears as soon as the XIXth century if one takes account of the production of a minority of some discrete breeders such as the Belgian Parmentier and the French Laffay. The cultivars bred by Parmentier and even more those of Laffay project on the left half of the map (Table 2). These breeders had succeeded to obtain varieties showing a typical character of *R. chinensis i.e.* the presence of a crimson border on young follicles: 'Brennus' n°148 in Table 1, 'Darius' n°193, 'Panachée Superbe' n°13 and 'Duchesse de Montebello' n°200 from Laffay, as well as 'Cardinal de Richelieu' n°183, 'Dumortier' n°71, 'Belle Doria' n°172 and 'Moïse' n°172 from Parmentier present these morphological characters and are placed in the area of *R. chinensis* on the PCA map of the present study. This movement is still more evident with the creation of the 'English Roses'. Ten of them considered to have a typical Gallica morphology were included in the collection of the 'Roseraie de la Cour de Commer' and in our sampling. All these roses have Hybrid Teas in their parentage and present the character of crimson edges of the young follicles. But only 'Chaucer' repeats its bloom again in the season. Later David Austin and others have obtained more efficient recurrence. However the imprint of the chinensis genome is perfectly obvious by the location of these genotypes on the PCA map. In our molecular typing analysis they display some unique PCR fragments shared with *R. chinensis* (for instance fragment 'P498 V'). They score relatively fewer PCR fragments than the domesticated Gallicas. We suggest that crossing tetraploid Gallicas with diploid Chinenses or highly inbred Hybrid Teas produced some lower diversity of the RAPD fragments in the progeny.

The control of pollination (at least partial) allowed more meticulous breeders like Parmentier and Laffay to create real hybrids between Gallicas and Chinenses as soon as before 1850. Recurrence is a recessive character (SEMENIUK 1971a, b; DE VRIES and DUBOIS 1984; DEBENER 1999): its introgression required backcrosses with Chinenses. Such crosses were repeated at will later when all rose breeders learned to control pollination rigorously, and to use the laws of genetics.

The positions of varieties on the PCA map identify the working methods used to breed them. The roses from Laffay and Parmentier fall very differently from those from Descemet and Vibert on the map (in the left half for the former, in the right half for the latter). This provides arguments for the identification sometimes doubtful of their origin. For example JOYAUX (1998) initially attributed 'Château de Namur' (n°185) to the French breeder QUÉTIER (1932). The distribution of this variety in Belgium especially and its position in close proximity of *R. chinensis* on our PCA map strengthens the hypothesis that the Belgian breeder Parmentier created it, instead of Quétier. Similarly 'Oeillet Flamand' (n°63 and 235) was given for a creation of VIBERT (1845) with the question of its 'Flamand' denomination. Its place on the PCA map among roses obtained by Parmentier suggests that this denomination would concern a creation of the latter sold on the market by Vibert after he bought its copyright.

At last, the results of molecular typing suggested us to revise the documentation sources concerning some homonym cultivars: among those named 'Estelle' (n°84 and 115), only the variety 84 is a Gallica while the n°115 was revised as a Perpetual Hybrid, explaining why their electrophoretic patterns differ so much

(Fig. 2, right lanes). A similar revision led to the conclusion that for cultivars of 'l'Enchanteresse' (n°158 and 12), only cultivar n°158 is well identified. The two specimens n°7 and n°65 of 'Nouvelle Pivoine' are two cultivars grafted from the same original variety from the Roseraie de l'Haÿ-les-Roses (Val de Marne, France). The low distance between their points of projection (couple n°14 on the upper right quarter of the PCA map) results from experimental errors (errors of appreciation of the presence/ absence of PCR fragments, imperfect PCR reproducibility) as well as from a possible genetic drift between cultivars after grafting (this genetic drift is at the origin of variants or 'sports' of the same varieties).

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