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The domestication process of the Modern Rose: genetic structure and allelic composition of the rose complex

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Abstract Genetic variability among 100 old cultivated rose varieties from 13 horticultural groups was estimated by arbitrary primed (AP) PCR. Using five long (20-mer) PCR primers, 58 polymorphic DNA fragments were produced, of which 55 were highly discriminant, allowing differentiation of the quasi-totality of the 100 cultivars. A dendrogram was constructed displaying the relative genetic similarities between cultivars estimated from the presence/absence of PCR fragments. It shows the relationships between the Chinese and European founder roses, hybrid groups of the first (Bourbons, Noisettes, Portlands) and second (Hybrid Perpetuals and Teas) generations, and the most modern Hybrid Teas, produced during the history of domestication. Principal components analysis (PCA) of the same data demonstrates the occurrence of a continuous gradient of the European/Chinese allele ratio, and a considerable reduction of genetic variability superimposed with the progress of domestication. The two complementary analyses are in good agreement with the horticultural literature. They also give access to DNA fragments potentially linked to genes involved in the control of the main morphogenetic characters of various groups.

Keywords Genus *Rosa* · Domestication · Introgression · Genealogy · Principal component analysis

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Introduction

While the genus *Rosa* comprises more than 100 botanical species (Crépin 1866; Rehder 1940), only 7–10 of them were used to create the Modern Rose we know today (Wylie 1954; Maia and Vénard 1976). The most recent Hybrid Teas are the final result of a process of domestication that occurred during the XIXth and in the early part of the XXth century, the centre of which was principally located in Europe. Very numerous crossings and hybridisations were performed between rose founder species of European origin on the one hand and of Chinese origin on the other. This domestication has allowed the introgression into modern roses of important horticultural characters such as winter hardiness, resistance to pests, floral complexity and flower doubling brought by European roses, while recurrent flowering or perpetuity as well as colour brightness came from their Chinese counterparts.

The basic genetics of several characters has been documented in the literature, especially of those concerning winter hardiness, recurrent flowering, ‘‘moss’’ and ‘‘dwarf’’ characters, double flowers and flower colour (Svejda 1979; Marshall et al. 1983; De Vries and Dubois 1984; Dubois and De Vries 1987; Grossi 1998; Debener 1999; Raymond 1999). Many of these traits are essentially under monogenic control, but they often show interactions with minor modifier genes that modulate their expression, explaining the many continuous physiological and morphological variations observed among the horticultural groups of roses created in the course of their domestication.

In the present work, we describe the genetic diversity of the domestic rose complex by DNA analysis of 100 cultivars of Old Roses. They were chosen to represent the main horticultural groups that marked the successive

stages of the domestication history. The rose is a semi-woody perennial plant with a life span that largely exceeds a century ; it can be maintained and reproduced through vegetative culture (grafting, cutting and / or layering). Many private or public collections give access to most of the original cultivars that marked the history of the Modern Rose creation. We have taken advantage of these features to analyse the genetic structure and relationships of the main rose horticultural groups. We have detected different balances of various alleles in each of these groups. We demonstrate a continuous shift of allele ratio superimposed on a significant reduction of allelic diversity during the process of domestication, from the initial founder groups (Gallicas, Damascenas, Chinenses) to the most modern ones (Teas and Hybrid Teas).

Materials and methods

Plant material

Neighty eight cultivars of roses grown in the Roseraie of the Val de Marne in l'Haÿ-les-Roses (France) have been chosen among horticultural groups of Albas (3 cultivars), Bourbons (9), Centifolias (14), Chinenses (8), Damascenas (8), Gallicas (13), Multifloras (3), Noisettes (11), Perpetuals (10), Portlands (5), Sempervirens (2), Teas (7) and Hybrid Teas (5); in addition, two modern Hybrid Tea roses (Anna and Virginia, Pekmez-France) were added to the samples (Table 1).

Table 1 Rose cultivars. List of the rose cultivars harvested in may 1988 at the Roseraie du Val de Marne (L'Haÿ-les -Roses, France), sorted by horticultural group and alpha-numerical names

ID no.	Group	Cultivar	Created	ID no.	Group	Cultivar	Created
1	Gallica	Assemblage de Beautés	1823	51	Perpetual	La Reine	1842
2	Gallica	Belle Flore	1826	52	Perpetual	Miss House	1838
3	Gallica	Belle sans Flatterie	1820	53	Perpetual	Olivier Metra	1844
4	Gallica	Chloris	1800	54	Chinensis	Bengale Animé des Anglais	1832
5	Gallica	Duc de Bordeaux	1820	55	Chinensis	Bengale Pourpre	1827
6	Gallica	Fanny Bias	1819	56	Chinensis	Bengale Sanguin	1838
7	Gallica	Grand Cramoisi de Vibert	1818	57	Chinensis	Catherine II	1832
8	Gallica	Impératrice Joséphine	1770	58	Chinensis	Common China	1789
9	Gallica	Octavie	1800	59	Chinensis	Cramoisi Supérieur	1832
10	Gallica	Perle de Weissenstein	1773	60	Chinensis	Gloire des Rosomanes	1825
11	Gallica	*R. gallica versicolor*	1800	61	Chinensis	*R. chinensis semperflorens*	
12	Gallica	*R. officinalis*		62	Bourbon	Acidalie	1838
13	Gallica	*R.semperflorens*		63	Bourbon	Appoline	1848
14	Centifolia	Aristobule	1840	64	Bourbon	Bouquet de Flore	1839
15	Centifolia	Blanchefleur	1835	65	Bourbon	Desgache	1840
16	Centifolia	C.bullata (pink)	1809	66	Bourbon	Georges Cuvier	1843
17	Centifolia	Cent Feuilles Descemet	1810	67	Bourbon	Maréchal du Palais	1846
18	Centifolia	Chapeau de Napoléeon	1827	68	Bourbon	Mme Desprez	1831
19	Centifolia	Crested Provence (pink)	1827	69	Bourbon	Pierre de St-Cyr	1838
20	Centifolia	Cristata	1827	70	Bourbon	Proserpine	1841
21	Centifolia	De Meaux	1814	71	Noisette	Aimée Vibert cl.	1841
22	Centifolia	Duc d'Angoulême	1827	72	Noisette	Bougainville	1824
23	Centifolia	Duchesse d'Angoulême	1827	73	Noisette	Caroline Marniesse	1848
24	Centifolia	Mignonne Charmante	1814	74	Noisette	Céline Forestier	1842
25	Centifolia	Oeillet	1800	75	Noisette	Champney's Pink Cluster	1811
26	Centifolia	Old Black	1796	76	Noisette	Desprez à Fleurs Jaunes	1835
27	Centifolia	Petite de Hollande	1800	77	Noisette	Isis	1853
28	Damas	Bernard	1846	78	Noisette	Jacques Amyot	1850
29	Damas	Botzaris	1856	79	Noisette	La Biche	1832
30	Damas	Dom Pedro	1833	80	Noisette	Lamarque cl.	1830
31	Damas	Kazanlik	1689	81	Noisette	Philomèle	1844
32	Damas	L'Amitié	1850	82	Sempervirens	Félicité Perpétue cl.	1827
33	Damas	Leda (pink)	1827	83	Sempervirens	Flore	1829
34	Damas	Mme Hardy	1833	84	Multiflora	Bijou de Lyon	1882
35	Damas	Red Damask	1800	85	Multiflora	De la Grifferaie	1845
36	Alba	Cuisse de Nymphe	1802	86	Multiflora	Euphrosine	1895
37	Alba	Maiden's Blush	1797	87	Tea	Emilie Dupuy cl.	1870
38	Alba	Maiden's Blush Great	1797	88	Tea	Maréchal Niel cl.	1861
39	Portland	Achille Gonod	1854	89	Tea	Mme Falcot	1858
40	Portland	Coelina Dubos	1849	90	Tea	Mme Mélanie Willermoz	1846
41	Portland	Dembrowsky	1849	91	Tea	Mme Trifle	1870
42	Portland	La Quatre Saisons Continue	1811	92	Tea	Perfection de Montplaisir	1871
43	Portland	Mme Knorr	1855	93	Tea	Président	1860
44	Perpetual	Baronne Prévost	1842	94	Tea Hybrid	Anna (Pekougel)	1988
45	Perpetual	Caroline de Sansal	1849	95	Tea Hybrid	Gloire Lyonnaise	1884
46	Perpetual	Céline	1825	96	Tea Hybrid	Henry Benett	1872
47	Perpetual	Comte Odart	1850	97	Tea Hybrid	Jean Lorthois	1879
48	Perpetual	Docteur Marx	1842	98	Tea Hybrid	La France	1867
49	Perpetual	Duchesse de Galliera	1847	99	Tea Hybrid	Melle Brigitte Violet	1879
50	Perpetual	Gerbe Rose cl.	1849	100	Tea Hybrid	Virginia (Pekwhina)	1990

DNA extraction

Leaves were harvested, washed, deep-frozen to –70°C, ground under liquid nitrogen with a mortar and pestle, suspended in extraction buffer (50 mM Tris · HCl, pH 8 ; 100 mM NaCl ; 50 mM EDTA) and lysed by the addition of SDS to a 1% final concentration. After de-proteination by phenol – chloroform (1/1) and centrifugation, DNA was precipitated with ethanol, re-suspended in extraction buffer, treated with RNAse (50 µg/ml, 60 min, 37°C) and de-proteinated a second time. DNAs were stored at 4°C in 1 mM Tris-HCl, pH 8.0, 10 mM EDTA (TE) buffer

PCR amplifications

PCR amplifications were performed in a Perkin-Elmer 9600 thermocycler using the Klen *Taq* polymerase (Ab Peptides, St Louis Mo., USA) according to manufacturer's recommendations, in reactions of 25 µl containing 50 ng of initial DNA. The primers used were the 20-mers ''P495'', ''P496'', ''P497'', ''P498'' and ''P499'' developed by Debener and Mattiesch (1998). Temperature cycles were the following: 3 min at 94°C (initial melting), 35 cycles of (1 min, 94°C; melting) / (1 min, 55°C; annealing) / (1 min, 72°C; elongation) – (2 min, 72°C; final elongation). The PCR-amplification products were fractionated by 2% agarose-gel electrophoresis, and visualised by bromide-UV illumination.

Data analysis

Data were analysed by estimation of the relatedness between cultivars using the ''RAPDistance'' package from Armstrong et al. (1994). This software offers 18 methods for the calculation of similarity between pairs of cultivars, and gives access to several methods of dendrogram construction. We chose the index of Apostol (1993) (a simple matching method; d = (*n*11 + *n*00) / *n*) to calculate distances between plants since this method presents two interesting specificities: it calculates similarities by taking account of shared present fragments (*n*11) as well as of shared absent ones (*n*00). Thereby, it manages the fact that various allelic forms of homologous PCR fragments for different cultivars may migrate at different levels of the electrophoregrams; it also provides similarity indexes having the same mathematical formulation as the canonical distances used by the principal component analysis (PCA) (Thioulouse et al. 1996). This allows the rigorous comparison of results represented by the dendrogram constructed using the index of Apostol, with complementary results from PCA, since both methods calculate distances through similar procedures.

The dendrogram was constructed from the Apostol distance matrix by the UPGMA method (Unweighted Pair-Group Method of Arithmetic Averages) (Sokal and Sneath 1963) from the ADE package (Thioulouse et al. 1996). The PCA of presence / absence tables of PCR fragments was performed with the corresponding module of the same ADE package.

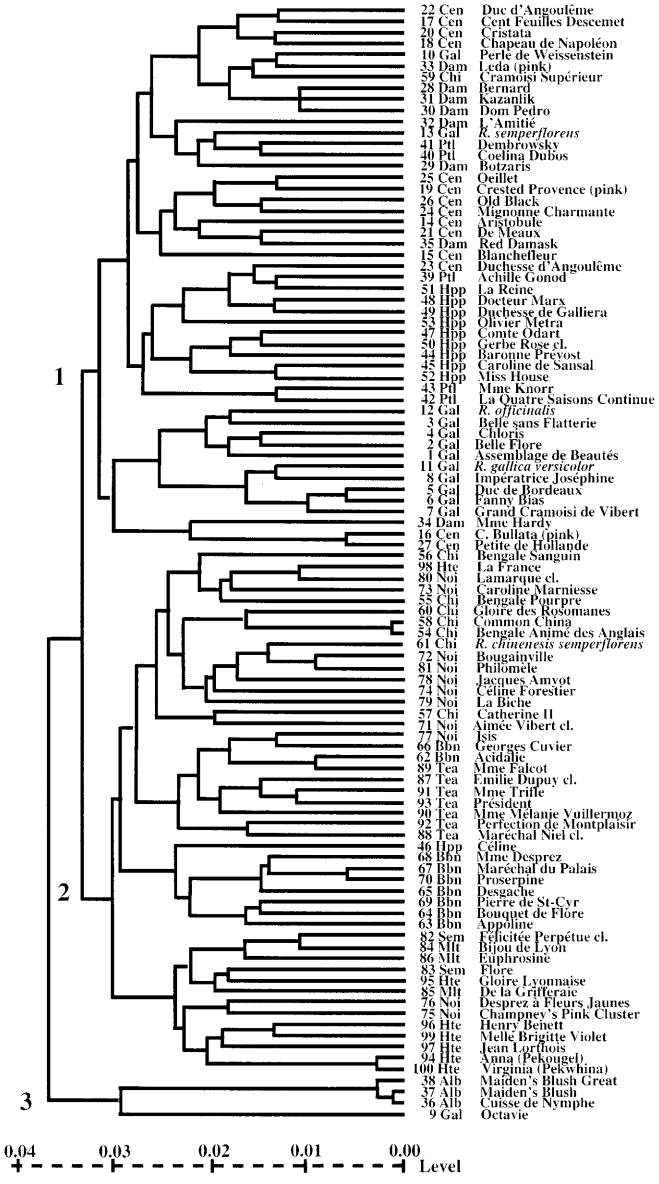

Fig. 1 UPGMA dendrogram. The UPGMA dendrogram was obtained from cluster analysis of 100 samples of old and modern rose varieties, using the Apostol distance measure. *Alb*=Albas, *Bbn*=Bourbons, *Cen*=Centifolias, *Chi*=Chinenses, *Dam*=Damascenas, *Gal*=Gallicas, *Hpp*=Hybrid Perpetuals, *Hte*=Hybrid Teas, *Mlt*=Multifloras, *Noi*=Noisettes, *Ptl*=Portlands, *Sem*=Sempervirens, *Tea*=Teas

Results

PCR amplifications

A previous preliminary work using classical short 10-mer RAPD primers gave us reasonably reliable, reproducible and significant estimates of genetic distances between modern cultivars (Piola et al. 1999). We decided, however, to use the longer primers (20-mers) described by Debener and Mattiesch (1998) with an AP-PCR (arbitrary primed – PCR) amplification protocol (De Vienne 1998). These PCR conditions give complex and very reproducible banding patterns with the various rose cultivars, due to a primer annealing temperature much higher (55°C) than that allowed in the case of classical RAPD (35°C) thus providing elongation complexes for the *Taq* polymerase with superior stability and reproducibility. We observed that, with only five of the primers designed by Debener and Mattiesch (P495, P496, P497, P498 and P499), the patterns obtained were sufficiently polymorphic and discriminant to differentiate almost all of the 100 cultivars analysed here (two cases of non-discriminated cultivars are commented below in the ''Discussion''). Seven, 15, 15, 7 and 14 (i.e. a total of 58) PCR fragments were produced respectively with each primer, 55 of them being informative and discriminant.

Dendrogram analysis of the PCR data

A matrix of distances between cultivars was produced from the original presence / absence data of PCR fragments, using the simple matching (or Apostol) distance index. The matrix was processed by the UPGMA method of the ADE package to provide the dendrogram presented in Fig. 1. The phenogram includes two major clusters.

The first (1) contains essentially roses of European origin such as the ''Gallicas'', the ''Centifolias'' and the ''Damascenas'', together with some of the first- and second-generation hybrids formed between European and far-eastern roses, such as the ''Portlands'' and the ''Hybrid Perpetuals''.

The second cluster (2) contains the "Chinenses", some of their first generation hybrids such as the ''Bourbons'' and the ''Noisettes'', second generation hybrids such as the ''Teas'' and the most modern ''Hybrids Teas'', together with the ''Sempervirens'' and the ''Multifloras''.

Finally the ''Albs'' roses form a third, minor cluster (3) distinct from the two previous ones and associating a Gallica rose (Octavie) amongst them.

The main horticultural groups thus appear well clustered and homogeneous:

(1) the ''Gallicas'' are particularly well grouped since 10 of the 13 cultivars in this group form a unique branch in cluster 1;
(2) the ''Hybrid Perpetuals'' (nine cultivars) form another unique branch contaminated only by one ''Portland'' and one ''Centifolia'' in cluster 1;
(3) the ''Chinenses'', ''Bourbons'', ''Noisettes'', ''Teas'' and ''Hybrid Teas'' in cluster 2 form fairly well individualised branches; and
(4) of the 14 the ''Centifolias'' analysed here, two groups of four and seven cultivars form two branches in cluster 1, interspersed among various European roses including ''Damascenas'' and ''Portlands''; the two groups of the ''Portlands'' and of the ''Damascenas'' are particularly heterogeneous, scattered between various groups of European origin in the cluster 1.

Principal components analysis (PCA)

The primary presence / absence data of PCR fragments obtained for each of the five primers provided five distance tables that were analysed by the Canonical module of the ADE package. The matrix of vectorial correlations between the tables shown below (expressed in 1 / 1000 values),

(1) 1000
(2) 909 1000
(3) 920 901 1000
(4) 946 914 943 1000
(5) 936 906 946 955 1000

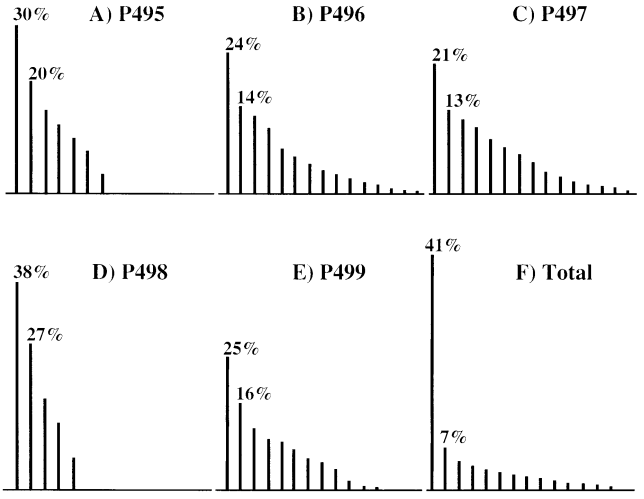

Fig. 2 Structure of the PCR data tables. Eigenvalues associated with the tables of the PCR fragment presence/absence data obtained for each of the five PCR primers (*A*=P495, *B*=P496, *C*=P497, *D*=P498 and *E*=P499), and with the table obtained for cumulated data (*F*). The relative inertia on the two first axes has been indicated on the graphs

displays very strong correlations between pairwise tables, since correlation values are all higher than 900/1000. The graphs of the eigen-values and of their related inertia for the five PCR primers (Fig. 2 A, B, C, D and E) demonstrate that the five tables all have a uni-dimensional structure since their first axis always bears the highest inertia.

Moreover, the interstructure between these tables is also mainly expressed by the first axis, which bears more than 94% of the total inertia. The global matrix of the cumulated data was processed in the same way by PCA (Fig. 2 F); here, again, a very strong uni-dimensional structure is evident, with the first axis representing more than 40% of the inertia and the second one less than 7%. Owing to this uni-dimensional structure, each cultivar can be characterised by a score (comprised between –1 and +1) represented on the single abscissa axis (Fig. 3), instead of the usual PCA representations on two axes or more. Thus PCA provides information complementary to that provided by the dendrogram.

This shows that the European founder groups (the Centifolias, Damascenas and Gallicas) have particularly variable and heterogeneous, but essentially positive, scores (represented on the right positive part of the axes). By contrast, modern roses (the second generation Teas and particularly the very modern Hybrid Teas) have rather homogeneous and negative scores (represented on left negative of the graphs). The Chinenses founders and the hybrids produced during the initial cycles of hybridizations between Chinese and European roses (Portlands, Bourbons, Noisettes, and Hybrid Perpetuals) have intermediate scores (represented at the centre of the graphs around null-value scores). PCA in fact demonstrates the occurrence of a continuous gradient of scores, with a regular decrease of values in per-

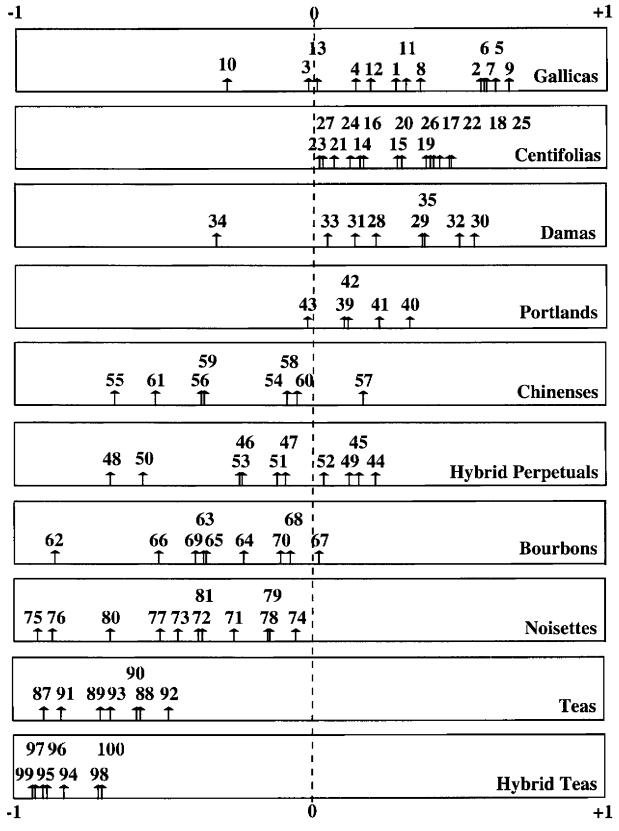

Fig. 3 Principal component analysis of the PCR data. The PCA scores of the various rose cultivars, indexed by their ID numbers, have values ranging from –1 to +1. The cultivars have been sorted by horticultural group. Only the first axis of the PCA has been represented since it bears most of the inertia

fect synchrony with the progress of the successive stages of rose domestication.

Discussion

Structure of the PCR data

The diversity of PCR fragments obtained with the five long primers is sufficient to differentiate most of the genotypes analysed. The PCA structure of the canonical distances calculated between cultivars provides information about the effectiveness of exploration of the genome by AP-PCR. The very strong correlations between the distance tables obtained for the five primers indicate that, in the different plants, the various regions of the DNA sampled by each primer diverge to the same extent and provide data of similar structure. Each of the primers contributes in equal part to complete the description of the cultivars. These features are consistent with the hypothesis of a totally random probing of the genome by the PCR reaction. Debener and Mattiesch (1998) already reported that long primers favour PCR-amplification of non-repeated sequences, while moderately or repeated sequences are amplified preferentially by short RAPD primers (N'Goran et al. 1994). Our data confirm that the amplifications performed here provided non-redundant and perfectly additive information, probably due to the fact that PCR involved a high proportion of non-repeated regions of the genome, allowing random and general analysis.

Only two cases of non discriminated cultivars were observed.

(1) ''Maiden's Blush'' (cultivar no. 37) and ''Cuisse de Nymphe'' (no. 36) in the ''Albas'' group. In the horticultural literature Testu (1984) and Austin (1992) have described these two roses as a single and unique cultivar with synonymous denominations, respectively English and French. Their proposal is consistent with our observation.
(2) ''Common China'' (no. 58) and ''Bengale Animé des Anglais'' (no. 54) belong to the group of the ''Chinenses''. Their present non-discrimination confirms the failure of Raymond (1999) to distinguish these two cultivars using 30 morphological, three colorimetric, 16 chemical (flavonoids) and three isoenzymatic characters.

By contrast, Anna and Virginia were clearly discriminated. These are two modern Hybrid Teas registered by Pekmez (France), Virginia being a derived white sport of Anna, a pink original variety. Several PCR fragments differentiate them, indicating that Virginia might result from the accumulation of successive mutations largely silent in Anna, with only one of them having modified the flower coloration; an alternative possibility is that a single mutational event had a major effect on the pigment synthesis only, but modified a significant part of the genome structure.

UPGMA analysis

The genealogical analysis and its dendrogram representation illustrate genetic distances between horticultural groups and varieties, in good correspondence with the history of rose domestication (Wylie 1954; Maia and Vénard 1976). The very different types of European and Chinese rose founders are placed together with their more closely related groups in the two main clusters. First-generation hybrids produced by crossings between the ''Chinenses'' and the European roses during the early half of the XIXth century, are split themselves between the two main branches of the UPGMA tree : the ''Portlands'' and the ''Hybrid Perpetuals'' are placed in cluster 1 with the European roses, while the ''Bourbons'' and the ''Noisettes'', created by hybridisations of a similar nature, are placed together with the ''Chinenses'' in cluster 2. To further explain some features of the dendrogram, it must be also remembered that:

(1) the ''Chinenses'' group itself is of heterogeneous origin, comprising not only Chinese botanical roses but also ''Bengal'' hybrid varieties of the first generations;

(2) the ''Multifloras'' and ''Sempervirens'' were used for introgression of some of their horticultural characters into the ''Hybrid Teas''; and
(3) the ''Albas'' are European hybrids between *Rosa gallica* and *Rosa canina* varieties that were not involved in the major steps of modern rose creation (Maia and Vénard 1976).

The organisation of the dendrogram corroborates and confirms the chemotaxonomical classification of domesticated roses proposed by Raymond (1999) on the basis of the biochemical analysis of flower pigments (anthocyanins and flavonols) . It is also in perfect agreement with the description of the groups in the horticultural literature which presents the ''Bourbons'' and the ''Noisettes'' as very recurrent but sensitive to the cold, characters typical of the Chinese botanical roses. Whereas, by contrast, the ''Portlands'' and the ''Hybrid Perpetuals'' are winter hardy and resistant to pests, but poorly recurrent (contrary to the denomination of this last group denoting an intention of the rosierists rather than a real selection success). These are typical characters of the European roses. These genetic and biological features must result from different ratios of Chinese and European alleles introgressed into the various biological groups formed during the first hybridization cycles.

The genetic distances between cultivars and groups provided here by the UPGMA method are thus in good agreement with other classifications and seem to be realistic. The robustness of the dendrogram was demonstrated by the fact as few as two primers (P497 and P498) were sufficient to generate the two main branches related to the chinese founders on one hand, and the european founders on the other. The UPGMA method calculates ''ultra-metrical'' distances between individuals, i.e. distances equal to the sum of lengths of the branches connecting these individuals. It is well-adapted to represent the structure and relationships within the rose complex, since this complex results from recombinations produced by a domestication process. Methods that would minimise the length of the trees (like the Neighbour Joining method for instance) in order to give the most-parsimonious representation of evolutionary processes seem less favourable in the case of our study. UPGMA permits the identification of the large groups of recombinants, even if it does not allow accurate tracing of the parentages that have produced the various individual cultivars.

PCA analysis

PCA offers a representation complementary to UPGMA trees. It illustrates particularly well some aspects of the genetic variability within horticultural groups, owing to the very strong uni-dimensional structure of the presence/absence PCR data and to their ordination on a single axis. The sorting out of cultivars by horticultural groups displays a remarkable gradient of the scores attributed to each plant, starting from positive and very heterogeneous values for ancient founder roses, to reach final homogeneous and negative values for modern roses. This gradient demonstrates the effects of selection for a limited array of morphological traits, resulting in the retention of only a small number of alleles during the process of rose domestication. These alleles probably originate from ''Chinenses'' for characters concerning colour and recurrence, and from European groups for those concerning hardiness and flower complexity. Our analysis illustrates the limited variability among the modern cultivated varieties and the reduced genetic basis of the Hybrid Tea group (Debener et al. 1996), and justifies the search for new aesthetical traits, as promoted recently by several breeders (Austin 1992).

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References

Apostol BL, Black NC IV, Miller BR, Reiter P, Beaty BJ (1993) Estimation of the number of full sibling families at an oviposition site using RAPD-PCR markers : application to the mosquito *Aedes aegypti*. Theor Appl Genet 86: 991–1000

Armstrong JS, Gibbs AJ, Peakall R, Weiller G (1994). 'The RAPDistance package' ftp://life.anu.edu.au/pub/software/RAPDistance http://life.anu.edu/molecular/software/rapd.html

Austin D (1992) Old Roses and English Roses. The Antique Collector's Club, London UK, pp 1–205

Crépin F (1866) Etudes sur les Roses. Bull Soc Roy Bot Belg 5: 13–27

Debener T, Mattiesch L (1998) Effective pairwise combination of long primers for RAPD analysis in roses. Plant Breed 117: 147–151

Debener T (1999) Genetic analysis of horticulturally important morphological and physiological characters in diploid Roses. Gartenbauwissenschaft 64: 14–20

De Vienne D (1998) Les marqueurs moléculaires en génétique et biotechnologies végétales. D. de Vienne (ed) INRA Editions, Paris, pp 1–200

De Vries DP, Dubois LAM (1984) Inheritance of the recurrent flowering and moss characters in F_1 and F_2 Hybrid Tea × *Rosa centifolia muscosa* (Aiton) Seringe populations. Gartenwissenschaft 49: 97–100

Dubois LAM, De Vries DP (1987) On the inheritance of the dwarf character in Polyantha × *Rosa chinensis minima* (Sims) Voss F_1 populations. Euphytica 36: 535–539

Grossi C (1998) Université de Lyon-I. Analyse comparée de la biodiversité au sein d'une collection de rosiers sauvages et de rosiers cultivés : contribution à la compréhension du processus de domestication. Thèse de Doctorat (PhD thesis)

Maia N, Vénard P (1976) Cytotaxonomie du genre *Rosa* et origine des rosiers cultivés. In: FNPH (ed) Travaux sur le rosier de serre. INRA Editions, Paris, pp 7–20

Marshall HH, Campbell CG, Collicut LM (1983) Breeding for anthocyanin colors in *Rosa*. Euphytica 32: 205–216

N'Goran JAK, Laurent V, Risterucci AM, Lanaud C (1994) Comparative genetic diversity studies of *Theobroma cacao* L. using RFLP and RAPD markers. Heredity 73: 589–597

Piola F, Heizmann P, Jay M (1999) L'origine des roses modernes : une histoire ancienne et complexe. Bull l'assoc ''Les Amis de la Roseraie du Val de Marne'' 34: 10–11

Raymond O (1999) Université de Lyon-I. Etude multivariée de la diversification au niveau d'un centre de domestication du Rosier. Thèse de Doctorat (PhD thesis)

Rehder A (1940) Manual of cultivated trees and shrubs, hardy in North America, 2nd edn. The McMillan Co., New York

Sokal RR, Sneath PH (1963) Principles of numerical taxonomy. WH Freeman and Company, San Francisco London

Svejda F (1979) Inheritance of winter hardiness in roses. Euphytica 28: 309–314

Testu C (1984) Les roses anciennes. Flammarion, La maison rustique, Paris

Thioulouse J, Chessel D, Dolédec S, Olivier JM (1996) ADE-4 : a multivariate analysis and graphical display software. Stat Comput 7: 75–83 http://biomserv.univ-lyon1.fr/ADE-4.html

Wylie A P (1954) The history of garden roses. J Roy Hort Soc 79: 555–571; 80: 8–24; 80: 27–87