Rosa Taxonomy and Hierarchy of Markers Defined by ACT STATIS

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The ACT STATIS method, a multi-table comparison, was applied to 62 *Rosa* species to be clustered into four sections (Carolinae, Cinnamomeae, Pimpinellifoliae and Synstylae); the data sets were dealing with morphology (15 criteria), anthocyanin pattern (10 compounds), flavonol heteroside pattern (26 compounds) and superoxide dismutase isozyme (SOD) polymorphism (11 bands). This method appeared very powerful to recognize the rose sections and to set up a marker hierarchy which places at the first level the flavonol heteroside pattern, then the morphological data, the SOD isozyme data and finally the anthocyanin pattern. The correlation studies between the markers underlined the relatively common view by means of flavonol patterns and the morphological features.

Introduction

For a long time and currently taxonomists dealing with the genus *Rosa* used the morphological features for classification purpose; so Rehder (1940) divided this genus into four subgenus (*Hultemia*: 1 species, *Platyrodhon*: 1 species, *Hesperhodos*: 2 species and *Eurosa*: 120 species) and subdivided the subgenus *Eurosa* into ten sections (Banksianae; Bracteatae; Caninae; Carolinae; Chinenses; Cinnamomeae; Gallicanae; Laevigatae; Pimpinellifoliae; Synstylae).

Later on other criteria were used to confirm the taxonomy and to support some more precise taxonomic points such as seed morphology (Buth and Misri, 1984), flower colour (Yokoi, 1975), pollen exine (Ueda and Okada, 1994) or chromosome number (Lata, 1982).

More recently another important marker was developed within the genus *Rosa*, based on the chemical compounds relevant for phenolic metabolism. This marker has been used for a long time especially for the flavonol heteroside compounds in several taxonomic proposals (Breitwieser and Ward, 1993; Neuwhof *et al.*, 1990), even in the genus *Rosa* (Harborne, 1967). In this genus, Van Sumere *et al.* (1993) proposed a phenolic fingerprinting to recognize the rose cultivars. Biolley *et al.* (1992, 1994) underlined the extraordinary diversity of the phenolic patterns within the genus *Rosa*. Mikanagi *et al.* (1990, 1995) emphasized a good agreement between the morphology and chemical markers within some wild rose species. Finally, Raymond *et al.* (1995) used this marker for phylogenetic aspects. About the anthocyanin compounds, studies are scarce in the *Rosa* genus and apparently demonstrate a low taxonomic power.

The third kind of data currently used in the study of plant biodiversity consists of proteins and more especially isozyme polymorphism on various taxonomic levels: population (Chung *et al.*, 1991; Gebczynski *et al.*, 1993), species (Sanchez-Yelamo, 1992) or cultivar (Wendel and Parks, 1983). In the genus *Rosa*, the first work dealing with isozyme polymorphism, was carried out by Kuhns and Fretz (1978 a, b); another contribution came from our work on 25 cultivars (Grossi *et al.*, 1997).

The fourth and new descriptor relates to the study of DNA polymorphism. Interesting results were obtained with *Citrus* cultivars (Matsuyama *et al.*, 1992), with *Dendranthema grandiflora* (Wolff and Peters-Van Rijn, 1993) or with *Rubus* cultivars (Nybom and Hall, 1991). About roses only a few studies dealt with cultivar recognition (Vainstein and Ben-Meir, 1994; Torres *et al.*, 1993; Hubbard *et al.*, 1992). More recently Debener *et al.* (1996) and Millan *et al.* (1996) confirmed by Random Amplified Polymorphic DNA (RAPD) methods the taxo-

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nomical relationships of ten rose species, based on the morphological and cytological criteria. In these different approaches the authors gave conclusions mainly referring to their own result and just compared them with conclusions from other authors; nobody attempted to directly compare several data sets taking into account different biological aspects.

Our study describes 62 species of the genus *Rosa* under four data sets referring to morphological features, anthocyanin patterns, flavonol heteroside patterns and super oxide dismutase isozyme polymorphism, respectively; these 62 species belong to four major sections of the *Eurosa* subgenus: Carolinae, Cinnamomeae, Pimpinellifoliae and Synstylae. Our set of statistical treatments is new for the plant kingdom: the objective is:

– to propose a synthetic or global taxonomy taking into account simultanously four data sets, by means of ACT STATIS method (L'Hermier des Plantes, 1976; 'Analyse conjointe de tableaux-structuration des tableaux à trois indices de la statistique'),

Table I: List of *Rosa* (*R.*) species used for the present study.

CAROLINAE	PIMPINELLIFOLIAE	SYNSTYLAE
1 *R. carolina* L.	41 *R. altaica* Thory	49 *R. arvensis* Huds.
2 *R. foliolosa* Nutt.	42 *R. foetida* Herrm.	50 *R. filipes* Rehd. et Wils.
3 *R. nitida* Willd.	43 *R. gracilipes* Chrshan	51 *R. helenae* Rehd. et Wils.
4 *R. rousseauriorum* Boiv.	44 *R. hugonis* Hemsl.	52 *R. henryi* Boulang.
5 *R. virginiana* Mill.	45 *R. pimpinellifolia* L.	53 *R. longicuspis* Bertol.
	46 *R. primula* Boul.	54 *R. luciae* Franch. et Rochebr.
	47 *R. sericea* Lindl.	55 *R. luciae fujisanensis* Makino
	48 *R. xanthina* Linfl.	56 *R. moschata* Herrm.
CINNAMOMEAE		57 *R. mulliganii* Boulang.
6 *R. acicularis* Lindl.		58 *R. multiflora* Thunb
7 *R. albertii* Regel		59 *R. rubus* Lev. et Van.
8 *R. amblyotis* Mey.		60 *R. sempervirens* L.
9 *R. arkansana* Porter		61 *R. setigera* Michaux
10 *R. blanda* Ait.		62 *R. soulieana* Crep.
11 *R. caudata* Baker		
12 *R. cinnamomea* L.		
13 *R. davurica* Pall.		
14 *R. elegantula* Rolfe		
15 *R. farreri* Stapf.		
16 *R. fedtschenkoana* Regel		
17 *R. forrestiana* Boulang.		
18 *R. giraldii* Crep.		
19 *R. hemsleyana* Täckholm		
20 *R. hissarica* Slob.		
21 *R. holondonta* Stapf.		
22 *R. huntica* Chrshan.		
23 *R. johannensis* Fern.		
24 *R. lacerans* Boiss. et Buhse		
25 *R. lactibracteata* Boulang.		
26 *R. maretti* Lév.		
27 *R. moyesii* Hemsl. et Wils.		
28 *R. multibracteata* hemsl. et Wils.		
29 *R. nanothamnus* Boulang.		
30 *R. nutkana* Presl.		
31 *R. oxyodon* Boiss.		
32 *R. pendulina* L.		
33 *R. piptocalyx* Juz.		
34 *R. rugosa* Thunb.		
35 *R. rugosa alba* Thunb.		
36 *R. rugosa rugosa* Thunb.		
37 *R. setipoda* Hemsl. et Wils.		
38 *R. swenginzowii* Koehne		
39 *R. webbiana* Wall.		
40 *R. willmottiae* Hemsl.		

– to establish a hierarchy of these various markers with respect to a taxonomic proposal applied for a collection of *Rosa* species.

Materials and Methods

Samples and experimental

62 roses species were collected at the "Roseraie du Parc de la Tête d'Or" (Lyon, France) during the period from April to July 1993. They belong to 4 sections Carolinae (5 species / 5 species belonging to this section), Cinnamomeae (35 species / 48), Pimpinellifoliae (8 species / 10), and Synstylae (14 species / 23). The list was given in Table I.

Table II. Correspondancy of the variable number and the identity of the variable.

Modalities of the morphological character

Character	Caption	Modalities				
		a	b	c	d	e
1	habit	setting	hanging	shrub		
2	stem color	red	braun	green		
3	shoot color	red	green			
4	leaf color	yellow-green	medium green	dark green	blue green	
5	leaf brillance	mat	glossy	blue green		
6	minimum number of leaflets	3	5	7	9	
7	maximum number of leaflets	7	9	11	13	
8	spine shape	straight	curved	feathered		
9	spine abundancy	1	2	3		
10	inflorescence	corymb	solitary			
11	hips shape	pyramidal	ovoid	rounded	bottle	
12	hips abundancy	1	2	3		
13	hips color	red	orange	black		
14	style	short	exersted			
15	ploidy	2×	4×	5×	6×	8×

Structure of the anthocyanins

Peak	Compounds
1	cyanidin-3,5-diglucoside
2	pelargonidin-3,5-diglucoside
3	peonidin-3,5-diglucoside
4	cyanidin-3-monoglucoside
5	pelargonidin-3-monoglucoside
6	pelargonidin substitued
7	unidentified
8	peonidin substitued
9	cyanidin substitued
10	peonidin substitued

Structure of the flavonol glycosides

peak	compounds	peak	compounds
1	unidentified	14	kaempferol-3-glucoside or glucuronide
2	unidentified	15	quercetin-4′-glucoside
3	unidentified	16	kaempferol-4′-glucoside
4	quercetin-3-sophoroside	17	kaempferol-3-xyloside
5	unidentified	18	unidentified
6	unidentified	19	kaempferol-3-arabinoside
7	kaempferol-3-sophoroside	20	kaempferol-3-rhamnoside
8	quercetin-3-rhamnosylglucoside	21	unidentified
9	unidentified	22	kaempferol substitued
10	quercetin-3-glucoside	23	quercetin acylated
11	quercetin-3-galloylglucoside	24	kaempferol substituet
12	kaempferol-3-rhamnosylglucoside	25	kaempferol acylated
13	quercetin-3-arabinoside	26	unidentified.

Two kinds of organs were collected:

– firstly, the flowers were taken at the first decolled petal. The pigments (anthocyanins) and the copigments (flavonol heterosides) were extracted and analysed by HPLC as described by Biolley *et al.* (1994). The various phenolic compounds are listed in Table II;

– secondly, SOD isozymes were extracted from the youngest leaves according to the procedure described by Grossi *et al.* (1997). Electrophoresis was performed as described by Grossi *et al.* (1998). Other isozyme systems such as leucine amino peptidase and esterase developed within the *Rosa* genus did not appear appropriate for our problem.

Finally, 48 modalities refering to 15 characters directly read in the nursery, composed the morphological set: stem, leaf, spine, hips and ploidy, were used. All the modalities are presented Table II.

Theoretical background of the data analysis and procedure

ACT STATIS was introduced by L'hermier des Plantes (1976) and developed by Escoufier (1973, 1977, 1980); Robert and Escoufier (1976) and synthetized by Lavit (1988, 1994). The major advantage of this k-tables method is to define in the same time the structure of each matrix X_k and the co-structure between the K matrixes (Fig. 1).

ACT STATIS procedes in 5 steps.

Firstly, it analyses separatly the K tables.

Secondly, it creates either the matrix of vectorial variance / co-variance (Vav/Covv) or the matrix of vectorial correlation (Rv) to measure the co-structure between tables X_k and X_j.

Thirdly, after diagonalization of the previous matrix, the compromise matrix UD can be built.

The fourth step is the diagonalization of the compromise matrix UD which allows to obtain a reference map.

For the last step and to understand the compromise map, many complementary drawings can be made:

* The eigenvalues diagram of each separate analysis and the eigenvalues diagram for the compromise analysis: the larger the difference between eigenvalues is, the more the data are structured. Generally, a break in the slope of the eigenvalues representation marks this fact.

* The variables map in the compromise analysis: it explains what variables are responsible for the structure of the compromise map.

* The individual map in the compromise analysis. In order to know how each feature can reconstitute the compromise, Thioulouse *et al.* (1995) proposed to project the individuals defined by the table k on the compromise map. The distance between the species position on the compromise map and the one of the factor map of block k corresponds to the mismatch of the compromise building by each block.

* Quantification of the typological values of the descriptors: the problem of these previous drawings is that they do not give a numerical value which could quantify the adequacy between the map given by a given descriptor and the compromise map. Thioulouse *et al.* (1995) suggests the typological value ($\cos^2(\omega_k)$) to measure the adequacy.

Procedure:

For morphological data, the set is made of 48 modalities representative of 15 variables (Table II). Before the analysis, this table has to be modified as a disjonctive table.

For anthocyanin set, there are 10 chemical variables translated into relative percentage of each compound occuring in petal extracts (Table II).

For the flavonol set , there are 26 chemical variables noted just like anthocyanins (Table II).

For super oxide dismutase data, there are 11 isozymes encoded 0 for the absence and 1 for the presence.

On the whole, 62 species were described by each one of these 62 features. All the data matrice are not shown in this paper but are deposited at the laboratory.

The four tables have been coded into fuzzy variables so that each criteria could be compared to each other; all these tables were condensed and therefore ready for statistical analysis as shown in Figure 1. The ACT STATIS method allowed firstly to display the organisation of plant collection seen through each kind of markers, and secondly to reach the compromise matrix built from all the markers together. Furthermore, with a vectorial correlation matrix, rather than the vectorial variance / co-variance one the weight of each marker

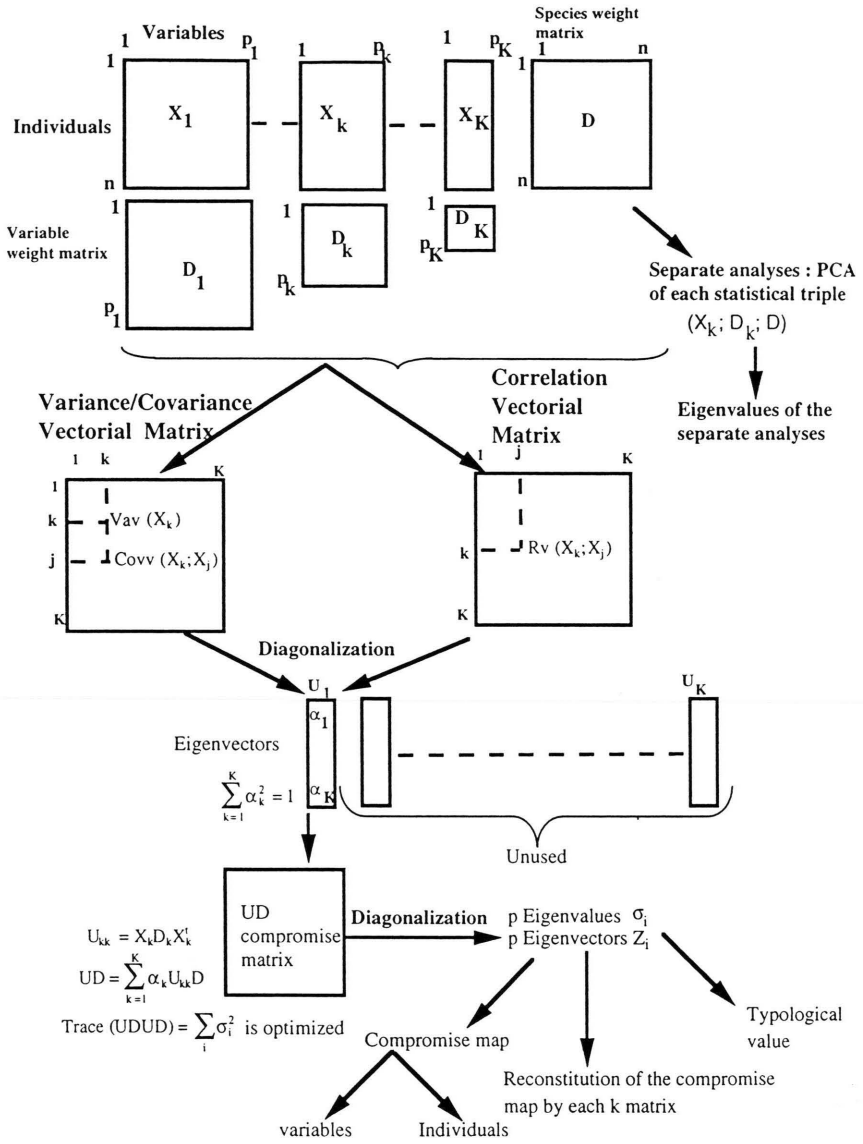

Fig. 1. Summary of the ACT STATIS theory.

group could be reached. This procedure was performed by the mean of ADE.4 program (Thioulouse *et al.*, 1995).

Results and Discussion

ACT STATIS as a taxonomical method

On the F1*F2 compromise map (Fig. 2), the sections Pimpinellifoliae and Synstylae are clearly distinguished, while a mixed group put together the Carolinae and Cinnamomeae sections. The main traits responsible for this organisation can be detected in Fig. 3 which shows the relative weight of each marker with regard of the first two axes of this compromise ordination (the most contributing variables are underlined by an arrow and numered as indicating in Table II).

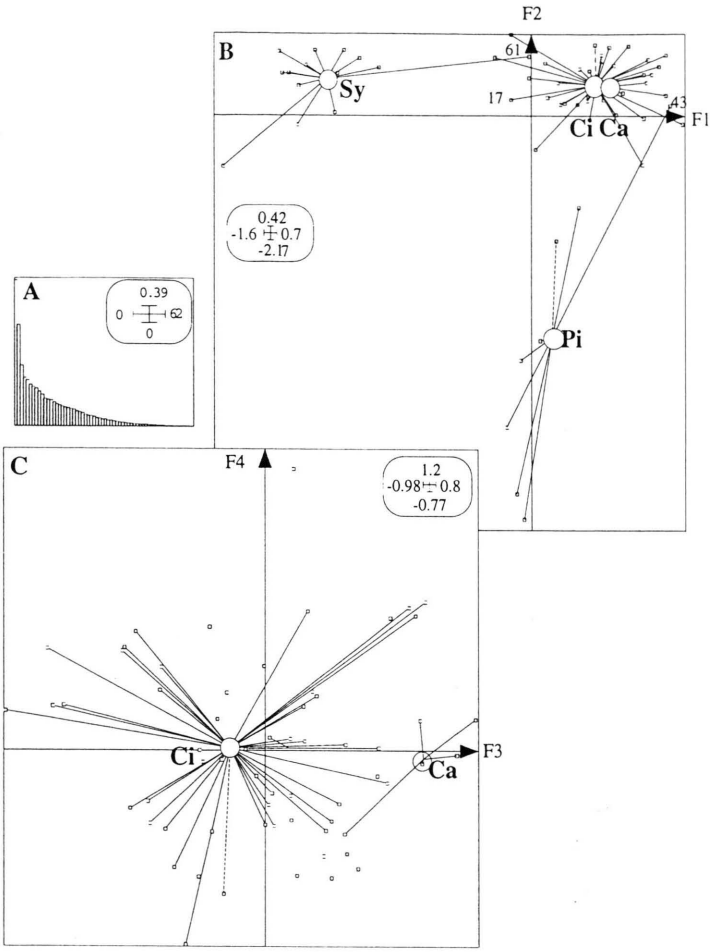

Fig. 2. The compromise analysis: eigenvalues and species map.
A = Eigenvalues;
B = F1*F2 species map for Ca = Carolinae, Ci = Cinnamomeae, Pi = Pimpinellifoliae, Sy = Synstylae;
C = F3*F4 species map of the sections Cinnamomeae and Carolinae.

– The Pimpinellifoliae are generally defined by the flavonol heterosides based on the 4'-O-glycosylation (peaks 15 and 16) (Harborne, 1967), by a lack of SOD band 11, by a lot of bands in SOD isozyme system (about 8 bands per species) and by black hips (13c) and a high number of leaflets (6d, 7c and 7d) (Fig. 3).

– The Synstylae are generally characterized by the absence of anthocyanins, by a 3-O-glycosylation (rhamnose or glucose) of kaempferol (peaks 14 and 20), by a low number of bands in SOD isozyme system (about 4 bands per species) and by exerted styles (14b), orange hips (13b) and curved spines (8b).

The Carolinae and Cinnamomeae share several chemical features. The species of these sections are able to synthetize anthocyanins (Fig. 3). In both sections, the main flavonol markers consist of quercetin and kaempferol sophorosides (peaks 4 and 7, Mikanagi *et al.*, 1990, 1995). However it is possible to distinguish Carolinae from Cinnamomeae with axe n°3 of the compromise ordination as showed in figure 2 (bottom map); this sharing is due to a large presence in Carolinae section, of a particular anthocyanin (the peak n°6) and a low leaflet number (7a); and to the presence in the Cinnamomeae section, of the high ploidy level (15b, c, d, e). At last, the SOD band n°11 is only

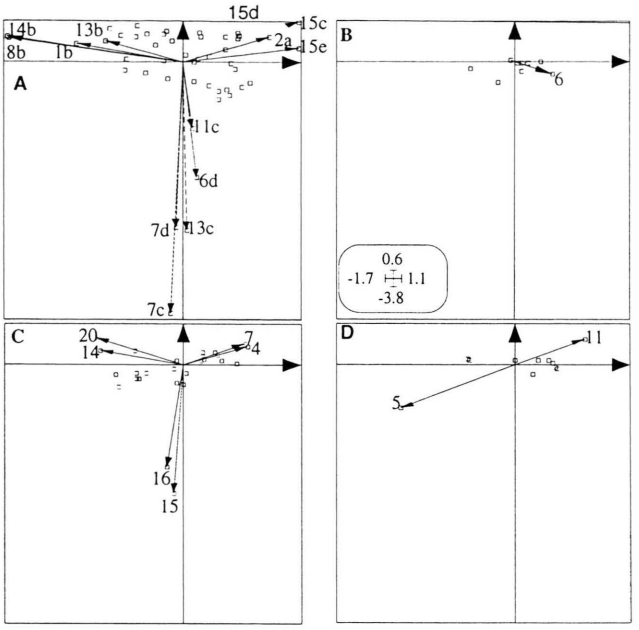

Fig. 3. Compromise analysis, map F1*F2: projection of all variables for each descriptor. The most contributing variables are underlined by an arrow
A = morphological descriptor;
B = anthocyanin descriptor;
C = flavonol descriptor;
D = SOD isozyme descriptor.
For each descriptor, the numbers and the letters are corresponding to the variables and the modalities respectively, as explained in Table II.

detected in several species of the Cinnamomeae section.

Our conclusion about Pimpinellifoliae and Synstylae joined as well that of Rehder (1940), Wylie (1954), Beales (1985), Phillips and Rix (1988) based on morphological features as that of Hurst (1925), Rowley (1967), Maia and Venard (1976) based on the cytological data. However, our procedure gives more information on the main splitting markers; moreover that allows to give a true taxonomical reality for the Carolinae which were up to now distinguished with great difficulty from Cinnamomeae.

Furthermore, the ACT STATIS procedure leads to underline very interesting and new taxonomic problems with some species such as *R. gracilipes* (species 43), a Pimpinellifoliae showing a great affinity with Cinnamomeae; the same remark concerns *R. setigera* (species 61), a Synstylae sharing many features with the Cinnamomeae (Grossi *et al.*, 1998). For the last one this species is peculiar by its geographical origin (Lewis, 1958) and its position is probably to be revised even if De Vilmorin and Simonet (1935) demonstrated that the cytological features of *R. setigera* were similar to that of the Synstylae.

ACT STATIS as a method for the hierarchy of the markers

The second advantage of ACT STATIS method in comparison of other statistical methods such as Principal component analysis, Correspondance analysis, Multiple correspondances analysis or Coinertia analysis, is the quantification of the typological value of each marker with the help of some drawings or indices. According to a phenotype – genotype scale, the morphological markers will be studied first, next the anthocyanins and flavonol heterosides which are the phenotypic expression of the phenol metabolism and finally, the isozymes of SOD which are the more genotypic markers.

The morphological data (Fig. 4A) which are the first studied by the taxonomists, give the second most structured among the four tables, with a typological value appreciated by ($\cos^2(\omega_k)$) equal to 0.395. The main information is contained between the first two axes such as shown by the eigenvalues of the separate analysis (Fig. 4Aa). Finally, the ordinations of the species simultaneously obtained from the morphological matrix and from the compromise matrix, are close together as shown by the very short trajectories (shorter the arrows are, better the adequacy between the specific marker and

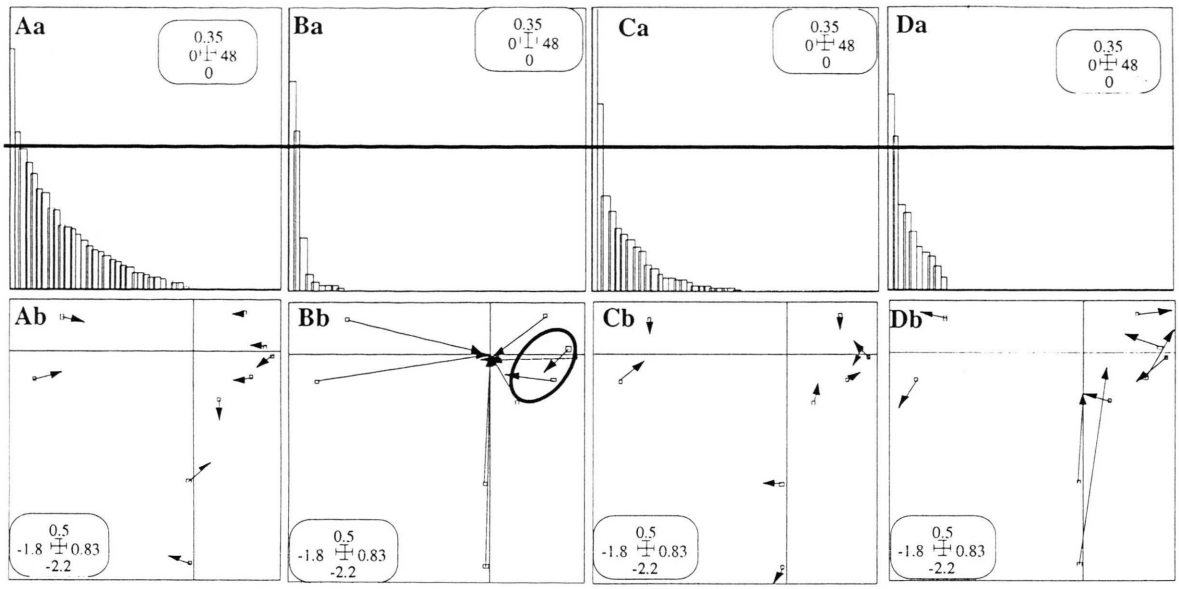

Fig. 4. Structure of each matrix and their contributions to the compromise analysis.
Aa = morphological descriptor: eigenvalues of the separate analysis;
Ba = anthocyanin descriptor: eigenvalues of the separate analysis;
Ca = flavonol descriptor: eigenvalues of the separate analysis;
Da = SOD isozyme descriptor: eigenvalues of the separate analysis.
For the graph a block, the solid horizontal trait allowed to determine how many axes (in upper part) are important for taxonomical purpose: two for each data group.
For each following maps, only, some average and significant examples have been retained for illustration
Ab = morphological descriptor: comparison between compromise and separate analysis;
Bb = anthocyanin descriptor: comparison between compromise and separate analysis;
Cb = flavonol descriptor: comparison between compromise and separate analysis;
Db = SOD isozyme descriptor: comparison between compromise and separate analysis.
For the graph b block and for the selected samples, the start of the arrow is indicative of the species location in the compromise map, the end is indicative of the species location in the map of the matrix k.

the compromise is; Fig. 4Ab). To clarify the figure, only few representative and significant samples have been retained in order to illustrate the present situation.

The anthocyanin content (Fig. 4B) appeared as very poor with regard of its typological value ($\cos^2(\omega_k) = 0.017$); that is supported by the very long trajectories between location in compromise map and location in map based on anthocyanin features. Besides, most of arrows converged to the point (0, 0) indicative of a short range of anthocyanin variation (Fig. 4Bb); however, two samples on the graph and four on the complete analysis belonging to the Carolinae section, made exception because of particular anthocyanin (n°6) discriminating for this group.

The flavonol heterosides table (Fig. 4C) is the most structured one. The typological value is the highest one with ($\cos^2(\omega_k) = 0.65$); furthermore, the eigenvalues of the separated analysis present an information mainly supported by first two axes (Fig. 4Ca). Consequently, the agreement between both matrix compromise and flavonol respectively, is very good: very short arrows (shorter than in morphological map) joined the two locations of each sample (Fig. 4Cb).

Finally, the SOD isozyme marker (Fig. 4D) appeared less performing than the flavonol and morphological markers; its adequacy index is only equal to 0.210 versus 0.395 and 0.605 for morphological and flavonol data, respectively. However, while three sections are overlapping on a relative low range of variability, shown by a convergence of arrows in the right part of the graph, 2 samples on the graph and 13 on the whole analysis belonging to the Synstylae section, were clearly discrimi-

nated by this marker (left part and top of the Fig. 4Db).

In conclusion, ACT STATIS established the hierarchy of markers used for a taxonomic study of the *Rosa* genus: the most powerful descriptor is the flavonol, then the morphology, then the SOD isozyme and finally the anthocyanins. Thus, it appeared the first two phenotypic markers were very powerful to reveal the taxonomic organisation of a collection of *Rosa* species.

Another advantage of ACT STATIS consisted of the correlation between matrix as in co-inertia analysis. For our experimental context, the best correlation is between morphology and flavonol (0.454) and the worst one between SOD and anthocyanin (0.062). But even if these two correlated markers (morphology, flavonol) are on the same direction, each one keeps its own message; for example, *R. forrestiana* is clearly belonging to Cinnamomeae section in the compromise map (n°17 in the Fig. 2B); the morphological features would show this species was closed enough to the Pimpinellifoliae section on the same way, the flavonol patterns would indicate some affinities with Synstylae section. Consequently, this statistical procedure was able to lead to strong and well balanced taxonomy and on the same time, to give some subtle evolutionary trends based on a particular marker.

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