# Genetic Diversity in *Rosa* as Revealed by RAPDs

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## SUMMARY

We aim to study the variability within genus *Rosa*. To accomplish this we have analyzed a plant material collection (109 accessions) including all sections but one, as well as many intermediate forms and hybrids. We also aim to study the consistency of the groups considered within section *Caninae* ('caninae', 'rubiginosa' and 'tomentosa') as well as of the subgenus *Hulthemia*.

A dendrogram was constructed based on RAPDs data. The variability found in the dendrogram was discussed according to sectional status and geographic origin. Our results indicate that there is no clear distinction between *Caninae* groups when many intermediate forms are considered. Besides, the subgenus *Hulthemia* seems to merit just a sectional status as proposed by other authors for other subgenus.

The heterogeneity found in the dendrogram with respect to sectional status suggests the lack of clear reproductive barriers as is common with long lived woody perennial plants. Sect. *Cassiorhodon* may be considered as the Type of the genus since it shows the widest geographical distribution, the widest crossing ability within the Genus and it appears in most groups of the dendrogram suggesting to be the most representative Section.

## KEY WORDS

*Rosa* L., geographic distribution, taxonomy, genetic resources, molecular markers, RAPDs

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## INTRODUCTION

*Rosa* is one of the most economically important genera within ornamental horticulture. Although a limited number of the 110 *Rosa* spp. (around 10%) has contributed to the modern cultivars, extensive variability is still available for breeding. *Rosa* species are grouped taxonomically into 4 subgenera (*Hulthemia, Platyrhodon, Hesperhodos* and *Rosa*) (Rehder, 1940). *Rosa* is the largest and the most important one with 115 species distributed into 10 to 12 sections (Klastersky, 1968; Rehder, 1940; Gudin, 2000). The remaining subgenera contain 1-2 species each.

Many studies emphasize the taxonomic complexity of *Rosa*, not only among species but also concerning modern cultivated roses. In the taxonomic treatment, Latin names are used for botanic species, old and new garden and interspecific hybrids (for example, *R. noisettiana, R. borboniana, R. portlandica, R. pernetiana*) (Gudin, 2000). The taxonomic difficulty of this group may be in part explained by the phylogenetic proximity among species within the genus, complicated by the intense breeding over the past two centuries.

Previous work on the subject can be classified in three main groups: (1) strictly taxonomic using traditional tools (e.g., Floras), (2) studies using molecular markers, and (3) studies involving interspecific crosses.

Taxonomic studies. Most *Rosa* sections include diploid and tetraploid species with higher degrees of ploidy (5x, 6x, 8x) possible. Synonymies are common as well as intermediate forms, usually considered in literature as natural spontaneous hybrids, although in many cases their hybrid nature was never demonstrated. For example, in *Flora Iberica* (Silvestre and Montserrat, 1998) up to 60 hybrids are mentioned. *Flora Europeae* (Klastersky, 1968) contains many references to hybrids as well (for example, between species of Sect. *Pimpinellifoliae* and *Caninae*). Assignments between multiple sections are not uncommon. For example, *R. nanothamnus* was included in Sect. *Pimpinellifoliae* by Boulenger as well as in Sect. *Caninae* (Roberts, 1975). An extreme case of morphological subdivision was done by Almquist (1916, 1919, 1920, in Gustafsson, 1944) who classified 53 species of *Rosa* on the Sweedish island of Yxland.

In this issue section *Caninae* is particularly difficult to classify. Several groups or clusters (up to three in *Flora Europaea*; Klastersky, 1968) are usually considered in this section, frequently subdivided in microspecies by different authors (Gustafsson, 1944). Taxonomic differences among these groups are very subtle.

Taxonomic studies with molecular markers. Current molecular rose research is helping traditional morphological and cytological studies by genomic analyses. Direct DNA-based diagnostic assays are informative because the markers are phenotypically neutral and not affected by environmental effects. The molecular approach opens possibilities for marker assisted selection in the introgression of species derived genes into the cultivated germplasm base (Debener et al., 1998, 1999; Rajapakse et al., 2001, Yan et al. 2005).

These tools were used to solve problems related with varietal identification (Hubbard et al., 1992 with RFLPs; Torres et al., 1993 with RAPDs; Kim and Byrne, 1996, with isozymes), and they are currently used in taxonomy and evolutionary studies. Research with mitochondrial and chloroplastic DNA probes (Matsumoto et al. 1997), and the matK sequence (Matsumoto et al. 1998) variation of species in four *Rosa* subgenera concluded that the present sections of the genus were consistent taxa. However, there was no discrimination among three of the subgenera and the genus *Agrimonia* nor among sections *Pimpinellifoliae, Bractaeatae, Laevigatae* and *Banksianae* within the subgenera Eurosa. Millán et al. (1996) using RAPDs, studied 35 rose species, 17 of them belonging to Sect. *Caninae* and the rest distributed among 6 sections of the *Rosa* subgenus. Structure for Sect. *Caninae* agreed with the treatment of genus *Rosa* by Flora Europaea (Klastersky, 1968), Sect. *Cassiorhodon* was the closest to *Caninae* and Sect. *Pimpinellifoliae* was closest to *Synstylae*. Reynders-Aloisi and Bolleareau (1995) and Debener et al. (1996) performed similar studies although the number of species considered in both cases was lower.

Jan et al. (1999) studied 119 accessions belonging to 8 sections (although unfortunately neither *Gallicanae* nor *Caninae* were included) as well as one species of Sbg. *Hesperhodos* and one of Sbg. *Platyrhodon*. They suggested: (a) both Sbg. *Hesperhodos* and *Platyrhodon* might be considered as sections; (b) Sect. *Carolinae* could be included in Sect. *Cassiorhodon* in agreement with other studies and crossability data; (c) Sect. *Bracteatae, Banksianae* and *Laevigatae* appear phylogeneticaly distant; (d) Sect. *Chinenses* and *Synstylae*, are both morphologically close and molecularly similar. More recently, Martin *et al* (2001) studied the relationships and allelic composition of 100 primitive rose cultivars, obtaining an excellent grouping of classical primitive roses as well as clear discrimination among cultivars.

Studies involving interspecific crosses within the genus *Rosa*. Crosses within and between sections show a long history of fertile interspecific hybrids (Gustafsson 1944, Fagerlind 1944, 1955, 1958, Lewis and Basye 1961, Roberts 1977, our unpublished data). Some species, as *R. alba* and *R. gallica* are allopolyploid, and Sect. *Caninae* as a whole seems to have a hybrid origin (Zielinsky, 1985; Wissemann, 1999). Several interspecific crosses have been recently studied within the section Caninae (Werlemark et al. 1999; 2001; Nybom et al. 2004). Additional proof of wide limits to interspecific cross ability is given by the breeding work during the last two centuries which has involved up to twenty species belonging to at least six sections and two subgenera. This indicates the complexity of the genus.

The main objective of this research is to study the genetic diversity in *Rosa* by using RAPD markers and cluster analysis. The diversity of this genus will be discussed considering botanical, geographical and cross ability data. Besides we will study the consistency of the groups considered within *Caninae* section.

## MATERIALS AND METHODS

### Plant material

One hundred and nine accessions with different geographical origins, representing 39 species, were chosen for the study. One of them belongs to the Sbg. *Hulthemia*, and the rest to nine Sbg *Rosa* sections (missing section *Laevigatae*) (Table 1). Wild accessions were collected by ourselves or kindly provided by Prof. Silvestre (University of Sevilla, Spain). The present study includes (1) taxa of doubtful determination listed on Table 1 as 'suspected'; (2) species of hybrid origin (*R. alba* o *R.* x *alba*, very likely resulting from a cross between a *Caninae* and a *Gallicanae*); (3) possible hybrids (or intermediate forms) *canina* x *arvensis* (Sect. *Caninae* and *Synstylae,* respectively) and *canina* x *rubiginosa* (Sect. *Caninae* and group 'canina').

A note on names. In the literature, *Cassiorhodon* and *Cinnamomeae* as well as *Chinenses* and *Indicanae* are given to the same section; we have adopted the first name in both cases. Similarly, the Type section is referred as *Rosa*, being sometimes *Cassiorhodon* (= *Cinnamomeae*), or *Gallicanae* which is the right name for the Type according to the actual botanical nomenclature rules. In the present work we have not used *Rosa* as a section name except as a proposal in our conclusions.

### DNA extraction and RAPD analysis

Young leaf tissue of mature plants was frozen in liquid nitrogen and stored at –80°C. DNA was extracted using the method described by Torres et al. (1993). In samples with high levels of secondary compounds we applied the protocol reported by Cheng et al. (1997) in order to avoid DNA degradation and subsequent inhibition of the PCR reaction. Coprecipitated RNA was eliminated adding 0.7 units of RNAse per sample. DNA was dissolved in TE, and the final concentration was determined by 0.8% agarose gel electrophoresis using known concentrations of λ phage uncut DNA as standard.

Six primers 10 bases in length (Operon Technologies, Alameda, Cal.) were chosen (Table 2). The selection was made from a pool of primers that gave strong and consistent amplification. Polymerase chain reaction (PCR) was carried out in 25 μL reactions containing 20-40 ng of plant genomic DNA, buffer (50mM KCl, 10 mM Tris-HCl, pH 9.0, 0.1% Triton X-100), 1.5mM $MgCl_2$, 100 mM of each dNTP, 2-4 mM of primer and 0.6 units of Taq DNA polymerase (Bioline). Amplification was performed in a Perkin Elmer Cetus 480 thermocycler as follows: 40 cycles of 1 min at 94°C, 2 min at 35°C and 2 min at 72°C. Cycling was concluded with a final extension at 72°C for 8 min. PCR amplification products were electrophoresed in 2% agarose gels. DNA was stained with ethidium bromide and photographed under UV light.

### Data analysis

All the plants were scored for presence or absence of RAPD fragments, and the data were entered into a binary data matrix as discrete variables ("1" for presence and "0" for absence of a homologous band). Two independent experiments were performed for each accession/primer combination. Only those amplified fragments that could be reproduced in a second experiment and clearly scored were considered in the study. Ambiguities were scored as missing data. Jaccard's coefficient of similarity was calculated, and the species were grouped by cluster analysis using the unweighted pair-group (UPGMA) method. Phenograms were produced as described by Sneath and Sokal (1973) using the NTSYS-pc package for numerical taxonomy and multivariate analysis systems (Rohlf 1989).

Table 1. Accessions, source, origin and botanical section of the species used in this study

| Subgenus/Section | Species/variety | Accession | Source[1] | Geographical origin |
|---|---|---|---|---|
| Sbg. *Hulthemia* | *R. hulthemosa* | 105 | RBG | Central Asia |
| Sbg. *Rosa* L. | | | | |
| Sect. *Banksianae* Lindl | *R. banksiae 'beijing'* Ait. | 95 | RBG | Asia |
| | *R. banksiae 'normalis'* Ait. | 96 | RBG | Asia |
| | *R. cymosa* Tratt | 100 | RBG | China |
| Sect. *Bracteatae* Thory | *R. bracteata* Wendl. | 80 | TAMU | S China |
| Sect. *Caninae* DC | *R. agrestis* Savi | 1-6 | wild | Europe |
| | *R. agrestis* Savi | 90 | RBG | Europe |
| | *R. agrestis* Savi | Suspected: 72 | wild | Europe |
| | *R. andegavensis* Bast | 7 | wild | Europe |
| | *R. canina* L. | 8-11 | wild | Europe |
| | *R. canina* L. | Suspected: 73-74 | wild | Europe |
| | *R. canina x arvensis* | 12-14 | wild | Europe |
| | *R. coriifolia* Fries. | 15-16 | wild | Europe |
| | *R. corymbifera* Borkh. | 17-26 | wild | Europe |
| | *R. dumalis* Bechst. | 27 | wild | Europe |
| | *R. elliptica* Tausch | 28 | wild | Europe |
| | *R. micrantha* Borr. ex Sm. | 29-36 | wild | Europe |
| | *R. nitidula* Bess. | 110 | RBG | Europe |
| | *R. pouzinii* Tratt | 39-51 | wild | Europe |
| | *R. pouzinii x sicula* | 52-54 | wild | Europe |
| | *R. rubiginosa* L. | 55-59 | wild | Europe |
| | *R. rubiginosa* L. | Suspected: 76-77 | wild | Europe |
| | *R. sicula* Tratt | 63-64 | wild | Europe |
| | *R. squarrosa (Rau) Boreau* | 65 | wild | Europe |
| | *R. villosa* L. | 66 | wild | Europe |
| | *R. vosagiaca* Desoirtes | 69-71 | wild | Europe |
| Sect. *Carolinae* Crép | *R. virginiana* Hermm | 68 | wild | North America |
| Sect. *Chinensis* Sér | *R. chinensis* Jacq | 98 | RBG | China |
| | *R. chinensis semperflorens* Jacq | 99 | RBG | China |
| | *R. gigantea* Collet | 78 | TAMU | SW China |
| | *R. mutabilis* | 108 | RBG | Asia |
| Sect. *Cassiorhodon* Dumort (=*Cinnamomeae* DC) | *R. blanda Ait.* | 79 | TAMU | North America |
| | *R. laxa* Retz | 83 | TAMU | North America |
| | *R. pendulina* L. | 112-113 | RBG | North America |
| | *R. rugosa* Thunb. | 85 | TAMU | North America |
| Sect. *Gallicanae* | *R. x alba 'Gil Blas'* L. | 92 | RBG | North America |
| | *R. x alba 'semiplena'* L. | 93 | RBG | North America |
| | *R. x alba 'suaveolens'*L. | 94 | RBG | North America |
| | *R. gallica* L. | 87-88 | CBN | North America |
| | *R. gallica incarnata* | 89 | CBN | North America |
| | *R. x alba* | 91 | RBG | North America |
| Sect. *Pimpinellifoliae* DC | *R. foetida* Herm. | 101 | RBG | Near East |
| | *R. hemisphaerica* Herm. | 104 | RBG | Asia |
| | *R. pimpinelifolia* L. | 37-38 | wild | Siberia |
| | *R. pimpinelifolia* L. | 114 | RBG | Siberia |
| | *R. pimpinellifolia* L. | Suspected: 75 | wild | Siberia |
| | *R. sericea* Lindl | 86 | TAMU | China. Himalaya |
| Sect. *Synstylae* DC | *R. multiflora 'wuci'* Thunb. | 84 | TAMU | Asia |
| | *R. sempervirens* L. | 60-62 | wild | Europa |
| | *R. setigera* Michx. | 115 | RBG | North America |
| | *R. brunonii* Lindl | 81 | TAMU | China. Himalaya |
| | *R. moschata* Herrm. | 107 | RBG | Near East |
| | *R. wichuraiana* Crép. | 116-117 | RBG | Asia |

[1]CBN – Conservatoire Botanique National, Alpin de Gap (FRANCE); RBG – Royal Botanical Garden, Madrid (SPAIN); TAMU – Texas A&M University

Table 2. Primers used in the work

| Primer | Sequence (5´to 3´) | Nº of polymorphic bands | Bands molecular weight (bp) |
|---|---|---|---|
| OPA-01 | CAGGCCCTTC | 10 | 1446–510 |
| OPA-02 | TGCCGAGCTG | 11 | 1266–320 |
| OPA-05 | AGGGGTCTTG | 2 | 670–615 |
| OPA-07 | GAAACGGGTG | 1 | 577 |
| OPA-08 | GTGACGTAGG | 9 | 931–499 |
| OPI-16 | TCTCCGCCCT | 11 | 1325–466 |

## RESULTS AND DISCUSSION

Forty four polymorphic markers generated from 6 primers were used to characterize the 109 rose accessions representing a total of 39 species. Although these primers yielded around 100 polymorphic bands we only selected those bands that consistently appeared in two repetitions. It is generally preferred to extend the number of primers instead of performing a replication of the work to select reproducible bands. However, reproducibility is as important as the number of markers. In fact, from our data more than half of the available markers were discarded when both replications were considered. Therefore we are confident that no artifacts have been included in the analyses and we consider that this work is fully comparable to those works using around 100 markers were no repetition of the work is performed.

All the 44 markers used in this work were polymorphic. The most frequent band was found in 97 out of 109 accessions studied. The highest number of bands per accessions was 24 (two times) while the most frequent number of bands was 21. Within the *Caninae* group, which is the group with a higher number of accessions, no monomorphic markers were found. The most frequent band appeared in 65 of 68 accessions. Only 10 bands were detected in at least 90% of the accessions studied. Therefore, we may conclude that the molecular markers used in this work are variable enough to study the genetic variability in *Rosa*.

### Study on the consistency of *Caninae* groups

The Caninae section is divided in three groups ('canina', 'rubiginosa' and 'tomentosa') according to Flora Europeae. One of the objectives of this work is to study the consistency of these groups.

A compact group (J, node m) is clearly perceived, including exclusively species from Sect. *Caninae* (Figure 1). Within group J, subgroup $J_1$ (node p) only contains accessions belonging to the 'canina' cluster (especially to *R. canina* itself), whereas in $J_2$ (node n) entries from the three clusters considered in *Flora Europaea* (i.e., 'canina', 'rubiginosa' and 'tomentosa') are completely intermingled, with no presence of any *R. canina* accession. A similar situation is outlined in subgroup $J_3$. The "suspected" forms of Sect. *Caninae* as well as the hypothetical natural hybrids (see Material and Methods and Table 1) are included in J as well, but without apparent connection to their hypothetical species. These results are in agreement with those obtained performing crosses among species of this section (Gustafsson, 1944; Blackhurst, 1948; Fagerlind, 1955). Moreover, the suggested natural hybrids *canina* (Sect. *Caninae*) *x arvensis* (Sect. *Synstylae*) are also placed in group J, (accessions 13 and 14 in $J_3$, and 12 in $J_2$). It could be that either they are not true hybrids or, alternatively, that they contain more genetic material from *Caninae* than from *Synstylae*, as in the case of *R. alba*. In fact, Zielinsky (1985), suggested that *R. canina* could act as a "buffer" in the genus due to its system of reproduction, allowing wide crosses with other species.

Our data do not support the existence of 'clusters' or 'groups' within Sect. *Caninae.* This may be explained by the inclusion of many intermediate forms in the study. Werlemark et al. (2001) studied interspecific crosses within *Caninae*. They show that the expression of characters as well as molecular marker inheritance is dependent upon the direction of the cross and on the species involved. Therefore this would lead to the existence of many intermediate forms within *Caninae* section that makes practically impossible the establishment of groups within it in agreement with our results.

Figure 1 seems to indicate that *Caninae* is a Sect. of recent origin, in agreement with Zielinsky (1985) and Ritz et al. (2005). Probably, *R. canina*, the *Rosa* species with the largest geographical distribution, might have evolved out of the proto-*Caninae* later on. These new forms colonised the Near East, part of North Africa and Europe without strong reproductive barriers. Therefore the whole *Caninae* may be considered as a young group still under evolutionary radiation and speciation, hence with many intermediate forms.

### Phenetic analysis considering botanical and cross ability data

The section *Caninae* (group J, node m) is separated at node i from group I which contain the four *R. alba* accessions. The proximity of both groups is in agreement with the recent origin of Sect. *Caninae* (Zielinsky, 1985), and with the suggested hybrid origin of *R. alba*. This result was already obtained by Millán et al. (1996), who additionally supported the hypothesis of the other *R. alba* parent being a *gallica*-like species. In the present work, this possibility is also suggested as group H, containing *R. gallica*, as the closest one to group I+J (i.e., *R. alba* + Sect. *Caninae*). *R. alba* is closer to *Caninae* than to *Gallicanae* because, being an hexaploid species, it might very likely received five genomes from the *Caninae* acting as maternal parental (which are tetraploid, pentaploid or hexaploid) and only one from the tetraploid *Gallicanae*.

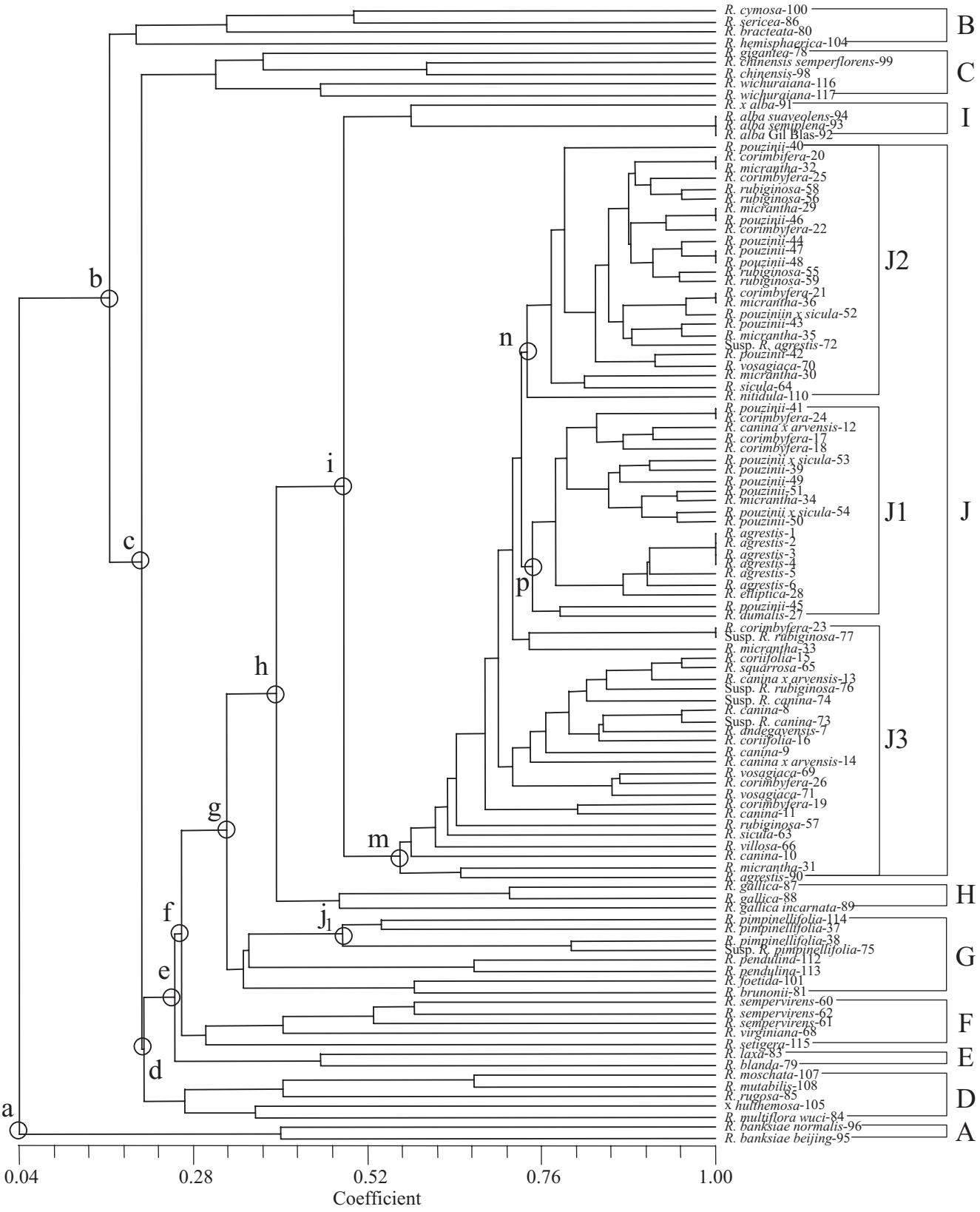


Figure 1.
Phenogram derived from analysis of 109 accessions. The different groups obtained are identified by capitals letters (from A to J) in the right part of the Figure. Besides, subgroups J1, J2 and J3 are also considered within the *Caninae* group (J). The splicing point of each group is encircled and identified by a letter to facilitate results interpretation.

There is a scarce similarity between the two species belonging to the Sect. *Banksianae*. *R. banksiae* lies in group A, the most dissimilar in the dendrogram (Fig. 1), and *R. cymosa* in B (the second one in divergence). Group B is very heterogeneous and included four species of different botanical sections: *Banksianae, Bracteatae* and *Pimpinellifoliae*. The former three have Chinese origin (*R. sericea* reaching the Himalaya region) and the latter is more cosmopolitan spreading from China to Western Europe.

Apart from the small group E (*Cassiorhodon*), the rest of the clusters are rather heterogeneous, displaying association among species of sections *Cassiorhodon, Synstylae* and *Pimpinellifoliae*. The same association has been described by Grossi et al. (1998) using flavonoid and isozyme polymorphism. The 'suspected *pimpinellifolia*' (75 in Table 1) lies in group G, mostly including *Pimpinellifoliae* species (node $j_1$), thus, it may be considered as a true *pimpinellifolia*. Group F contains Sect. *Synstylae* and *R. virginiana,* our only representative of Sect. *Carolinae*, a section that many authors (Lewis, 1957; Lewis and Basye, 1961; Jan et al., 1999, Kim, 1994) feel should be part of the section *Cassiorhodon*. Grossi et al. (1998) described *R. setigera* as more related to *Carolinae* than to other *Synstylae*. Our data support the association between *R. setigera* and *Carolinae* section. However, *R. setigera* appears grouped with other *Synstylae* species in agreement with Jan et al. (1999) while this species appears isolated from other *Synstylae* in the work by Grossi et al. (1998). In group D (*Cassiorhodon, Synstylae*) are included *R. hulthemosa* and *R. mutabilis*. The former belongs to Sbg. *Hulthemia*. A species belonging to a different Sbg. may be expected to appear separated from the other subgenus in the dendrogram. Since it is not the case, a sectional status could be more appropriate for Sbg. *Hulthemia* as suggested for *Platyrhodon* and *Hesperhodos* (Jan et al. 1999). This is also supported by the success in crossing species from *Hulthemia* with other species from Sbg. *Rosa*. For instance, the 'Hardy rose' ('*R. x hardyi*') was obtained from a cross between *R. clinophylla* (Sect. *Bracteatae*) and *Hulthemia persica* (*R. persica*, Sect. *Hulthemosa*) by J.A. Hardy about 1830 (Joyaux, 2001); *R. persica* is still being used in breeding (*inter alia:* Harkness, 1977).

*R. mutabilis* is currently considered as a member of Sect. *Chinenses* although its origin is obscure (Jacob *et al*, 1993). This fact, together with its close link to *R. moschata* (Sect. *Synstylae*), suggests that the status of *R. mutabilis* should be reviewed.

The section *Cassiorhodon* appears in different clusters in the dendrogram. This is the largest *Rosa* section showing a huge variability. For instance, Mikanagi et al. (2000) described 5 different groups in this section according to anthocyanins constituents. Moreover, Grossi et al. (1998) indicated that from a phylogenetic point of view, the largest section *Cassiorhodon* appears central to the evolution of the genus. Therefore, the wide distribution of *Cassbiorhodon* species in our dendrogram seems to adjust to the high variability found in this section.

Besides, the heterogeneity found in groups D, F and G, (i.e. different sections in the same group), is also supported by the general success in interspecific crosses in *Rosa*, both between and within sections. Some examples have been reported by Gustafsson (1944) with *Caninae*; Roberts (1977) with *Pimpinellifoliae*; Fagerlind (1948) within and between Sect. *Synstylae, Pimpinellifoliae, Cassiorhodon, Gallicanae* and Sbg. *Platyrhodon* (*R. roxburghii*); Fagerlind (1955) using species of Sect. *Caninae* as females and several species of both Sect. *Cassiorhodon* and *Pimpinellifoliae* as males; Lewis and Basye (1961) within and between Sect. *Bracteatae, Synstylae, Laevigatae, Cassiorhodon* and Sbg. *Platyrhodon* (considered by these authors as Sect. *Microphyllae*) and is also supported by our own results by performing crosses among species of Sect. *Synstylae*, *Cassiorhodon* and *Bracteatae.* Finally, the wide set of interspecific crosses leading to the modern rose cultivars, involving at least species from the Sect. *Chinenses, Cassiorhodon, Pimpinellifoliae, Synstylae, Gallicanae* and *Caninae* should be mentioned. Therefore, the heterogeneity found in the dendrogram in groups D, F and G is in agreement with the success obtained in interspecific crosses. It might also reflect a lack of resolution of the dendrogram. However, since we have avoided artefacts and the results fit well to reproductive behaviour of the species and to their geographical origin, we believe that the hypothesis of lack of strong reproductive barriers is more appropriate. The main conclusion obtained from hybridization studies is that there are no strong internal barriers to the genetic flow within the genus. Geographical isolation appears to be the only clear obstacle (Roberts, 1977). Thus, it is not surprising that many botanists suggest that the only valid name for modern roses is *Rosa hybrida.* This heterogeneity does not relate with Jan et al. (1999) where botanical sections are perfectly divided except *Carolinae* and *Cassiorhodon*. This may be explained by the study of different species in both works. Moreover, although some species are studied in both works they are normally different accessions with some occasional exceptions.

## Phenetic interpretation using geographical origins

The variability found in the dendrogram (Fig. 1) was also studied according to the geographic origin of the different species. To do this, we have constructed a schematic representation of the dendrogram (Fig. 2). Figure 2 shows the same groups described in Fig. 1 (from A to J). Within each group it is indicated the

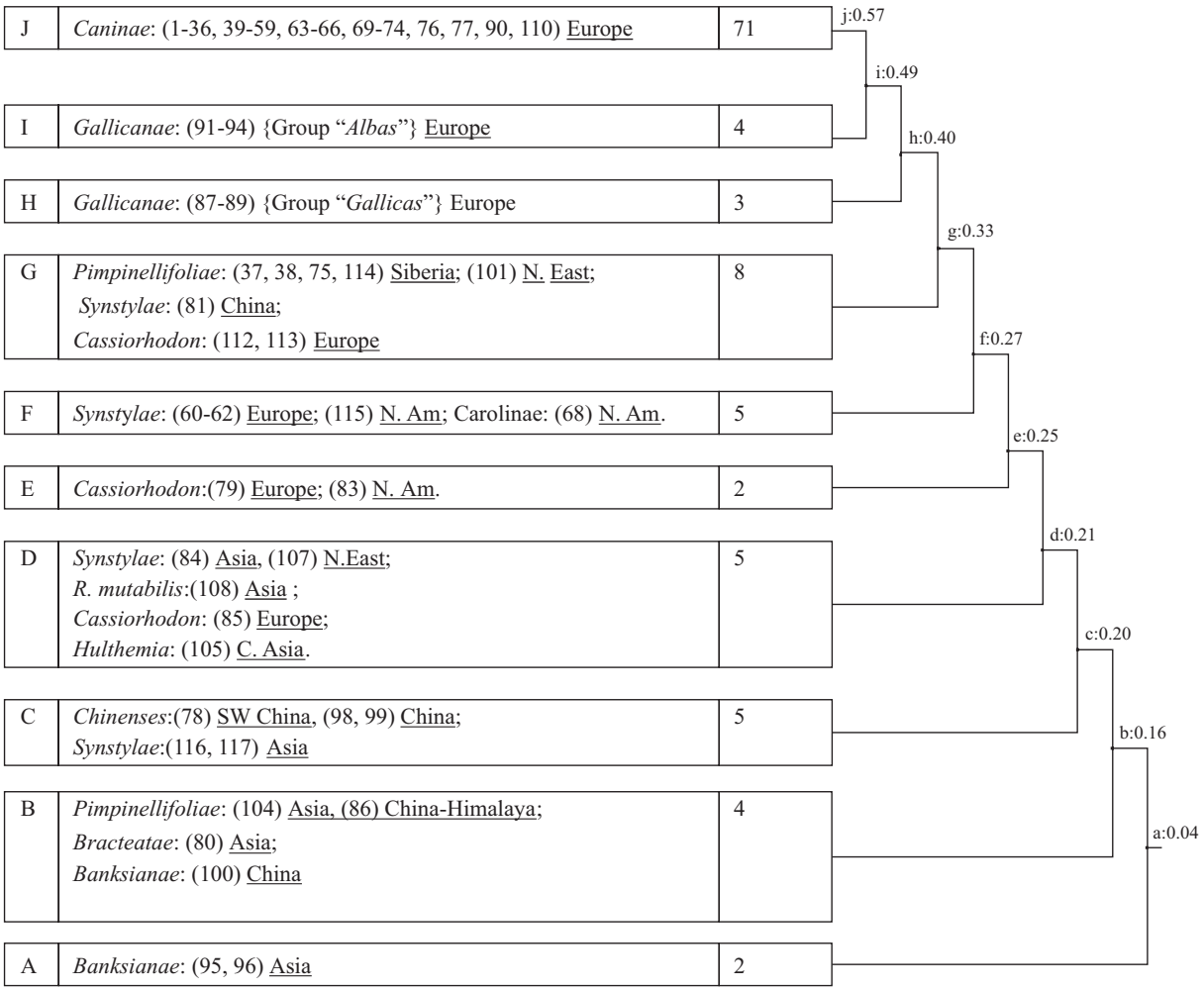


Figure 2.
Phenogram interpretation using geographic origin. This figure is a schematic representation of Figure 1 with additional information. Each box of this schematic phenogram contains a capital letter corresponding to the group identified in Figure 1, the accessions numbers included within each group, their section and their geographic origin. In the right, it is indicated the total number of accessions clustered in each group.

section(s), the accession numbers according to Table 1 and the geographic origin of the accessions joined in each group.

Considering the hypothesis that the dissimilarity in Fig. 2 correlates with the phylogenetic distances, *Banksianae* would then be the oldest section, and *Caninae* the youngest one. Fig. 2 shows that the most dissimilar species are from SW China, while the most similar ones are from Europe. Accordingly, Fig. 2 would suggest that the origin of the genus *Rosa* is in SW China, spreading towards both Europe and North America, being American roses more similar to the Asiatic than to the European ones. Taking into consideration the geographical distribution of the genus *Rosa* (Zielinsky 1985) (Figure 3) it may be hypothesized that the origin of the genus could be South and SW China, as it is there that Sect. *Banksianae* (very probably related to the most ancient forms), *Bracteatae, Sericeae, Chinenses*, *Cassiorhodon* and Sbg. *Platyrhodon* overlap. From the Chinese region, the ancestral *Rosa* forms seem to spread to the west, originating Sect. *Synstylae* and *Pimpinellifoliae,* Sbg. *Hulthemia* and the youngest sections *Gallicanae* and *Caninae* in the Near East, Caucasus, Europe and North Africa.

The evolutionary pathway, from the most ancient to the most recent sections, could be defined as follows: *Banksianae - Bracteatae-(Sericeae- Pimpinellifoliae) - Chinenses - (Cassiorhodon- Synstylae)- Gallicanae - Caninae*. Sect. *Carolinae* should merge into Sect. *Cassiorhodon* as also suggested by Jan et al. (1999), and Sbg. *Hulthemia* should receive a section status. The Chinese/European gradient described by Martin et al. (2001) in primitive cultivated forms and cultivars agrees with the spreading towards the West suggested by Figure 2.

Sect. *Cassiorhodon* is distributed along the whole *Rosa* area (Figure 3), shows the widest levels of crossability with other sections and appears in most groups in our dendrogram (Fig.1). All these data are

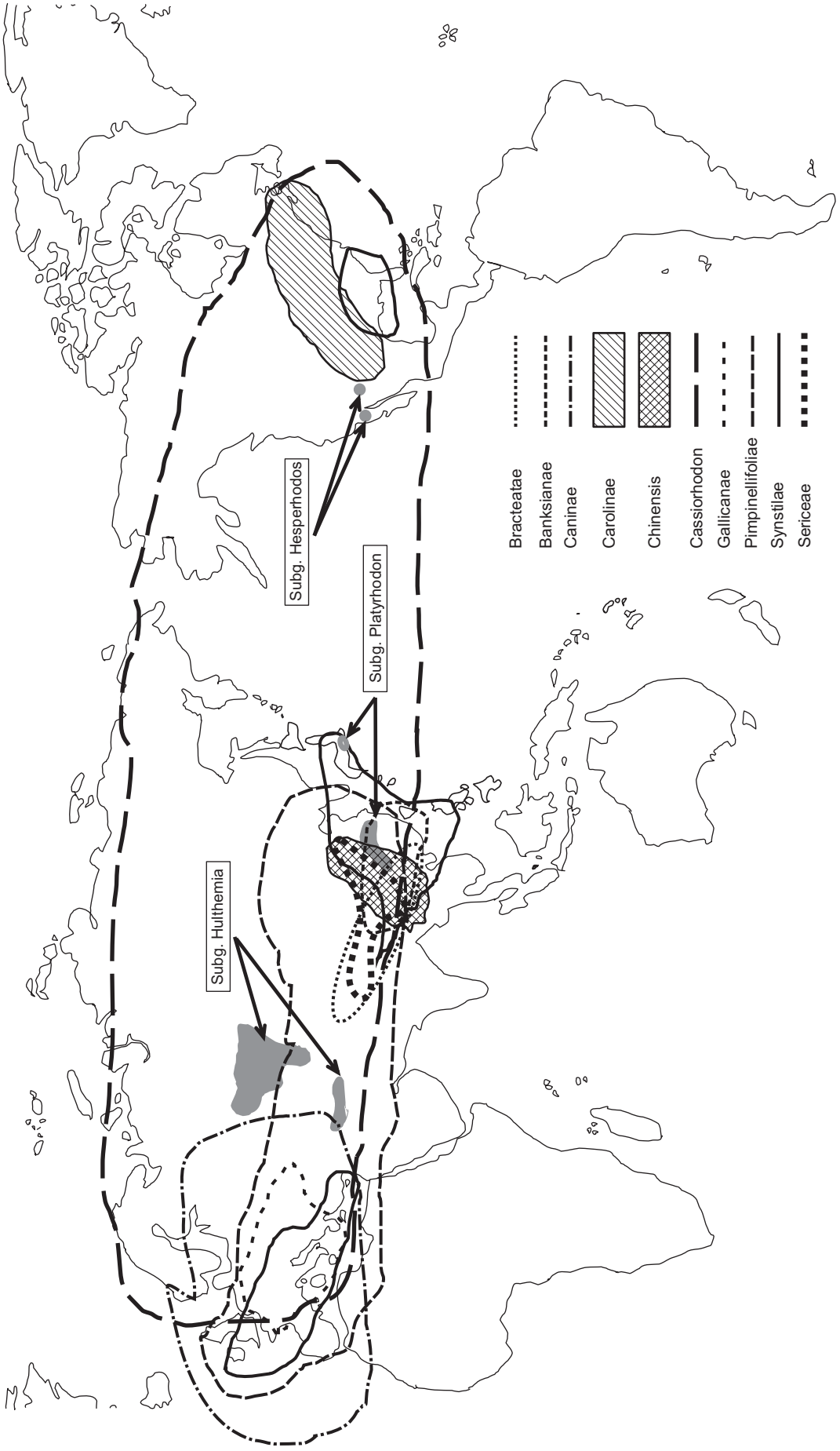


Figure 3.
Geographic distribution of the Genus *Rosa* (based in Klastersky, 1968 and Zielinsky, 1985).

consistent and suggest that Cassiorhodon is the most representative section of the genus and therefore it could be considered as the Type of the genus and, accordingly, be named Sect. *Rosa*, although the present time rules on botanical nomenclature still keep Sect. *Gallicanae* as the Type for the Genus. The idea of Wissemann (1999) that genome A (present in *R. gallica* and some *Caninae*) is an universal type should be considered in future works.

Some specimens of Sect. *Cassiorhodon* reached North America, originating the small Sbg. *Hesperhodos* (only described in SW USA, Fig. 3), Sect. *Carolineae* and some species of Sect. *Synstylae* (S.E. USA), the former likely being specialised forms of *Cassiorhodon*, in agreement with Gudin (2000). Thus, Sect. *Carolineae* and North American forms of *Synstylae* might be reconsidered within Sect. *Cassiorhodon*. The grouping of *Synstylae* with *Cassiorhodon* does not agree with Jan et al. (1999) although it makes sense considering the origin of American *Synstylae*. In fact, Grossi et al. (1998) explained the association between *Synstylae* and *Cassiorhodon* by the American origin of those *Synstylae* accessions.

## CONCLUSIONS

The existence of different groups within Sect. *Caninae* seems to be not very consistent since the inclusion of intermediate forms in the study lead to a lack of distinction between them.

Sbg. *Hulthemia* seems to merit just a sectional status since *R. hulthemosa* is grouped with Sbg. *Eurosa* and this species has been successfully crossed with other species of Sbg. *Eurosa*.

The heterogeneity found in the dendrogram (Fig. 1) with respect to the sectional status suggests the lack of clear reproductive barriers among sections as is common with long lived woody perennial plants. Our data seems to reflect more accurately the reproductive behaviour reported within the genus *Rosa* than the botanical sections mainly based on morphological characteristics and were many intermediate forms between sections are considered. Moreover, the description by botanists of many accessions characterized as possible hybrids or intermediate forms among sections are in agreement with the results obtained in the dendrogram.

Sect. *Cassiorhodon* may be considered as the Type of the genus since it shows the widest geographical distribution, the widest crossing ability within the Genus and it appears in most groups of the dendrogram suggesting to be the most representative Section. Thus, Sect. *Cassiorhodon* might be considered a better candidate than *Gallicanae* to define the Type section of the Genus, i.e., Sect. *Rosa*.

Sect. *Synstylae* has a large but scattered geographical distribution. The hypothesis of a multiple and independent origin of the *Synstylae* features should be considered in future works. If this is true, *Synstylae* species could simply be considered as specialised forms of other sections. Hence, North American *Synstylae* forms might be merged into Sect *Cassiorhodon*.

The conclusions of this work need further confirmation. Therefore, these conclusions may be considered as hypothesis in future works to advance in the comprehension of the genus *Rosa.*

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