The Diversity of Autochthonous Roses in Flanders (Belgium) in the View of the European GENEROSE Reference Framework

K. De Cock[1,2], K. Vander Mijnsbrugge[2], P. Breyne[2], H. Nybom[3], M.J.M. Smulders[4], J. Van Slycken[2] and J. De Riek[1]
[1]Institute for Agricultural and Fisheries Research (ILVO), Plant Unit – Applied Genetics and Breeding, Caritasstraat 21, B-9090 Melle, Belgium
[2]Research Institute for Nature and Forest, Kliniekstraat 25, B-1070 Brussel, Belgium
[3]Balsgård-Department of Crop Science, Swedish University of Agricultural Sciences, Fjälkestadsvägen 459 SE-291 94 Kristianstad, Sweden
[4]Plant Research International, PO Box 16, NL-6700 AA Wageningen, The Netherlands

Keywords: AFLP, *Canina*, dog roses, genetic variation, hybridisation, *Rosa*, taxonomy

Abstract

The subgenus *Rosa* is very intriguing but complex. Nowadays, many European taxonomists apply the taxonomical structure of Henker (2000), although other classifications and views are still considered. In order to gain insight in the taxonomical structure, 1,144 individuals belonging to 27 different wild rose species and some spontaneous hybrids were sampled in Belgium, France, Germany, The Netherlands and Scandinavian countries. Using AFLP analysis, the intra- and interspecific genetic variation was evaluated. PCO analysis supported the major subdivision of the subgenus *Rosa* in different sections and subsections according to Henker (2000). However, the position of *R. tomentella* in a separate subsection or within the subsection *Caninae* is questioned. In order to support the preservation and use of autochthonous genetic resources the genetic fingerprints of several populations were compared. It is concluded that wild populations of rare or locally prevailing species, accessioned from different regions, show genetic differentiation. However, for the common and well-dispersed species no differentiation was found.

INTRODUCTION

Since the beginning of the nineties, interest in autochthonous genetic resources increased in Europe. A plant is autochthonous if it is regenerated only naturally since the last glaciation or, if propagated artificially, only with strict local material (Kleinschmit et al., 2004). An autochthonous Swedish *R. canina* plant, for example, is considered allochthonous in Belgium. As a consequence of the local character of the plant, we assume that autochthonous individuals or populations are adapted to local ecological conditions. During the past century autochthonous plant populations were exposed to men-induced stress, such as increasing habitat fragmentation and import of allochthonous seeds of native species from foreign countries.

Several initiatives were taken to make an inventory for autochthonous genetic resources of woody species throughout Europe (Vander Mijnsbrugge et al., 2005). These inventories are only the first step in a global project to preserve and use these genetic resources. The inventory ordered by the Flemish community showed that Flanders, the densely populated Northern part of Belgium, still contains unexpected and interesting autochthonous genetic resources.

The complexity of the genus *Rosa* was already mentioned by Linneaus (1753) due to a lack of boundaries between the species and the absence of species specific characteristics resulting from interspecific hybridization (Wissemann and Hellwig, 1997). Determination of the status of hybrids is problematic due to a sympatric growth of putative parental species along with introgressants expressing mosaic characteristics which are not observed in the parental taxa (Rieseberg and Ellstrand, 1993; Werlemark and Nybom, 2001). In addition, the unique and mostly pentaploid chromosome constitution of the dog roses (Henker, 2000; Darlington and Wylie, 1961) and consequently the development of an alternative type of meiosis, named the *Canina*

meiosis (Blackburn and Heslop-Harrison, 1921; Täckholm, 1920, 1922) ended in the complex determination of wild individuals. Due to this *Canina* meiosis, descendents receive four chromosome sets from the maternal and only one from the paternal genome, making up a new pentaploid individual (Werlemark and Nybom, 2001).

The variety of classification systems and species concepts defined since Linneaus (1753) reflect the complexity of the subsection *Caninae*. Nowadays, the taxonomical structure of Henker (2000) is widely used on the continent, although sometimes small adaptations are made. He suggested the division of the subgenus *Rosa* into five sections: *Pimpinellifolia*, *Rosa*, *Caninae*, *Cinnamomeae* and *Synstylae*, and divided the section *Caninae* into the subsections *Trachyphyllae, Rubrifolia, Rubigineae*, *Vestitae*, *Tomentellae* and *Caninae* (Table 1).

In this paper we want to 1) quantify the genetic diversity within and between autochthonous sections, subsections and species in order to gain insight in the taxonomical structure of the wild European species, 2) investigate the intraspecific variation of populations sampled in different countries and regions in order to identify and characterize the more diverse and valuable populations.

The results presented in this paper are mostly from the EU-funded GENEROSE project (Van Huylenbroeck et al., 2005), focusing on the conservation and use of natural resources in the species *Rosa*. In addition, a closer look was taken at the genetic variation within and between the Flemish autochthonous species and populations.

MATERIALS AND METHODS

Plant Material

A total of 390 populations belonging to 27 different wild rose species and some spontaneous hybrids were sampled in Europe (Table 1). From each population 5 individuals were randomly sampled during spring and summer. Focussing on Flanders, a total of 481 Flemish autochthonous roses were analysed divided over 46 different populations, each containing 30 individuals, and belonging to 10 autochthonous species and one wild hybrid. The sampling sites were based on Thomaes et al. (2004).

Genetic Analysis

Young and fresh leaves were collected in the field and immediately frozen in liquid nitrogen, freeze-dried and stored under vacuum conditions. DNA was isolated using Qiagen DNeasy Plant Mini Kit (Westburg, Netherlands) according to the manufacturer's instructions. The AFLP reactions were performed according to Vos et al. (1995), with some modifications. Three hundred ng of DNA was cut with the restriction enzymes *Eco*I and *Mse*I (Life Technologies). Digestion and ligation of the adapters was performed in a single reaction for 4 hours at 37°C. Afterwards the samples were stored at 4°C. The preamplification step was performed with a *Eco*I primer and a *Mse*I primer containing one additional selective nucleotide. Final amplifications were performed with primers carrying three selective nucleotides. The three primer combinations used were selected based on their scorability, number of polymorphisms between species and individuals. Fragment separation was performed on the Global Edition IR^2 system van LI-COR (LI-COR). Polymorphic bands were scored in the range of 90 to 650bp.

Statistical Analysis

The binary output was imported in S-Plus 6.2 Professional (Insightful Corporation) and converted to a genetic distance matrix using the Jaccard coefficient, and a principle coordinate analysis (PCO) was performed. Based on the mean population frequency, a simulation was repeated 100 times in order to replace missing or not scorable markers. A comparison between three randomly chosen simulated datasets and the dataset including only the completely scored individuals showed comparable outcomes (Table 2). Further analyses were based on a randomly chosen simulated dataset.

RESULTS

Taxonomical Structure

1. The Subgenus *Rosa*. Using a simulated dataset, the first three components explained 41% of the variation present in the dataset (Table 2). Based on the first two axes (Fig. 1), the several sections and subsections within the subgenus *Rosa* could be recognized (Table 1). The first cluster was formed by the sections *Pimpinellifolia* (*R. spinosissima)*, and *Cinnamomeae* (*R. pendulina*, *R. majalis)*, the second contained individuals from the section *Caninae*, subsection *Vestitae* (*R. villosa*, *R. mollis*), and the section *Synstylae* (*R. arvensis)*. Another cluster contained individuals of the section *Rosa* (*R. gallica*). Finally, the section *Caninae* formed the largest and most dense cluster, with the major subsections *Rubigineae* (*R. rubiginosa*, *R. micrantha*, *R. agrestis*, *R. elliptica*, *R. inodora*), *Caninae* (*R. canina*, *R. corymbifera*, *R. dumalis, R. caesia, R. stylosa*, *R. subcanina*, *R. subcollina*, *R. montana*), *Tomentellae* (*R. tomentella)* and *Vestitae* (*R. pseudoscabriuscula*, *R. tomentosa, R. villosa*, *R. mollis*). The presence of the subsections *Trachyphyllae* and *Rubrifolia* in this dataset is too rare to be put in a global picture.

2. The Section *Caninae.* Focussing on the dense cluster, analyses were repeated only including the individuals of the largest subsections of the section *Caninae*, more specifically *Rubigineae*, *Vestitae*, *Caninae* and *Tomentellae*. The first three axes explained 40% of the variation present in the dataset. According to the first two components (Fig. 2a), only the subsection *Rubigineae* was subdivided.

Next, the subsection *Rubigineae* was excluded and the analyses were repeated. The subsection *Vestitae* was subdivided from the two large remaining subsections *Caninae* and *Tomentellae* (Fig. 2b). In this biplot, 27% of the variation was explained. The subsections *Caninae* and *Tomentellae* could not be subdivided using this method, although the first two axes also explained 27% of the variation.

Intraspecific Variation

For the most common and well-dispersed rose species in Europe, e.g. *R. canina* and *R. rubiginosa*, no intraspecific differentiation was found. In contrast, the less common and rather local species, e.g. *R. arvensis*, *R. gallica* and *R. jundzilii* showed intraspecific variation both between and within countries (Fig. 3). Furthermore, the populations of *R. arvensis* sampled at different regions within Belgium showed also a tendency towards genetic differentiation.

DISCUSSION

The large subdivision analyzed by AFLP agreed with that of Henker (2000), who based his structure on both the morphological similarities and dissimilarities between the different species and on their state of ploidy and type of meiosis. Focussing on the complex and polymorphic section *Caninae*, the large subsections are morphological easily recognised. The *Rubigineae* are characterized by very sticky leaflets with numerous glands that spread apple fragrance, while the leaflets of the *Vestitae* are conspicuously hairy on both sides. *R. tomentella*, the only representative of the subsection *Tomentellae*, has pubescent and glanded midribs and veins, mostly the lower surface of the leaflet is completely covered with glands and hairs. In contrast, within the subsection *Caninae* the leaflets vary from glabrous to hairy and different parts of the leaflets or hips vary from eglandular to sparely glanded (Henker, 2000). Consequently, the morphological differences between the two latter subsections are more subtle and the presence of *R. tomentella* in the subsection *Tomentellae* is sometimes questioned.

The subsection *Rubigineae* is the most distinct group within the section *Caninae*, followed by the *Vestitae*. However, it was not possible to distinguish between the sub-sections *Tomentellae* and *Caninae*. This clustering supports the subdivision of the section *Caninae* in several subsections, although subdivision of the subsection *Tomentellae* is not supported. Perhaps it is better to position *R. tomentella* within the subsection *Caninae* as some taxonomists already did (Thomaes et al., 2004).

The lack of intraspecific differentiation within common and well-dispersed rose species might be due to the presence of a meta-population where migration of pollen and seeds is possible among populations. Within some less common or locally abundant species, intraspecific variation was found between populations of different countries but also between different regions within one country. This intraspecific variation is worth to preserve if it is a local adaptation of the plant or of the population, if the introgression of maladapted (non-local) genes reduces the fitness of the local population or if the introduction of a non-local gene pool leads to a reduction of the species biodiversity.

ACKNOWLEDGMENTS

Thanks to all the lab technicians for their work in the lab, Paul Quataert and Pieter Verschelde for their statistical support and several colleagues for help with sampling. These studies has been carried out with financial support from the Flemish Community (Agency for Nature and Forest) and from the Commission of the European Communities, specific Research Programme "Quality of Life and Management of Living Resources", QLRT-2001-01278 "Genetic evaluation of European rose resources for conservation and horticultural use". It does not reflect its views and in no way anticipates the Commission's future policy in this area.

Literature Cited

Blackburn, K.B. and Heslop-Harrison, J.W. 1921. The status of the British rose forms as determined by their cytological behaviour. Ann. Bot. 35(85): 159-188.

Darlington, C.D. and Wylie, A.P. 1961. Chromosome atlas of flowering plants. George Allen & Unwin Ltd, London.

Henker, H. 2000. *Rosa*. In: Gustav Hegi, Illustrierte Flora von Mittel-Europa, Band IV, Teil 2C, Lieferung A.2. Auflage. Parey Buchverlag im Blackwell Wissenschaftsverlag GmbH, Berlin.

Kleinschmit, J.R.G., Kownatzki, D. and Gregorius, H.R. 2004. Adaptational characteristics of autochthonous populations-consequences for provenance delineation. Forest Ecology and Management. 197: 213-224

Linneaus, C. 1753. Species Plantarum. Holmiae. Tomus. 1: 491-492.

Rieseberg, L.H. and Ellstrand, N.C. 1993. What can molecular and morphological markers tell us about plant hybridisation? Crit. Rev. Plant Sci. 12(3)-213-241.

Täckholm, G. 1920. On the cytology of the genus *Rosa*. A preliminary note. Svensk Bot. Tidskr. 14: 300-311.

Täckholm, G. 1922. Zytologische Studien über die Gattung *Rosa*. Acta Horti Berg. 7(3): 97-381.

Thomaes, A., Vander Mijnsbrugge, K. and De Cock, K. 2004. Taxonomie, ecologie en verspreiding van inheemse rozen in Vlaanderen. Rapport IBW Bb R 2004.020. Instituut voor Bosbouw en Wildbeheer, Geraardsbergen.

Vander Mijnsbrugge, K., Cox, K. and Vanslycken, J. 2005. Conservation Approaches For Autochthonous Woody Plants in Flanders. Silvae Genetica. 54:4-5.

Van Huylenbroeck, J., Smulders, M.J.M, Debener, T., Nybom, H., Gudin, S., Cox, P., Crespel, L. and De Riek, J. 2005. GENEROSE: Genetic Evalutation of European Rose Resources for Conservation and Horticultural Use. Acta Hort. 690: 119-123.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau,M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research. 23(21): 4407-4414.

Werlemark, G. and Nybom, H. 2001. Skewed distribution of morphological character scores and molecular markers in three interspecific crosses in *Rosa* section *Caninae*. Hereditas. 134: 1-13.

Wissemann, V. and Hellwig, F.H. 1997. Reproduction and hybridisation in the Genus *Rosa*, section *Caninae* (Ser.) Rehd. Bot. Acta. 110: 251-256.

Tables

Table 1. Taxonomical overview of the European autochthonous species of the genus *Rosa* based on Henker (2000). Indicated are: P: ploidy (Henker, 2000; *: Darlington and Wylie, 1961); number of individuals sampled in Belgium (B), France (F), Germany (G), The Netherlands (N), Scandinavian countries (S).

Genus *Rosa*	P	B	F	G	N	S	Total
Section *Pimpinellifolia*							
R. spinosissima	4x	8	11	19	23	0	61
Section *Rosa*							
R. gallica	4x	0	39	10	0	0	49
Section *Caninae*							
Subsection *Trachyphyllae*							
R. jundzillii	6x	0	0	10	0	0	10
Subsection *Rubrifolia*							
R. glauca = *R. rubrifolia*	4x	1	7	8	0	0	16
Subsection *Vestitae*							
R. tomentosa	5x	3	4	0	56	0	63
R. pseudoscabriuscula	5x	23	0	5	1	0	29
R. sherardii	4x, 5x, 6x	0	1	11	10	6	28
R. mollis	4x, 5x, 6x	0	0	12	0	18	30
R. villosa	4x	2	2	0	0	0	4
Subsection *Rubigineae*							
R. rubiginosa	5x	25	5	18	35	43	126
R. micrantha	4x, 5x, 6x	6	0	5	14	0	25
R. elliptica	5x, 6x	0	4	5	2	0	11
R. agrestis	5x, 6x	10	9	0	10	0	29
R. inodora	5x, 6x	0	0	0	0	8	8
Subsection *Tomentellae*							
R. tomentella	5x	12	0	5	48	0	65
Subsection *Caninae*							
R. canina (*R. pouzinii*)	5x	30	12	51	72	42	207
R. corymbifera	5x	10	7	34	60	0	111
R. dumalis	5x, 6x	0	5	5	4	52	62
R. caesia	5x, 6x	4	2	1	3	4	14
R. subcanina	5x	2	0	1	6	0	9
R. subcollina	5x	0	0	0	11	0	11
R. montana	5x	0	10	0	0	0	10
R. stylosa	5x, 6x	4	0	0	0	0	4
Section *Cinnamomeae*							
R. pendulina	4x	0	2	10	0	0	12
R. majalis	2x, 4x, 8x	0	0	21	0	8	29
Section *Synstylae*							
R. arvensis	2x	15	6	36	12	0	69
R. sempervirens	2x*	0	8	0	0	0	8

Table 2. Comparison of the percentage of the variation explained by the first three components (Comp 1, 2 and 3) of the original and the randomly chosen datasets. Total: cumulative percentage of the first three components.

Dataset	Comp.1	Comp.2	Comp.3	Total
Original	18%	16%	11%	45%
Simulation 014	20%	11%	10%	41%
Simulation 048	20%	11%	10%	41%
Simulation 098	20%	11%	10%	41%

Figures

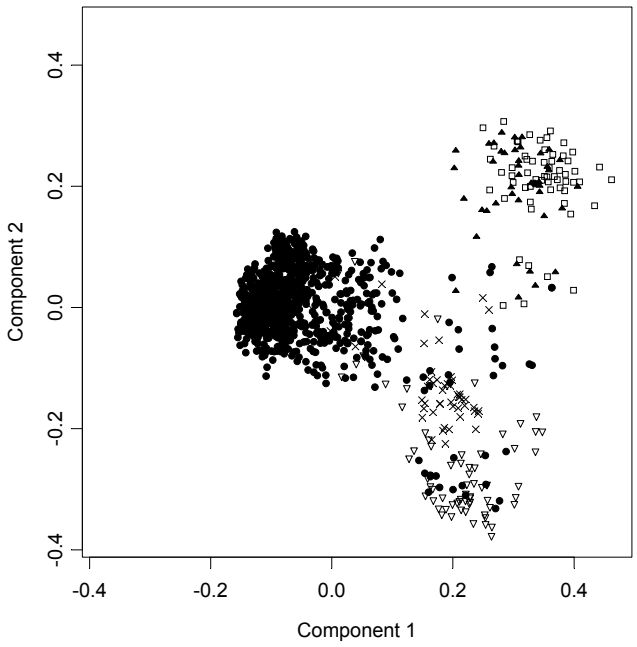

Fig. 1. Biplot of the first two Principle Components of the subgenus *Rosa* based on AFLP markers. Symb: □: *Pimpinellifolia*; X: *Rosa*; ●: *Caninae*; ▲: *Cinnamomeae*; ∇ : *Synstylae.*

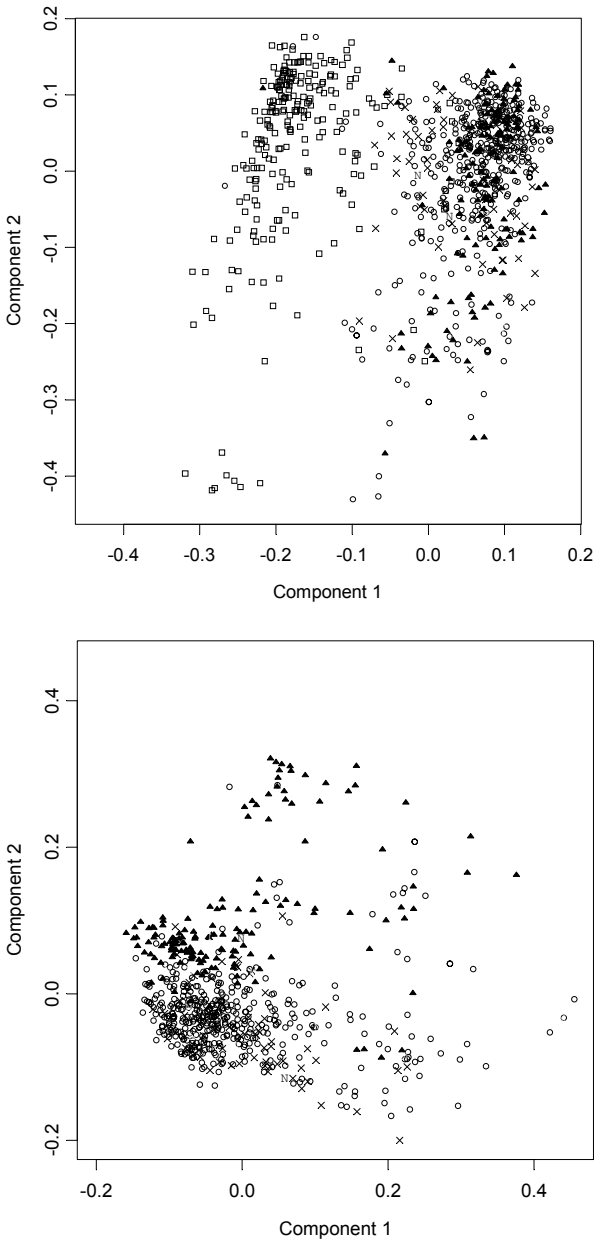

Fig. 2. Biplot of the first two Principle Components of (2a: above) the section *Caninae* and of (2b: below) the section *Caninae* without the subsection *Rubigineae* based on AFLP markers. Symb: ▲: *Vestitae*; ◘: *Rubigineae*; X: *Tomentellae*; ○: *Caninae*.

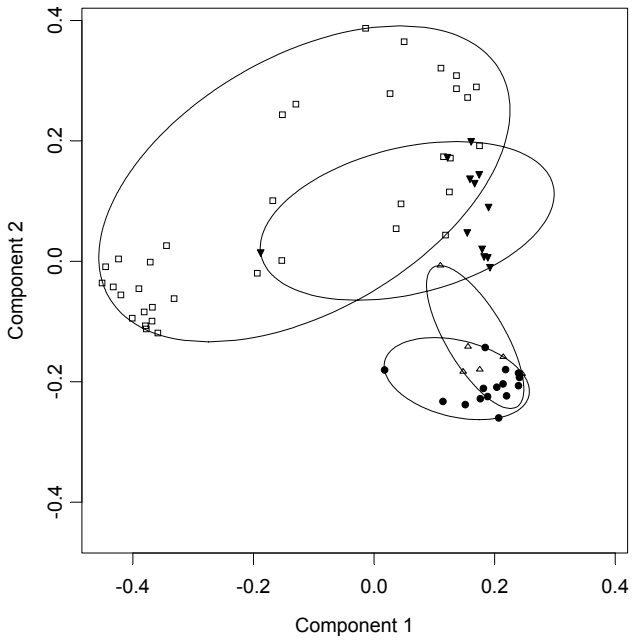

Fig. 3. Biplot of the first two Principle Components of *R. arvensis* showing intraspecific variation based on the origin of the individuals. Symb: Belgium: ●; The Netherlands: ▼; France: Δ; Germany: □.