Genetic variability and reproductive strategies in Nordic dogroses, *Rosa* section *Caninae*

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Doctoral thesis
Swedish University of Agricultural Sciences
Alnarp 2000

Acta Universitatis Agriculturae Sueciae
Agraria 257

ISSN 1401-6249
ISBN 91-576-5775-0

Abstract

Werlemark, G. 2000. **Genetic variability and reproductive strategies in Nordic dogroses, *Rosa* section *Caninae*.**
ISSN 1401-6249, ISBN 91-576-5775-0

In the present thesis, I investigate the morphological variation among and within Nordic dogrose species (*Rosa* section *Caninae),* and the transmittal of morphological characters and molecular markers to interspecific progeny plants. The occurrence of apomixis within the section is also investigated. All species within section *Caninae* are polyploid and characterised by their unique meiosis with unequal distribution of maternal and paternal chromosomes to their progeny. The pollen parent contributes only seven chromosomes, whereas the seed parent contributes 21, 28 or 35 chromosomes depending on ploidy level.

The dogrose species are morphologically rather distinct. Both reproductive and vegetative morphological characters could differentiate among the investigated taxa, with the exception of the two subspecies of *R. dumalis*, subsp. *corifoliia* and subsp. *dumalis. Rosa rubiginosa* appeared to be the most homogeneous of the species, both within and among populations, and *R. dumalis* the most heterogeneous, both within and among populations. *Rosa villosa* was heterogeneous among populations but showed high within-population homogeneity. Morphological characters could also separate interspecific hybrids from progeny groups representing the parental species and the influence from the seed parent was apparent as expected from the skewed distribution of chromosomes. The matroclinal inheritance of molecular markers is also very pronounced, since all but two maternal markers were transmitted to all the interspecific progeny plants. In contrast, only approximately half of the paternal markers were transmitted to the progenies. The degree of homology between the constituent genomes in the parents decide to what extent the genetic contribution of the pollen parent will be recognizable in the progeny plant, both in morphological characters and in molecular markers. The genomes could be separated by size polymorphism in their respective NOR sites. Apparently two of the five consituent genomes in one pentaploid plant were never involved in the bivalent formation. Apomixis appears to occur to a limited extent within the dogroses, indicated by elevated pollen viability compared to the experimentally derived hybrid plants, and a complete lack of paternal parent-specific molecular markers.

Key words: *Rosa* sect. *Caninae*, Rosaceae, genetic diversity, matroclinal inheritance, apomixis, RAPD, microsatellites, *in situ* hybridization

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I Werlemark G, Carlson-Nilsson U, Uggla M and Nybom H (1995) Effects of temperature treatments on seedling emergence in dogroses, *Rosa* sect. *Caninae* (L). Acta Agric. Scand: Sect. B, Soil and Plant Sci. 45: 278-282

II Nybom H, Olsson Å and Werlemark G (1996) Morphometric variation in Nordic dogroses (*Rosa* sect. *Caninae*, Rosaceae). Acta Univ. Ups. Symb. Bot. Ups. 31: 59-68

III Nybom H, Carlson-Nilsson U, Werlemark G and Uggla M (1997) Different levels of morphometric variation in three heterogamous dogrose species (*Rosa* sect. *Caninae*, Rosaceae). Pl. Syst. Evol. 204: 207-224

IV Werlemark G, Uggla M and Nybom H (1999) Morphological and RAPD markers show a highly skewed distribution in a pair of reciprocal crosses between hemisexual dogrose species, *Rosa* sect. *Caninae*. Theor. Appl. Genet. 98: 557-563

V Werlemark G (2000) Evidence of apomixis in hemisexual dogroses, *Rosa* section *Caninae*. Sex. Plant Reprod. 12: 353-359

VI Werlemark G and Nybom H. Skewed distribution of morphological character scores and molecular markers in three interspecific crosses in *Rosa* section *Caninae* (manuscript submitted)

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Preface

The dogroses, *Rosa* section *Caninae*, have been known as a distinct group since the Middle ages. Rosehips have been used for medicinal purposes, and in Sweden we have been making rosehip soup for more than one hundred years. Not very much is known about dogrose genetics, although their peculiar meiosis was discovered already in the 1920s (Täckholm 1920, Blackburn and Heslop-Harrison 1921). In the last two decades, the interest for dogroses as a new crop for domestication, has increased. The food industry imports large quantities of rosehips to supply the demand for rosehip soup. Consequently, a project was initiated in 1985 for developing Swedish dogrose plantations. This also triggered a new interest in the genetic effects of the odd meiosis, since intra- and interspecific crosses would be an important part of the breeding program. Since then, the world market price on rosehips has decreased and Swedish plantations for large scale production are at the moment not being persued. But the rosehips may have other qualities which will make them even more desirable. Recent research indicates e.g. that rosehips possess anti-inflammatory properties and might therefore be useful for therapy in patients with arthritis (Winther et al. 1999). The present thesis will hopefully be of use to all future breeders of this beautiful rose with its very own reproduction method.

Introduction

Dogroses, *Rosa* section *Caninae*, have been recognized as a well defined entity since the Middle ages, when they were grown in monasteries for medicinal purposes, taking both the hips and the seeds into consideration. Around the 13th century, dogrose species were being planted for hedges and in the second half of the 16th century, both the canina rose and the sweetbriar were mentioned in a book of herbs; "Kräuter-buch" by J. T. of Bergzabern (Krüssmann 1981). At the end of the 18th century, budding of ornamental roses was developed, and *R. canina* was found to be very suitable as a rootstock and has retained this function into the 21st century. *Rosa rubiginosa*, sweetbriar or eglantine, has been used both as rootstock, and as a seed parent in several interspecific crosses to develop roses for ornamental purposes (Krüssmann 1981).

The beauty of rose flowers has attracted the attention of many botanists throughout the years and their variability has resulted in prolific namegiving. Several botanists in the beginning of the 20th century were reluctant to recognize the existence of natural rose hybrids and gave specific rank to all identifiable entities. Thus the polymorphism within section *Caninae* inspired Almquist (1919) to name 352 different representatives (species, subspecies and varieties). However, modern classification only recognizes ca 20 different species which occur mainly in Europe, but also in North Africa and southwest Asia (Zielinski 1985). They are long-lived perennial shrubs, growing in dry woodland margins and disturbed habitats like roadsides and open pastures. They are self-compatible, but outcrossing and interspecific hybridization occur.

The *canina* meiosis

With the advances in the technology of microscopy in the beginning of the last century, the new field of cytology developed. This led to the discovery that the basic chromosome number in *Rosa* is seven (Täckholm 1920, Blackburn and Heslop-Harrison 1921), and that polyploid series of rose species are based on this number. Täckholm (1920) divided the genus *Rosa* into two very distinct groups; one group consisted of all species having a normal meiosis with bivalents only, and the other group consisted of the section *Caninae*. The species in this latter section are all polyploid with 2n = 28, 35 or 42, with 35 chromosomes as the most common, and they are characterised by a peculiar meiosis with both bivalents and univalents.

In the embryo mother cell (EMC) of a typical pentaploid dogrose species, the seven bivalents line up on the equator of the spindle while the remaining chromosomes occur as univalents at one of the spindle poles. The bivalent chromosomes separate as usual and move towards the poles, giving rise to two cells. The one closest to the micropylar end contains seven chromosomes from the bivalent formation together with all the univalents and the other cell, close to the

chalazar end, contains only seven chromosomes originating from the bivalents. During the second division, the univalents divide normally along with the chromosomes from the bivalents. This will result in a tetrad of two cells with 28 chromosomes, and two with only seven chromosomes. The tetraploid cell closest to the micropylar end develops into an embryo sac. In the pollen mother cell (PMC) the univalents lie around the edge of the equator region. When the bivalent chromosomes have separated in a normal manner, the univalent chromosomes move in to the region where the bivalents have been. Several of the univalents manage to reach the poles in time to be included in the daughter cells, but at the next division they lag behind. The result will thus be a tetrad with four cells containing seven chromosomes from the bivalent formation together with many micronuclei formed from the univalents. The meiosis in the PMC often fails which results in a lowered pollen viability (Matsson 1912, Heslop-Harrison 1958, Jicinska et al. 1976).

Täckholm (1920) believed that the dogrose species would be unable to maintain this odd chromosomal arrangement if they reproduced sexually, and therefore he assumed them to be apomicts. He explained the polymorphism within the section by a theory of hybridizations stemming from three original crosses: diploid (n = 7) X hexaploid (n = 21) to form the tetraploids, diploid X octaploid (n = 28) to form the pentaploids and diploid X decaploid (n = 35) to form the hexaploids. These hybridizations were thought to have taken place in the pre-Tertiary era and to have developed thereafter through mutations and further crossings into the multitude of forms we have presently (Täckholm 1920, Blackhurst 1948).

Another theory, that appeared shortly after, was Hurst´s once famous septet-theory (Hurst 1925). Hurst believed that the genus *Rosa* had evolved from a common northern decaploid ancestor. This ancestor would have had five different diploid septets of chromosomes, and where each septet gave raise to specific morphological characters. These septets were supposed to segregate as complete units with the bivalent pairing taking place between similar septets. As the species spread south, life conditions became less extreme, and the necessity of all the septets diminished, so that successively entire septets were lost. Species within the section *Caninae* would have evolved from hybridization between species with only one septet in common, resulting in the odd meiosis.

This theory was criticized by e.g. Erlanson (1938), Gustafsson and Håkansson (1942), and Blackhurst (1948). Gustafsson and Håkansson (1942) instead proposed that the dogroses had an internal autotriploid constitution, i.e. each species had three homologous genomes, e.g. A, A, A_1, C, D where the two As formed the bivalents, and the rest formed the univalents. This would explain the reciprocal differences in e.g. fertility that the authors had seen in their interspecific crosses (Gustafsson and Håkansson 1942, Gustafsson 1944). It does not, however, explain why there are no trivalents in the meiosis of the pure species, only bivalents. Blackhurst (1948) therefore suggested that the chromosome pairing was regulated

by a series of alleles. Each species was homozygous for a specific allele and heterozygosity at this locus caused a breakdown in the meiotic behaviour in the hybrids. Roberts (1975) postulated that meiosis-regulating genes had evolved to guide and regulate both the univalents and the bivalent formation in PMC and EMC, respectively. In his study on the species *R. nanothamnus* (1975), he advanced the theory that the meiosis-regulating genes operated by restricting the synapsis, i.e. the pairing of the chromosomes, rather than restricting the chiasmata formation and thereby preventing crossing-overs. An analogous regulation system occurs in e.g wheat, *Triticum aestivum*, where the Ph gene located on chromosome 5B has a major suppressing effect on homeologous pairing (Vega and Feldman 1998). It is also possible that there is a bivalent-promoting mechanism like the one existing in *Dahlia* (Gatt et al. 1999). In hybrids, a partial breakdown of this mechanism will allow homeologous pairing.

Only a few reproductive systems have been reported that are somewhat similar to that in the dogroses. One of them is found in *Leucopogon juniperus* (Epacridaceae) with 2n = 3x = 12 chromosomes and another in *Andropogon ternatus* (Poaceae) with 2n = 3x = 30 chromosomes. *Leucopogon juniperus* forms four bivalents and four univalents at the meiosis (Smith-White 1955). *Andropogon ternatus* has 20 chromosomes in their viable pollen cells, 10 from the bivalents and 10 from the univalents, and the egg cell contains only 10 chromosomes from the bivalents (Norrmann and Quarin 1987). Still another example is recorded from the hexaploid cytotype of the grass *Paspalum compressifolium*, which also has an irregular meiotic behaviour with about one third of the chromosomes associated as multivalents (Quarin et al. 1996).

There are no known diploid *canina* species, nor are there any meiotically normal polyploid species with the morphological characteristics of species belonging to the section *Caninae*. Thus all living forms exhibit a derived genetic system and the group must be fairly old (Grant 1971). Täckholm (1922) proposed that the ancestors of the *canina* group became extinct during Pleistocene, and that the hybrid complex has expanded since that period. The meiosis must have conferred selective advantage in order for it to survive and proliferate for such a long time.

Apomixis

In the beginning of the 20th century, it was generally believed that the species within section *Caninae* were able to produce seeds without prior fertilization. Studies of the flowering period and the apparent constancy within the species, led Matsson (1912) to postulate that the dogroses propagated by selfpollination. According to Matsson, this type of pollination led in its turn to apomixis, and some species were more inclined to propagate in this way than through sexual reproduction. Both Blackburn and Heslop-Harrison (1921) and Täckholm (1920) believed the dogroses to be apomictic. The explanation for the polymorphism which existed within the section was that numerous spontaneous interspecific hybridizations occurred in nature. Hurst (1932) called the dogroses "facultatively

sexual", since the progeny plants in most cases resembled their seed parent in all details, but frequent hybridizations sometimes resulted in extremely polymorphic characters in the progeny plants.

In contrast, Fagerlind (1940) failed to find unreduced embryo sacs in a rather limited material, and therefore concluded that dogroses propagated sexually by balanced heterogamy. Gustafsson (1937) initially thought that the dogrose species were pseudogamous, i.e. pollination is necessary for the development of the endosperm, but the embryo remains unfertilised. Later, he changed his mind, and wrote that no properly verified cases of pseudogamy had been found (Gustafsson and Håkansson 1942). He also stated that the progeny plants on which he had based his earlier assumptions, "had developed through the prevalence of the mother genes or incomplete emasculation". However, two years later, he added in a footnote, that plants within the same population could propagate by apomixis when crossed or selfed (Gustafsson 1944).

Kroon and Zeilinga (1974) made numerous interspecific crosses with Edelcaninas (commercial rootstocks, section *Caninae*) as seed parents and various rose species as pollen parents and also intraspecific Edelcanina crosses, and reported one-third of the progeny plants from the interspecific crosses to be of apomictic origin. In the intraspecific crosses, they instead found only approximately 6% apomictically derived plants. Unfortunately, they did not report any morphological data or statistical test results. Cole and Melton (1986) performed a small emasculation experiment with *R. rubiginosa* (= *R. eglanteria*) to test for apomixis, but none of the (very few) flowers produced any seeds.

A more elaborate crossing experiment with five German dogrose species, including *R. canina* and *R. rubiginosa*, was set up by Wissemann and Hellwig (1997), who produced more than 10,000 seeds from 900 crossings using autogamy (self-pollination within the same flower), geitonogamy (self-pollination within the same plant) and xenogamy (pollen from another plant). They also had some flowers which were emasculated without subsequent pollination, though they did not mention how many. They showed that all the dogrose species used in their study, were able to produce seeds by apogamy. However, they received very few seeds and the yield of viable seeds was only 5% of that achieved by xenogamy. This is in contrast to other studies showing a more or less normal seed set in apomictic taxa (Asker and Jerling 1992, Czapik 1994,). Furthermore, Wissemann and Hellwig (1997) only tested for autonomous apomixis, although many apomictic taxa within the Rosaceae family are known to propagate by pseudogamy (Gustafsson 1946, Campbell et al. 1991, Campbell and Wright 1996).

Contemporary research

The genus *Rosa* is economically one of the most important groups of ornamental plants. Species within the section *Caninae* are mostly used as rootstocks, and more rarely as seed or pollen parents in interspecific crosses even though they are

hardy and relatively tolerant towards various diseases. The dogroses have been included in some recent studies of genetic variation within *Rosa*. Debener et al. (1996) used RAPD (random amplified polymorphic DNA) markers to show that the species in section *Caninae* are well separated from species belonging to section *Cinnamomeae*. In particular, the two dogrose species *R. sherardii* and *R. villosa* were placed very close to each other in a cluster analysis of several wild rose species and some rose cultivars. Debener et al. (1997) later used *R. sherardii* as pollen parent in an interspecific cross with another member of section *Caninae*, i.e. *R. obtusifolia* Desv. to prove hybridity in the progeny plants using molecular markers. They did, however, not mention the existence of the *canina* meiosis in either of their studies. Millan et al. (1996) also used RAPD markers to show that Spanish dogrose species are closely related to each other and to *R.* X *alba*, a presumed cross between species from section *Caninae* and section *Gallicanae*. The systematic position of *R.* X *alba*, close to the species belonging to the *Caninae* section and separate from species in other *Rosa* sections, corroborated the hypothesis that a species from the section *Caninae* had acted as seed parent.

The Swedish rosehip research program

One of the traditional desserts in Sweden is rosehip soup. The rosehips used for this delicacy are nowadays imported, mainly from South America, but in 1985 a plant breeding program was initiated at Balsgård, Department of Horticultural Plant Breeding, Swedish University of Agriculture, in the south of Sweden. The aim was to develop varieties suitable for industrial cultivation in Sweden as well as growing techniques for the establishment of commercial plantings in Sweden. Calculations had shown that about 6500 tonnes of raw material, which is equivalent to 1200 ha of cultivation, would be needed to supply the demand (Olander 1986). This acreage could even increase in the future since other products like herbal teas etc. might be manufactured from the rosehips. To make a Swedish rosehip plantation feasible, the plants must have a large production of hips which ripen simultaneously and they must also be able to withstand machine harvesting without detrimental damage to the branches. The hips should have an attractive colour, contain high levels of vitamin C and of course have the particular aroma that people expect in their rosehip soup. This aroma is found mainly in species belonging to the *Caninae* section.

The rosehip breeding program can be divided into two steps; (1) to select superior genotypes in the field for immediate release as cultivars and (2) to use the most promising genotypes for intra- and interspecific as well as inter-sectional crosses. In order to start with the first step, plant material from nurseries and Botanical gardens was collected and a huge gene bank of wildgrowing material from the Nordic countries was initiated. Later, numerous intraspecific and interspecific as well as inter-sectional crosses were performed and the resulting progeny plants were evaluated with respect to their suitability for cultivation.

Aim of my thesis

In connection with the rosehip breeding program, certain questions were raised. The three specific questions concerning the aim of the following investigations are: (1) What influence does the *canina* meiosis have on the amount and distribution of genetic variation within the dogrose species? (2) How are morphological characters and molecular markers transmitted to the progeny plants? (3) What contribution does apomixis make to the genetic variation within the section?

Material and methods

Plant material

The plant material used for this thesis derives mainly from the collections of wild material made in the Nordic countries during 1987–88. The most common Nordic dogrose species are *R. canina* L., *R. dumalis* Bechst. (with subsp. *coriifolia* (Fr.) A. Peders. and subsp. *dumalis*), *R. rubiginosa* L., *R. sherardii* Davies (with varieties *umbelliflora* (Sw.) Herring and *venusta* (Scheutz) Herring) and *R. villosa* L. subsp. *mollis* (Sm.) Kell. and Gams. Of these, *R. villosa* is tetraploid, *R. canina, R. dumalis* and *R. rubiginosa* are pentaploid and *R. sherardii* is reported to be tetra-, penta- and hexaploid. When the ploidy level of the two populations of *R. sherardii* var. *venusta* used in the following investigations were assessed with flow cytometry, one of them showed to be tetraploid and the other pentaploid. The ploidy level of the other variety, var. *umbelliflora*, was never assessed. See Table 1 for details of which species and intra- and interspecific crosses were used in the different Papers.

Seed germination

In order to ascertain possible differences between the species in seed germination rate and preference for temperature treatment, a germination test was performed. A total of 50,000 seeds were sown with a maximum of 200 seeds/pot, and the pots were subsequently divided into two groups, which were subjected to two different temperature treatments. One group had 12 weeks at +20 °C followed by 12 weeks of +5 °C, whereas the other group had 24 weeks of +5 °C. Thereafter, all pots were taken outside for germination, emerging seedlings counted and removed and the pots with non-germinated seeds remained outside until October. During wintertime, both groups were kept in +5 °C and were then taken outside at the end of March, and the emerging seedlings were once again counted (Paper I).

Morphology

Morphological characters, both reproductive and vegetative, were used to score phenotypic variation within and between taxa as well as within and between progeny groups in Papers II, III, IV and VI. The reproductive characters consisted of ovary, sepal and pedicel characters, measured manually when the plant was in full bloom. One apical flower from an inflorescence was chosen from each plant in Papers II and III whereas mean measurements of three apical flowers/plant were used in Papers IV and VI. The vegetative characters consisted of leaf shape and leaf ser-

ration assessments on one leaf/plant in Papers II and III, and the mean shape of three leaves in Papers IV and VI.

The leaf characters in Papers II, III and IV were measured manually, whereas automated image analysis was employed in Paper VI. This is a fast and relatively novel way of scoring morphometric variation. The outline of a leaflet was recorded with a video camera linked to a computer via an analogue-to-digital converter and the shape of the image was described by coordinates of the image points (White et al. 1988). Different types of shape descriptors can be used to describe the shape of the stored outlines, and in Paper VI we chose elliptic Fourier coefficients, which gave us a total of 40 coefficients. The automated image acquisition and shape description procedures were carried out using the program ARBO written by R.J. White (White et al. 1988).

In Paper II, four growth-related characters were also measured, but since these showed very little significant differentiation among the species, they were not used further.

Table 1. Species and intra- and interspecific progeny plants used in the different papers

Paper I	Collection of dogrose seeds made in 1987 in the south of Sweden. Species included in this collection were *R. canina, R. dumalis* subsp. *coriifolia* and subsp. *dumalis, R. rubiginosa, R. sherardii* var. *venusta*, and *R. villosa* subsp. *mollis*. Included in the collection were also some seeds from a putative hybrid between *R. canina* and *R. dumalis*. Rosehips were collected from several individual plants per species, each species being sampled at 3–5 localities.
Paper II	Collection of dogrose seeds made in 1987–88 in the Nordic countries. Seeds from 1–3 species were collected represented by 1–4 plants each. The resulting seedlings were randomised and grown in an experimental field. Species included in this collection were *R. canina, R. dumalis* subsp. *coriifolia* and subsp. *dumalis, R. rubiginosa, R. sherardii* var. *umbelliflora* and var. *venusta*, and *R. villosa* subsp. *mollis*. In addition, one putative hybrid between *R. canina* and *R. dumalis*, and two putatitive hybrids between *R. sherardii* and *R. villosa* were included. The subsequent analyses were made on 555 seedling plants derived from 48 different localities.
Paper III	The material was obtained from the germination of seeds described in Paper I. Forty progeny plants, taken at random from progeny groups of *R. dumalis* subsp. *coriifolia* and subsp. *dumalis, R. rubiginosa*, and *R. villosa*, were randomised and grown in an experimental field. The subsequent analyses were made on 498 seedling plants derived from 10 different localities.
Paper IV	The parental plants used in a pair of reciprocal crosses between *R. dumalis* subsp. *dumalis* and *R. rubiginosa* were collected from two different locations in the south of Sweden, and the crosses were performed inside a greenhouse. Approximately 40 progeny plants from each cross were sampled for assessment of morphological characters and molecular markers.

Paper V The same plants as in Paper IV, with special emphasis on the five *R. dumalis* X *R. rubiginosa* and the four *R. rubiginosa* X *R. dumalis* progeny plants which did not receive any molecular markers specific for the respective pollen parent.

Paper VI Together with the rosehips collected in 1987–88, some root suckers were collected from each species, to be used later in intra- and interspecific crosses, performed inside a greenhouse. This study was based on progeny plants involving two species combinations; (A) Intraspecific crosses in *R. rubiginosa* (16 plants) and *R. sherardii* var. *venusta* (27 plants) and an interspecific cross between these two species (35 plants). The *R. rubiginosa* plants used for the intraspecific and interspecific crosses came from the same locality, whereas the *R. sherardii* plants came from two different localities, (B) Intraspecific within-population crosses of *R. sherardii* var. *venusta* (27 plants), selfpollinated *R. villosa* (27 plants), and interspecific crosses between these two species, *R. sherardii* X *R. villosa* (53 plants) and *R. villosa* X *R. sherardii* (6 plants). One *R. sherardii* plant was used for both the intra- and interspecific crosses, whereas two *R. villosa* plants from the same location were used for the intra- and interspecific crosses. The plants were planted in a randomised design.

Paper VII This study is based on plants of *R. dumalis* subsp. *dumalis*, *R. rubiginosa*, *R. sherardii* var. *venusta* and *R. villosa* used in papers IV and VI. In addition, 10 progeny plants from the *R. dumalis* X *R. rubiginosa* cross (Paper IV) (including the five plants which lacked pollen-specific RAPD markers), 10 progeny plants from the *R. rubiginosa* X *R. dumalis* cross (Paper IV) (including the four plants which lacked pollen-specific RAPD markers), 10 progeny plants from the *R. sherardii* X *R. villosa* cross (Paper VI) and 6 plants from the *R. villosa* X *R. sherardii* cross (Paper VI) were analysed.

Paper VIII Root tips and pollen cells from one pentaploid plant of *R. canina*, collected in Epping Forest, London, UK, and one "gynogenetic haploid" *R. canina* var. Pfänders (tetraploid), one plant each of an interspecific cross between *R. rubiginosa* and *R. sherardii* (Paper VI) and an inter-sectional cross between *R. dumalis* (Paper IV) and *R. rugosa*, cv. "Ottawa" (sect. *Cinnamomeae*, diploid), were used for chromosome preparations.

Molecular markers

The use of DNA markers permit studies of relatedness and variability among and within species without influence from environmental factors. One of the most used techniques for DNA studies is the PCR (polymerase chain reaction) procedure, and a simple way of employing the PCR technique without prior knowledge of the DNA sequences, is the RAPD (random amplified polymorphic DNA) method (Williams et al. 1990, Welsh and McClelland 1990). This method uses arbitrary decamer primers that amplifies both coding and non-coding regions of the genome resulting in markers that are dominantly inherited in a Mendelian fashion. The reliability of RAPD has been questioned, since small changes in the reaction conditions can influence the marker patterns and there is also competition for priming sites in the genome (Halldén et al. 1996). The advantages are that RAPD is simple to perform, does not require work with radioactivity, yields highly polymorphic data, and if reproducibility can be established, is sufficiently reliable (Devos and Gale 1992, Lashermes et al. 1996, Barcaccia et al. 1997).

RAPD was used to study the transmittal of molecular markers from seed and pollen parents to progeny plants in Paper IV and VI, and also to establish the variation among the parental plants. The RAPD study in Paper IV led to the discovery that certain progeny plants lacked all markers specific for the pollen parent and were therefore assumed to be of apomictic origin. An extended study with additional RAPD markers was conducted to ascertain the true origin of these plants (Paper V).

Microsatellite markers is another application of the PCR technique. Microsatellites or simple sequence repeats (SSR) are stretches of short tandemly repeated sequences dispersed in the genome. They are highly variable and co-dominantly inherited. Once the unique flanking sequences particular to each SSR locus have been characterised for primer development, assay is easy with PCR. Microsatellites were used in a preliminary study, to analyse the parental contributions in interspecific crosses (Paper VII).

In situ hybridization

The molecular techniques of fluorescent in situ hybridization (FISH) and genomic in situ hybridization (GISH) have enabled major advances in the cytogenetic studies of plant chromosomes. They can be used for physical mapping of sequences to their chromosomal region, identification of chromosomes or chromosome segments, and to provide indicators of evolutionary rearrangements in the genome (Schwarzacher and Heslop-Harrison 2000). FISH was applied to root tips of *R. canina* to ascertain the amount and size of the normal rDNA sites. For comparison, a "gynogenetic haploid" *R. canina*, i.e. a plant derived from pollination with irradiatied pollen and embryo rescued, was analysed. This plant had a chromosome range of 2n = 27–30 chromosomes. FISH was also applied to pollen grains of these two plants, to see which of the genomes took part in the bivalent formation. GISH was applied to the interspecific and inter-sectional hybrids to further try and separate the genomes from each other and to see which genomes participated in the bivalent formation.

Male and female fertility

The reproductive potential in offspring from interspecific dogrose crosses has been reported to range from more or less complete sterility to almost normal fertility (Gustafsson 1944). Therefore, both pollen and seed characters were characterised in a pair of reciprocal interspecific crosses between *R. dumalis* and *R. rubiginosa* in Paper V, to assess the occurrence of meiotic irregularities in both PMC and EMC. Pollen viability was checked with cotton blue (aniline blue lactophenol) stainability. Preferably three flowers from each plant were analysed and the mean value was taken. Stained and non-stained pollen grains were easy to discriminate, since the stained grains were large and dark blue, whereas the non-stained grains were small, misshapen and transparent. Pollen viability was also analysed in the *R. rubiginosa* X *R. sherardii* progeny plants and their parents in Paper VI.

Interspecific hybrids in dogroses are reported to have very large rosehips, but few seeds compared to their respective parental plants (Gustafsson 1944, Halásová 1988). Therefore, 30 rosehips (when available) were picked from each of the progeny plants derived from reciprocal crosses between *R. dumalis* and *R. rubiginosa*, and also from the two parental plants. The seeds were counted, seeds and fruit flesh were weighed separately, and in the following analysis mean number of seeds/hip, mean seed weight and mean weight of fruit flesh/hip were used (Paper V).

Statistics

The statistical analyses in Paper III were performed with SPSS, Systat and Superanova statistical program packages, whereas only SPSS was used in the remaining Papers. Relationships between taxa and/or progeny groups based on reproductive and vegetative characters were assessed with the help of one-way analyses of variance or Student´s *t*-tests. All pairwise comparisons of taxa or progeny groups were performed with a Scheffé a posteriori test ($p<0.05$) which compensates for groups with unequal sample sizes, but tends to underestimate deviation from the null-hypothesis, i.e. that the groups do not differ. Correlation tests were performed to estimate the association between morphological characters. Variation within progeny groups was assessed by calculating the coefficient of variance. Canonical variates analyses (CVA), which aims to obtain maximum separation among predetermined groups, were used to assess the relationships between Nordic dogrose taxa, differentiate between intraspecific populations and discriminate between progeny groups and groups representing the parental species.

Results and discussion

Seed germination

Dogrose species used for rootstocks are usually propagated by seed, and seed propagation may also become an alternative for setting up large plantations of dogroses for commercial rosehip production. It has long been known that most of the germination in these species is achieved in the second year (Matsson 1912, Crocker and Barton 1931, Rowley 1956, Kroon and Zeilinga 1974), and considerable efforts have therefore been made to speed up the process. The most commonly practised method is to apply sulphuric acid prior to stratification in order to diminish the thickness of the pericarp around the seed (Roberts 1979). However, there are reports that this treatment is superfluous (Suszka and Bujarska-Borkowska 1987) and that a simple temperature treatment is sufficient to achieve satisfactory results.

The different temperature treatments applied in Paper I agreed with previous investigations in that most of the germination occurred during the second year. Also, the seeds which were treated with a period of warm temperature in the beginning of the stratification had a higher germination percentage compared to the other group with only cold treatment. There were some differences in

germination among the species. In contrast to all other species, *R. rubiginosa* germinated best during the first year and had a very low germination in the second year. Maybe only few seeds were viable and these germinated already in the first year. In contrast, Uggla and Nybom (1999) reported that *R. rubiginosa* as seed parent in both intra- and interspecific crosses showed a high seed germination. Highest total percentage of seed germination was found in *R. dumalis* subsp. *coriifolia* with 42.6%, followed by *R. dumalis* subsp. *dumalis* with 28.6%. *R. sherardii* var. *venusta* had the lowest germination percentage; only 8.5%, which is even lower than that found in the presumed hybrid *R. canina* X *R. dumalis*. The embryo in *R. sherardii* is reported to be in deeper dormancy compared to other species within the genus *Rosa* (Jackson and Blundell 1963), which could explain its low germination.

Genetic variation within and among species

Variation among species assessed with manually scored reproductive and vegetative characters in Paper II, showed that *R. rubiginosa* was a very well defined taxon and clearly separated from all the other species, with the possible exception of *R. sherardii*. Only three of the 16 analysed characters showed highly significant differences ($p<0.001$) among the 14 populations collected in the Nordic countries. The *R. rubiginosa* progeny plants, derived from a collection of three populations in the south of Sweden, showed very little differentiation both within and between the different offspring families (Paper III). Comparison of these three populations yielded significant variation only in leaflet shape. Another study using RAPD markers and Fourier coefficients of leaflet shape, similarly showed *R. rubiginosa* to be a very homogeneous and well-defined species (Olsson et al. 2000).

By contrast, *R. dumalis* appeared to be very heterogeneous both within and between the different populations (Papers II and III). The two characters which are commonly used to separate the two subspecies *dumalis* and *coriifolia* from each other, i.e. leaf pubescence and compact growth form in subsp. *coriifolia* (Nilsson 1967, Malmgren 1986), were not included among our chosen characters. Consequently, the subspecies overlapped considerably in a CVA based on morphological characters (Fig. 1) (Paper II). Of the 16 analysed characters, 15 showed significant variation (within both subspecies) among the eight (subsp. *coriifolia*) and 22 (subsp. *dumalis*) studied populations, respectively. Pairwise comparisons indicated that the variation was evenly distributed among the different populations. Studies made on offspring families, showed that these also differed from one another, regardless of whether they came from the same or from different populations (Paper III). These two subspecies could not be separated with molecular RAPD markers (Olsson et al. 2000) and therefore Olsson (1999) suggested that they should be treated at lower rank than that of subspecies.

Rosa dumalis is commonly separated from the relatively similar *R. canina* by the shape of the flower disc and style head arrangement. Nilsson (1999) mentioned the "*canina* type" with a wide, more or less conoidal disc compared to the "*dumalis*

type" with a narrower, flat to slightly concave disc. Zielinski (1985) regarded *R. canina* as the only good species among the *Caninae* taxa. He claimed that all other taxa, commonly treated as species, were segregants and belonged to different hybrid swarms. Precisely why he regarded *R. canina* in particular, to be a good species is somewhat unclear since he also mentioned the "great polymorphism" caused by introgression in this species. In Paper II, *R. canina* appeared to be a distinct species, separated with several characters from *R. villosa* and *R. dumalis*, and with little intraspecific variation. A study of genetic diversity in Nordic dogroses assessed with Fourier coefficients of leaflet shape and manually scored reproductive characters, showed that *R. canina* together with *R. sherardii* and *R. villosa* had very high levels of between-population variability, with no indication of geographic structure (Olsson, manuscript submitted). The two species *R. canina* and *R. dumalis* were also investigated with molecular markers, and found to be completely overlapping (Olsson et al. 2000). Accordingly, Olsson et al. (2000) placed them together in a *R. canina* group.

Rosa sherardii appeared to be a very well-defined species, although the analysed plant material was quite limited (Paper II), with few significant differences among the populations. The varieties, var. *umbelliflora* and var. *venusta*, could be discriminated with the morphological characters, but the former overlapped with *R. villosa*, whereas var. *venusta* was more distinct (Fig. 1). These two *R. sherardii*

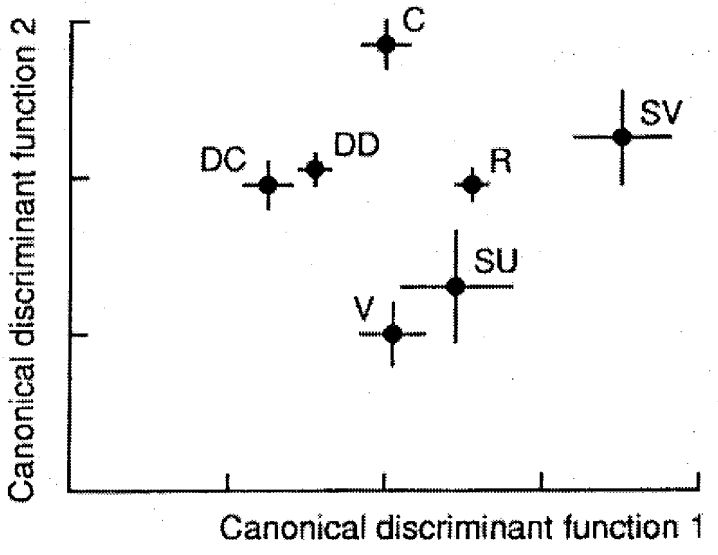

Fig. 1. Stepwise canonical discriminant analysis calculated on seven characters for the discrimination between seven different taxa of the genus *Rosa*. Centroids for the different taxa (C *R. canina*, DC *R. dumalis* subsp. *coriifolia*, DD *R. dumalis* subsp. *dumalis*, R *R. rubiginosa*, SU *R. sherardii* var. *umbelliflora*, SV *R. sherardii* var. *venusta*, V *R. villosa* subsp. *mollis*) plotted on the first two discriminant functions together with their mean errors.

varieties were also separated in a study using Fourier coefficients of leaf shape (Olsson, manuscript submitted), and they have different distributions in the Nordic countries. Variety *umbelliflora* is found along the east coast of Sweden as far north as Uppland, whereas var. *venusta* is more commonly found on the west coast, in Denmark, and in southern Norway (Nilsson 1967). Two *R. sherardii* var. *venusta* plants with different ploidy levels collected at two different locations, showed only 3% DNA marker polymorphism. It is not possible to say whether this difference was due to the differences between the genomes *per se* or if it was due to within-populational diversity caused by e.g. genetic drift. Either way, the results imply very little variation among the *R. sherardii* genomes.

Rosa villosa showed pronounced between-population variation (Paper II), but high levels of within-population homogeneity (Paper III). This species was also well separated from the other species, with the exception of the above-mentioned *R. sherardii* var. *umbelliflora*. However, when *R. sherardii* and *R. villosa* were investigated with molecular markers, they were completely unseparable (Olsson et al. 2000) and therefore placed together in a *R. villosa* group. In a study by Debener et al. (1996) of genetic distances between rose genotypes, *R. villosa* and *R. sherardii* were placed closer together than was a pair of sibling cultivars of *R. hybrida*. There was also very little RAPD marker polymorphism between *R. sherardii* and *R. villosa* (7.6%), and very few *R. villosa* specific markers were found, indicating a close relationship between the genomes in these two species (Paper VI). *Rosa villosa* also shared all its analysed microsatellite alleles with *R. sherardii*, whereas the latter species had additional alleles as well (Paper VII). This may indicate that *R. sherardii* has originated from a hybridization between *R. villosa* (or a close relative) and another species, where *R. villosa* acted as seed parent.

Transmittal of character scores and markers

R. dumalis X *R. rubiginosa* and its reciprocal

The progeny groups of *R. dumalis* subsp. *dumalis* X *R. rubiginosa* and its reciprocal could be separated from each other with four out of seven morphological characters (Paper IV). Unfortunately, there were no progeny groups representing the parental species. Still, the morphological differences between the two offspring groups suggest a strong matroclinal (offspring very similar to seed parent) inheritance (Fig. 2). Gustafsson (1944) made a pair of reciprocal crosses between *R. rubiginosa* and *R. canina,* and reported indications of matroclinal inheritance. Kroon and Zeilinga (1974) reported from their crossings between Edelcaninas and different *Caninae* species, that the expression of the pollen parent is often obstructed by the heterogamic reproduction, leading to very matroclinal offspring.

When the progeny groups of *R. dumalis* and *R. rubiginosa* were studied with RAPD markers, pronounced matroclinal inheritance was indicated since all but one of the seed parent-specific markers were transmitted to all progeny plants. In

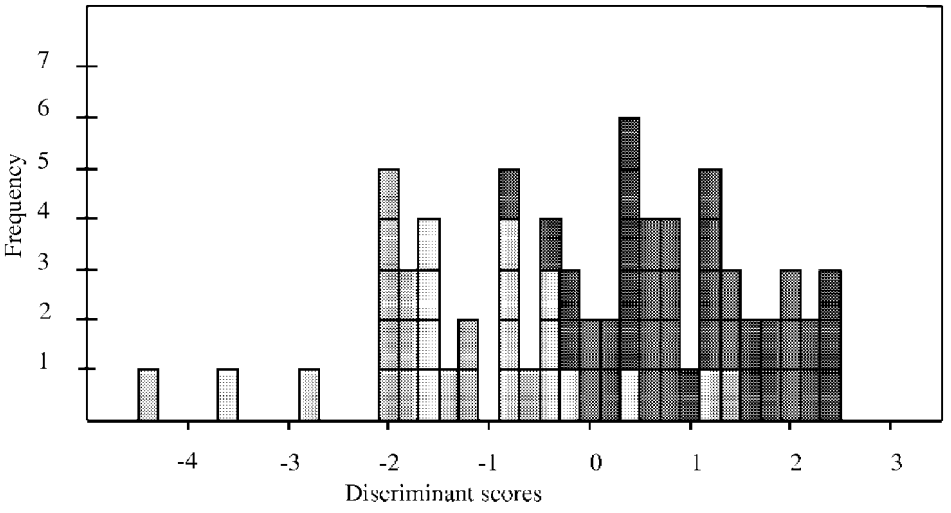

Fig. 2 Discriminant analysis calculated on seven morphological characters for discrimination between a pair of reciprocal crosses. *R. dumalis* X *R. rubiginosa*, *R. rubiginosa* X *R. dumalis*

contrast, only half of the pollen parent-specific markers were transmitted, and none of them reached all the progeny plants (Fig. 3).This means that the parental plants in the crosses between *R. dumalis* and *R. rubiginosa*, must have been heterozygous for these markers, i.e. the markers occurred in one to four of the genomes, but not in all five of them. The results from Paper VIII also indicate that two of the genomes in a pentaploid plant, never participated in the pollen meiosis, and that markers occurring on these genomes would never be transmitted to the progeny plants. There was also a difference in the number of pollen-specific markers each progeny group received; *R. dumalis* X *R. rubiginosa* offspring received an average of 3.2 markers, whereas *R. rubiginosa* X *R. dumalis* offspring received an average of 2.7 markers. Even in an interspecific cross, *R. dumalis* apparently gives rise to more heterogeneous offspring than does *R. rubiginosa*.

When these progeny groups were studied with microsatellite markers, the matroclinal inheritance was even more pronounced, since none of the alleles specific for the pollen parent were transmitted to the ten analysed *R. dumalis* X *R. rubiginosa* progenies (Paper VII). In the reciprocal cross only one allele from the most polymorphic of the analysed loci, was transmitted to six of the ten progeny plants.

The progeny plants of both *R. dumalis* X *R. rubiginosa* and its reciprocal as well as the parental plants were studied for male and female fertility in the form of pollen viability and fruit characters (Paper V). The pure species had a pollen viability of 20–30%, whereas most progeny plants had a pollen viability of <10%. Some progeny plants showed the same pollen viability as the pure species and were therefore assumed to be of apomictic origin. A further indication of apomixis

in these plants was that most of them lacked RAPD markers specific for the pollen parent. They were also separated from the other progeny plants in a CVA based on pollen and fruit characters.

The low pollen viability in the pure species indicates that the *canina* meiosis causes disturbances in the PMC, and these disturbances become even more pronounced in the interspecific hybrids. Both progeny groups produced a high amount of seeds and normal-sized hips, in contrast to previous reports of fewer seeds and greater amount of fruit flesh in hybrid plants (Gustafsson 1944, Halásová 1988). Maybe the high chromosome number in an EMC counterbalances the meiotic disturbances in the interspecific cross to a higher extent than in the PMC with its two genomes. This is also indicated in the "gynogenetic haploid" *R. canina* (Paper VIII). Pollen from this plant was completely sterile, whereas some maternal fertility still existed. Gustafsson (1944) found differences in fertility in his pair of reciprocal crosses of *R. canina* and *R. rubiginosa*. When the latter acted as seed parent, the progeny plants were fully fertile in contrast to the progeny plants from the reciprocal cross which were highly sterile. Jicinska (1976) used different species in section *Caninae* as seed parents and *R. rugosa* as pollen parent, and reported the resulting hybrids

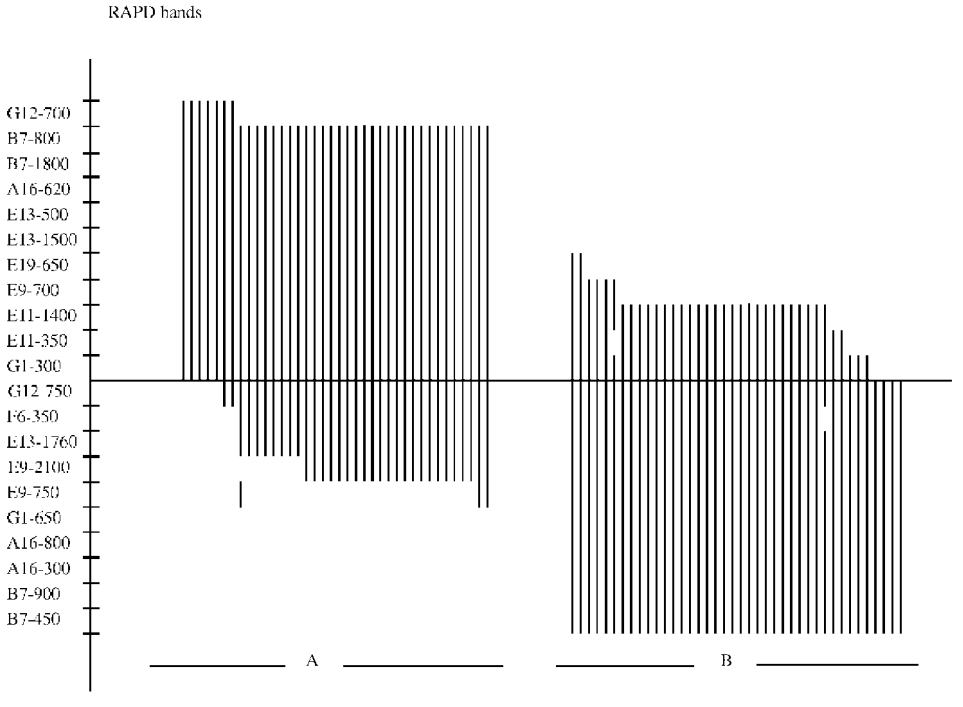

Fig. 3. Distribution of RAPD bands in interspecific progeny plants between *R. dumalis* and *R. rubiginosa*. The bands above the horisontal line are specific for *R. dumalis* and the bands below the line are specific for *R. rubiginosa*. Each vertical line represents one progeny plant. A = *R. dumalis* X *R. rubiginosa*, B = *R. rubiginosa* X *R. dumalis*.

in these inter-sectional crosses to be fully viable and fertile. This is in contrast to Gustafsson´s (1944) inter-sectional crosses between *R. canina* and *R. rugosa*, where the hybrids certainly were more vigorous than the seed parent, but had very few seeds. Fertility differences in the progeny, measured in amount of seeds, probably depends largely upon genome compatibility between the two parental species.

R. rubiginosa X *R. sherardii*

Progeny plants from the *R. rubiginosa* X *R. sherardii* var. *venusta* cross were assessed with reproductive and vegetative characters together with two progeny groups representing the parental species (Paper VI). The groups representing the pure species were obtained from a selfpollination of the same *R. rubiginosa* plant as used in the interspecific cross, and a within-population pollination of *R. sherardii*, unfortunatly from another population than the one involved in the cross. These two populations of *R. sherardii* had different ploidy levels, but showed very little variation in RAPD markers.

Both reproductive and vegetative characters separated the two parental groups. In a CVA based on reproductive characters, the hybrid group was placed adjacent to the *R. sherardii* group, whereas it was placed next to the *R. rubiginosa* group in the analysis based on vegetative characters.

Pronounced matroclinal inheritance of the RAPD markers was seen in this cross just as in the previously described reciprocal crosses between *R. dumalis* and *R. rubiginosa*. Thus all maternal markers were transmitted to all progeny plants. Half of the pollen-specific markers were transmitted to all but one of the progeny plants in this cross. *Rosa sherardii* must have been homozygous for these markers; i.e. the markers occured in all five genomes, or, alternatively, they occurred at least in the two genomes which paired in the pollen meiosis. Debener et al. (1997) made a cross between *R. obtusifolia* as seed parent and *R. sherardii* as pollen parent, where 90% of the pollen specific markers were transmitted to at least one of the five progeny plants. In combination with *R. obtusifolia*, *R. sherardii* appears to be more heterozygous.

Male fertility was studied in the *R. rubiginosa* X *R. sherardii* cross too, and here 34 out of 35 progeny plants had a very low pollen viability indicating a hybridogenous origin. Only one plant had the same pollen viability as the parental species, and was therefore assumed to be of apomictic origin. This plant also lacked all pollen-specific markers and was furthermore assigned to the *R. rubiginosa* group in a CVA based on the reproductive characters.

R. sherardii X *R. villosa* and its reciprocal

The reciprocal crosses between *R. sherardii* var. *venusta* and *R. villosa* were also compared with two progeny groups representing the parental species (Paper VI). Here, the parental species groups were obtained from a within-population pollina-

tion of *R. sherardii* plants, of which one was subsequently used in the reciprocal interspecific crosses, and a selfpollination of *R. villosa* from the same population as the plant used in the interspecific crosses. The two progeny groups representing the parental species were well separated with both reproductive and vegetative characters, although only 7.6% of the obtained RAPD markers separated the parental plants from each other. The Fourier coefficients also separated the two hybrid groups from the groups representing the parental species, whereas the reproductive characters were unable to separate *R. sherardii* X *R. villosa* from *R. sherardii*. A possible explanation for this difference in resolution between the two

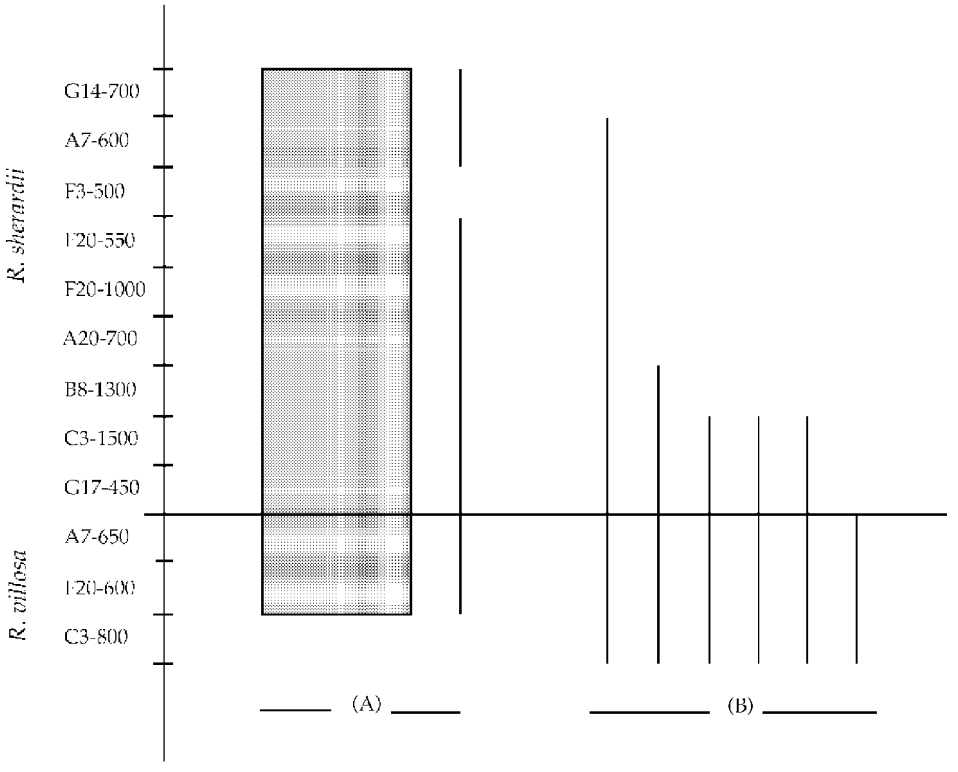

Fig. 4. Distribution of RAPD markers in progenies of (A) *R. sherardii* X *R. villosa* and (B) *R. villosa* X *R. sherardii*. The markers above the horizontal line are specific for *R. sherardii* and the markers below the line are specific for *R. villosa*. The rectangle in (A) represents 19 *R. sherardii* X *R. villosa* progeny plants with exactly the same RAPD markers and the vertical line represents the deviating progeny plant. The six vertical lines in (B) represent the six *R. villosa* X *R. sherardii* progeny plants.

characters sets could be the difference in number of variables. The reproductive character set had only five variables, whereas the Fourier coefficient data set consisted of 40 variables.

The same pronounced matroclinal inheritance of RAPD markers was apparent in these two reciprocal progeny groups as in the previously described crosses; all maternal markers were transmitted to all but one of the progeny plants. All *R. sherardii* X *R. villosa* progeny plants received two out of three paternal markers, in contrast to the *R. villosa* X *R. sherardii* progeny plants, which had a more uneven distribution of paternal markers (Fig. 4). One of the progeny plants from the latter cross never received any molecular markers from its pollen parent and was therefore assumed to be of apomictic origin. In a CVA based on reproductive characters, this plant was placed close to the progeny group of *R. villosa* seedlings. Unfortunately, this plant was never assessed with vegetative characters due to powdery mildew, and it died before it could be checked for pollen viability. The analysed microsatellites markers showed a completely matroclinal inheritance in these two reciprocal crosses (Paper VII). None of the pollen specific markers were transmitted to the progeny plants.

Character and marker assessment

The morphological characters utilized in our investigations, have shown significant differences not only between species but also between the hybrid progeny groups and the progeny groups representing the parents. Reproductive characters, e.g. the ovary characters, are generally considered to be less influenced by the environment than are vegetative characters, probably because of a higher selection pressure. Therefore, reproductive characters are of higher taxonomic value. In accordance with this, the reproductive characters in Papers II and III (ovary and sepal characters), showed more than twice as much interspecific differentiation as did the vegetative characters (manually scored leaflet shape).

Several factors can bring about more or less matroclinal progeny plants in species with a regular meiosis. The seed parent may influence its offspring through the endosperm which contains more maternal than paternal material, through inheritance of plastids and mitochondria, and through phenotypic effects mediated by the environment. Several species also resemble the seed parent when they are juveniles, but change in morphology as they get older (Roach and Wulff 1987). These mentioned causes for matroclinal inheritance seldom show any deviation from the phenotype expected in Mendelian-inherited nuclear genes.

Accordingly, in studies of hybridogeneity in *Forsythia, Prunus* and *Rhododendron*, Melville (1960) could not find any evidence for the leaf shape being inherited from either parent. Rather, the offspring seemed to display a mixture of the parental features depending upon dominance effects.

In a review paper by Rieseberg and Ellstrand (1993), a surprisingly high number of different hybrids displayed transgressive characters. This was also seen within our dogrose crosses as all interspecific progeny plants had more glandular hairs on the ovary and pedicel than did either of the parental plants. Similarly, Blackhurst (1948) found a majority of the obtained hybrids to have more dense armature than either parent in crosses between *R. rubiginosa* as seed parent and various *Rosa* species as pollen parents. However, Gustafsson (1944) and Jicinska (1976) claimed that the shape and quantity of the prickles was patroclinally inherited. Leaf characters were reported to be maternally inherited (Gustafsson 1944, Jicinska 1976) and the results of Paper VI also point in this direction. Whether any characters are more prone to matroclinal or patroclinal inheritance in dogroses is questionable. Most probably, the levels as to which characters are displayed in hybrid offspring plants, depend mainly upon which species acts as seed parent and thereby delivers the major part of the genetic material.

The distribution of molecular markers can be expected to deviate from Mendelian inheritance in interspecific hybrids also when the parental species have a normal meiosis (Zamir and Tadmor 1986). Departures from Mendelian ratios can indicate linkage between the molecular markers and different distorting factors occurring before, during and after the actual meiosis. But the segregation distortion found in the dogroses is far greater than that previously reported in any species, and it is most likely a reflection of the *canina* meiosis. Actually, the skewed distribution of molecular markers in the present studies was rather expected since four of the five (or three of four in the tetraploids) genomes are inherited from the seed parent. Nevertheless, a 1:1 inheritance of paternal markers would be expected if one assumes that the same two genomes make up the bivalent formation in the paternal parent and the RAPD marker occurs in one of these genomes but not in the other. Still, all but three markers deviated from the expected 1:1 distribution ($p<0.001$) in offspring from the *R. dumalis* X *R. rubiginosa* cross and its reciprocal (Paper IV). This deviation also existed within the microsatellite markers, since only one of eight alleles was transmitted from the pollen parent to the progeny plants in this reciprocal cross (Paper VII).

When mitotic metaphases were studied in progeny from an interspecific *R. rubiginosa* X *R. sherardii* cross and in a pentaploid *R. canina* plant, five rDNA loci (NOR sites) were revealed, one for each genome as previously reported for the genus *Rosa* (Ma et al. 1997) (Paper VIII). There was a considerable size polymorphism among these loci, with one very large locus, one very small and presumably inactive locus, and three homologous intermediate-sized loci. When pollen meioisis of the *R. canina* plant was studied, it was ascertained that the largest and the smallest of these loci did not participate in the bivalent formation. It was, however, not possible to be certain which two of the remaining three loci pair to form bivalents. A tetraploid *R. canina*, derived through pollination with irradiated pollen and embryo rescue, showed that the bivalent formation in PMC failed without the presence of a male genome. This would then imply that the

pollen-transmitted genome in a normal pentaploid, is in some way predestined to participate in the bivalent formation.

Apomixis

In the study of a pair of reciprocal crosses between *R. dumalis* and *R. rubiginosa*, nine of the progeny plants (approximately 10%) did not receive any pollen parent-specific RAPD markers which was taken as an indication of apomixis (Paper IV). Also one plant in the *R. rubiginosa* X *R. sherardii* combination and one in the *R. villosa* X *R. sherardii* combination (Paper VI) lacked everyone of the *R. sherardii* pollen parent-specific markers. Therefore an extended study was initiated with more RAPD markers as well as analyses of pollen viability and seed characters (Paper V). Most apomictic species appear to have a hybrid origin and the pollen viability is reported to be very low due to irregular meiosis even if seed set is more or less normal (Asker and Jerling 1992, Czapik 1994). In contrast, interspecific tetraploid hybrids in pseudogamous *Rubus* species usually have a higher pollen viability than their respective parents (Gustafsson 1946, Nybom 1988).

The present study showed that the dogrose species normally have 20–30% pollen viability, whereas the true hybrids, i.e. plants which had received pollen parent-specific markers (PM plants = pollen parent-specific markers), had a pollen viability of <10%. All five *R. dumalis* X *R. rubiginosa* progenies which lacked pollen parent-specific markers (NPM plants = no pollen parent specific markers), had a pollen viability equal to both parents i.e. >20%. In contrast, two of the four *R. rubiginosa* X *R. dumalis* NPM progenies had an intermediate pollen viability of ca 14% and the other two plants had the same viability as the PM plants. When assessed with microsatellite DNA markers (Paper VII), all four plants showed the same pattern as with RAPD markers i.e. no transmittal of pollen parent-specific markers. The two *R. rubiginosa* X *R. dumalis* NPM plants with low pollen viability may not be of apomictic origin, but they seem to diverge in chromosomal distribution from the other PM plants in this combination.

Some characteristics, that are very typical of taxa with apomictic propagation, also agree with the characteristics of section *Caninae*. So far, almost all apomictic taxa have proved to be polyploid even if their sexual relatives are diploids (Asker and Jerling 1992). Apomictic plant groups are highly polymorphic and form numerous microspecies, which often make taxonomic treatments difficult and controversial (Czapik 1994). Apomicts are often found in marginal or peripheral habitats and apomictic reproduction is thus often encountered in weeds and colonising species, like *R. rubiginosa*. This species was introduced as an ornamental in Australia by the end of the 19th century and some 20 years later it was declared one of the worst weeds in New South Wales (Hatton 1989). It is therefore not so strange that many authors have believed the dogroses to be apomictic. Both molecular marker distribution and the variation found in pollen viability seem to indicate that facultative apomixis does occur in section *Caninae*. Its contribution to the already low intraspecific genetic variability is probably not substantial, since

a large part of the genomic constitution is already locked up in a permanent heterozygous condition (Grant 1971). Also, the presently studied plants of apomictic origin, derive from artificial interspecific crossings. So far, there are no reports in dogroses on to what extent apomixis occurs in nature, and whether it does occur at all when pollinations take place within a species.

The archesporal tissue in Rosaceous species is generally multicellular, enabling multiple embryo sacs to form simultaneously (Gustafsson 1946). Meiosis is often attempted, even in the apomictic species, and sometimes results in the successful formation of reduced embryo sacs. However, unreduced embryo sacs may subsequently develop, suppressing the possible reduced ones. A substantial role of pollen competition in limiting gene flow has been demonstrated in sexual species, where con-specific pollen shows faster pollen tube growth than pollen from other species (Arnold et al. 2000). In a competitive situation, con-specific pollen will therefore achieve a proportionally higher fertilization success than pollen from other species even though the species in question may be perfectly cross-compatible. In a facultatively apomictic species, con-specific pollination could therefore be expected to result in fertilization of reduced embryo-sacs whereas interspecific pollination might, to a higher extent, trigger the development of unreduced embryo-sacs. This hypothesis is in good accordance with the findings of Kroon & Zeilinga (1974), who report mainly sexual seed set after intraspecific pollination in dogroses as opposed to one third apomictically derived seedlings after interspecific pollination.

Conclusions

The section *Caninae* is well separated from other sections in the genus *Rosa*, indicated by both morphological characters and molecular markers (Grant 1971, Millan 1996). The species within the section are morphologically rather distinct (Papers II, III, VI), but recent studies with molecular markers have shown that some species overlap considerably with each other (Papers VI, VII, Olsson et al. 2000). The species also differ in the amount and partitioning of variation, with *R. dumalis* being the most heterogeneous and *R. rubiginosa* the most homogeneous species. *Rosa villosa* differs between populations, but is fairly homogeneous within each population.

When studying the progeny plants from interspecific crosses with molecular markers, it is very obvious that matroclinal inheritance plays a major part in differentiation, which is to be expected considering the *canina* meiois. Each progeny plant inherits the majority of its genetic material from its seed parent, and varying degrees of homology between the constituent genomes in the parents decide whether the genetic contribution of the pollen parent will be recognizable in the form of deviating morphological characters. A study of the pollen meiosis showed that two of the five genomes in a pentaploid species, do not participate in the bivalent formation (Paper VIII). It is also suggested that the pollen transmitted genome is in some way predestined to be involved in this formation.

Several authors have mentioned the abundance of dogrose hybrids in nature (Täckholm 1920, Melville 1975, Graham and Primavesi 1993), and the interspecific crosses made in these investigations show that most of the dogrose taxa can hybridize with each other. But because of differences in flowering phenology and perhaps also interspecific pollen competition, the species have different inclinations to hybridize in nature. The most homogeneous of the dogrose species, *R. rubiginosa*, is in full bloom a few days after the majority of the other *canina* species and therefore very little, if any, foreign pollen is available for hybridization.

When planning future collections for plant breeding purposes, the differences in variability among the dogrose species must be considered. To obtain maximum variability in *R. dumalis* it is necessary to collect several plants from several locations, whereas a few plants from a few locations will suffice for *R. rubiginosa*. *Rosa villosa* should be collected from several locations, but a few plants from each location is sufficient. The inheritance of specific characters, valuable in a future plant breeding program, will have to be assessed for each interspecific cross, since each species combination shows unique patterns in the transmittal of characters.

Recent advances in different molecular techniques will now, or in the near future, make it possible to further study the peculiar *canina* meiosis. With genome *in situ* hybridization, the hybridizing genomes may be identified and followed through the generations. More details on the inheritance of genomes could also be gained with more *Rosa* specific microsatellite loci primer pairs. Molecular techniques can also be used to ascertain the differences among the genomes, both within and between the species, and thus make it possible to establish relationships and study the evolution within the section.

Acknowledgements

To everybody´s surprise, not least my own, this thesis is completed. In the beginning, I thought it was an unreachable goal, like the stars in the sky, but now I am here! Of course, I could never ever have managed to reach this place in life all of my own, a lot of people have helped me with all the steps on the way. Therefore all these people deserve a heartfelt thank you, thank you, thank you and some more than others.

First and foremost, my supervisor, Hilde Nybom, who shoved me, kicking and screaming, forward. Thank you Hilde, I could never have managed this without you. Thank you also, Björn Salomon, assistant advisor, for hints and "outside comments". At the lab, Helena Persson has been a true support of help, advice, and supplier of firemen, thereby making the lab work a lot more agreeable. Also, Igor Bartish gave lots of tips concerning interpretation of RAPD bands and other technical stuff. Members of our small, but quite active rose group Ulrika Carlson-Nilsson and Madeleine Uggla always have lots of interesting debate going on all the time; thank you for taking time to talk things through, and of course for

collecting the material and making all the rose crosses on which this thesis depends. Åsa Olsson is also part of this rose group, although she is at the university of Lund. She deserves a special thank you for helping me with the automated image analysis. Yoong Lim and Andrew Leitch at Queen Mary and Westfield College, London University, deserve a lot of gratitude for letting me come and study the technique of painting chromosomes, and for taking such good care of me while I was there.

A lot of people have been involved in taking care of the prickly plants out on the fields and helping me with the measurements; thank you all. Bengt-Olof Bjurman always answered all hysterical screams when the computer did not want to do what I wanted it to do, thank you. Stefan Andersson at the university of Lund, has helped me a lot with different kinds of statistical advice, thank you. Kimmo Rumpunen also deserves a lot of gratitude for technical assistance at the last possible moments.

There are of course also people outside the academical world which have suported and cheered me on. Of these, I will just mention my father, who has always stood by me, no matter what has happened. Ett stort tack till min pappa för att du alltid finns där och stöttar mig, även om nyponens kärleksliv inte röner något större intresse från din sida. And of course there are also my very own canines; Mandis, Kevin and Norton, always with me.

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