Sexual Preferences Linked to Rose Taxonomy and Cytology

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Keywords: apomixis, breeding system, chromosome number, compatibility, meiosis, pollen, Rosa

Abstract
The genus Rosa is usually subdivided into four subgenera, the largest of these is subgenus Rosa with 10 sections. Most of the genetically analysed rose species appear to be sexual and diploid (2n = 14) or tetraploid (2n = 28) although there are a few triploid (2n = 21), hexaploid (2n = 42) and octaploid species (2n = 56). The diploid species are usually self-incompatible whereas the polyploids are self-fertile. Pollen stainability is usually high in all species with even ploidy levels, i.e. 2x, 4x or 6x. Rose species are usually sexual and have a regular meiosis but there is one deviating section, Caninae, which harbours the so-called dogroses. Most of these are 5x but there are some taxa with 4x and 6x. Only seven chromosomes (derived from seven bivalents) are transmitted through the pollen grains, whereas egg cells contain 21, 28 or 35 chromosomes (derived from seven bivalents and 14, 21 or 28 univalents) depending on the ploidy level. Apomixis occurs occasionally in the dogroses and genetic selfing is probably common since these taxa are self-fertile. Interspecific hybridization takes place spontaneously among rose species at all ploidy levels and is used as a potent tool in plant breeding. Information about compatibility, breeding system, pollen viability, chromosome number and inheritance is important for optimal utilization of crosses in rose breeding.

INTRODUCTION
The genus Rosa is distributed throughout the colder and temperate regions of the Northern hemisphere but also occurs in warmer areas in New Mexico (USA), Iraq, Ethiopia, Bengal and southern China. This large genus has caused considerable dispute among taxonomists, from the humble beginnings with a total of 10 species recognized in 1561 to later publications where almost every identifiable unit was given species rank resulting in many hundreds of species. According to Shepherd (1954), already Linnaeus realized some of the difficulties involved in rose taxonomy since he very succinctly stated that: ‘The species of Rosa are very difficult to determine and those who have seen few species can distinguish them more easily than those who have examined many’.

For many years, Rehder’s classification system (Rehder, 1940) was widely accepted and it is still used as a basis for more modern treatises (e.g. Wissemann, 2003). According to this system, the genus Rosa is divided into four subgenera; Hulthemia, Platyrhodon, Hesperhodos and Eurosa. The first three subgenera contain only one or two species each. By contrast, subgenus Rosa (Rosa is the correct name for the former subgenus Eurosa according to the nomenclatural code) contains a large number of species (approx. 120 species according to Rehder) that cover a wide range of genetic adaptations.

In addition to the systematics of wild rose species, there is also a horticultural classification of cultivated roses and some of their ancestors (Cairns, 2003). In the present contribution, we will mainly treat the wild species, with emphasis on taxonomy, cytology and ‘sexual preferences’.

TAXONOMY
Traditionally, characters used for defining rose species have been based on morphology, especially hip shape as well as presence and form of prickles, hairs and...
glands. In the notoriously problematic section *Caninae* (‘dogroses’), the commonly used characters appear to be closely correlated, resulting in two basic types, L-type and D-type, as well as several intermediate forms. Roses of the L-type exhibit a lax growth habit, deciduous sepals and a narrow stylar orifice (diameter below 1 mm) on their hips. Roses of the D-type show a dense and compact growth habit, have persistent sepals and a wide hip orifice. The co-occurrence of these character states has been a well-known fact among rose taxonomists for many years but the genetic background is still not known. Ritz and Wissemann (2003) report that these characters (growth habit, sepal behaviour and size of orifice) not only appear to be strongly linked but that they also may be inherited mainly from the paternal parent.

In the last two decades, various molecular marker-based methods have been applied for discrimination among taxa and assessment of genetic variability. These include DNA markers, isozymes and some secondary substances. Among the latter, polyphenolic compounds (e.g. flavonols and anthocyanins) as well as hydrolysable tannins have some discriminatory power but sensitivity to environmental influence is a serious drawback. Lately, very promising results have been reported with various DNA-based marker methods. This success stems mainly from the practically unlimited number of markers available and from the fact that they are not influenced by the environmental conditions where the plants grow.

For phylogenetic and phylogeographic analyses, DNA sequencing information from carefully identified genes (and especially their introns) is usually the preferred method. In roses, various sequences have been used (e.g. Matsumoto et al., 1998; Wissemann, 2002; Wu et al., 2001) but their interpretation was sometimes problematic (Ritz et al., 2005). In contrast, phenetic analyses, i.e. a representation of present-day patterns of similarity among taxa, are very amenable to various DNA fingerprinting methods (Nybom, 2003). Several smaller studies have been performed using RAPD, in which dendrograms constructed for intra- and interspecific analyses of 5-10 species have generally shown a good correlation with previous classifications based on morphology and caryology (Debener et al., 1996; Millán et al., 1996). In these analyses, samples belonging to the same species usually grouped together.

Jan et al. (1999) conducted a large RAPD-based phenetic analysis of 119 accessions, representing 36 species (comprising one species each from the subgenera *Platyrrhodon* and *Hesperhodos*, and 34 species from a total of 8 sections of subgenus *Eurosa*). They used 10 primers, which together yielded 213 polymorphic RAPD markers. All accessions could be distinguished, and almost all species were grouped into their respective sections by a cluster (UPGMA) analysis. Apart from the surprisingly isolated section *Bracteatae*, a major division occurred between Asian sections on the one hand and primarily North American sections on the other hand. It was suggested that the two subgenera *Platyrrhodon* and *Hesperhodos* should be placed within subgenus *Eurosa*, since they grouped together with the North American *Eurosa* sections *Carolinae* and *Cassiorhodon*.

Species in the taxonomically rather isolated section *Caninae* constitute a close-knit group with overall low levels of diversity when analysed together with samples from other sections (Debener et al., 1996; Millán et al., 1996). Within this section, taxa that are reasonably well distinguished using classical morphological characters or image analysis of leaf shape form fewer and larger groups when analysed with DNA markers (Olsson et al., 2000). These results suggest that overall genetic differentiation, as measured with DNA markers, may not be well reflected in a taxonomical system that is based on the manifestation of a small set of genes that only govern a few morphological traits.

**CYTOLOGY**

The basic chromosome number in roses is 7, and the chromosomes are generally small and almost metacentric. Polyploidy is common in *Rosa* and has pronounced effects on fertility and character inheritance. In addition, some rose taxa are characterized by a unique meiosis. Consequently, it is important to have some basic knowledge about the
cytology of the species we are interested in.

Chromosome Number

Large compilations of chromosomal counts in roses have been published by e.g. Rowley (1967) and Wissemann (2003). The few species in subgenus *Hulthemia*, *Hesperhodos* and *Platyrrhodon* are all diploid. In subgenus *Rosa*, the large section *Synstylae* contains almost exclusively diploids, as do also the small sections *Laevigatae* and *Bracteatae*. Diploids also dominate in the large section *Pimpinellifoliae* and the small sections *Carolinae* and *Banksianae*. The large section *Cinnamomeae* contains mostly 2x and 4x species, but there are also some 6x and 8x species. The small section *Indicae* contains three species, one of which (*R. chinensis*) has been denoted as 2x, 3x and 4x. Section *Gallicanae* (synonym *Rosa*) contains only one truly wild species, *R. gallica*, which is a tetraploid. Usually, a number of tetraploid (occasionally triploid) taxa described from cultivated material are also treated within this section. The taxonomically difficult section *Caninae* differs from all the others by containing mainly 5x taxa although some 4x and 6x taxa also occur.

Meiotic Behaviour

Most rose species are diploid and have a regular meiosis with 7 bivalents. Usually these are ring bivalents with one cross-over in each chromosome arm. In addition, there is a considerable number of polyploid species. These species are probably mainly allopolyploid since they also have regular bivalent formation at meiosis.

By contrast, the species in section *Caninae* are characterized by the peculiar *canina* meiosis described 80 years ago (Täckholm, 1922). Regardless of ploidy level (usually 5x, but some 4x and 6x taxa also occur), only 7 bivalents are formed in the first meiotic division. The remaining chromosomes occur exclusively as univalents. These univalents are not included in viable pollen grains, which contain only the 7 divided bivalent chromosomes. In contrast, all the univalents are transmitted to one of the daughter cells in the female meiosis, and are eventually included in the viable egg cells, which therefore contain 21, 28 or 35 chromosomes in the different ploidy levels. The resulting seedlings obtain the full chromosome number but only 15-25% of these chromosomes are inherited from the pollen parent, the rest is from the seed parent. To some extent, we may regard dogroses as diploidized (a highly regular bivalent formation takes place), yet the maternally inherited passenger genomes have probably retained much of the progenitor species since they have evolved mainly by mutation and selection instead of by sexual recombination.

In a set of recent dogrose studies (Nybom et al., 2004a; and manuscript submitted), 7 dogrose plants, representing five taxa, were analysed for microsatellite DNA variability at 12-20 loci. In the four pentaploid species *R. caesia*, *R. dumalis*, *R. sherardii* and *R. rubiginosa*, several microsatellite DNA loci were found to contain four simultaneously appearing alleles, but never five. Correspondingly, there were up to three but never four alleles in the loci of the tetraploid *R. villosa* subsp. *mollis*. In addition, numerous seedlings derived from interspecific cross-pollinations were analysed, some of which had the maximum number of alleles for that ploidy level. These analyses were carried out both qualitatively and quantitatively, taking relative ratios between allele peaks within the same genotype and locus into account (MAC-PR approach, Esselink et al., 2004; Nybom et al., 2004b). The results suggest that bivalent formation takes place mainly between chromosomes that carry identical alleles. In all likelihood, all four pentaploid species can therefore be regarded as having four different but homeologous genomes, one of which is diploid and three that are haploid. Bivalent formation takes place primarily between the two members of the diploid genome, whereas the three haploid genomes are transmitted only maternally. Correspondingly, tetraploid species appear to have one diploid and two haploid genomes. Alleles residing on the bivalent-forming genome relatively seldom occur on the other genomes, suggesting pronounced differentiation between biparentally and uniparentally inherited chromosomes.
Our proposed model for genomic configuration is corroborated by another study, in which the nucleolar organiser region in dogrose chromosomes was studied cytologically. Each of the five genomes in *R. canina* appears to have a single ribosomal DNA locus as evidenced by consistently finding five loci in FISH investigations (Lim et al., 2005). Two of these loci deviate morphologically by being either very small or very large. It is apparent that two of the seven bivalents formed in meiosis involve pairing between two of three homologues with 5S and 18S-5.8S-26S rDNA loci and between two of three homologues with only 5S rDNA.

The exact mechanism governing the *Canina* meiosis is still unknown, but cytological analysis (Lim et al., 2005) has shown that chromatid segregation takes place in the univalents in anaphase I. Normally chromatid segregation does not occur until anaphase II, as is also true for the bivalent-forming dogrose chromosomes. Some kind of genetically determined mechanism obviously distinguishes those chromosomes destined to participate in bivalent formation from those destined to be univalents.

**Pollen Viability**

In a large experimental study, Ueda and Akimoto (2001) analysed pollen viability (by staining with acetocarmine) in a wide range of species in subgenus *Rosa*. Species with even ploidy levels (i.e. 2x, 4x or 6x) generally proved to have at least 75% pollen stainability, suggesting that they have a regular meiosis and are fully fertile. The few exceptions consisted of the diploid species *R. mulliganii* (42%), *R. palustris* (64%) and *R. filipes* (70%), the tetraploid *R. multibracteata* (9%), *R. setipoda* (14%) and *R. arkansana* (53%), and the hexaploid *R. spaldingii* (4%). There were also three pentaploid species, all belonging to section *Caninae*, with only 30-45% pollen stainability.

In a review of previous pollen viability publications, the range for diploid species was 51-99%, sometimes with considerable variation also within species (Spethmann and Feuerhahn, 2003). Only three tetraploid species were listed; *R. gallica* with 19-50%, *R. pimpinellifolia* with 25-98% and *R. mollis* with 19-40%. The latter of these belongs to section *Caninae*. Five pentaploid species, all belonging to section *Caninae*, had 0-70%, and three hexaploid species (none of them belonging to section *Caninae*) had 7-100%.

When analysing species in section *Caninae* and their offspring, *R. dumalis* and *R. rubiginosa* (both 5x) were found to have 22 and 26% pollen stainability, respectively (Werlemark, 2000). By contrast, offspring obtained by interspecific crosses between these two species had only c. 5% pollen stainability. In another study, *R. rubiginosa* had 19-26% pollen stainability, *R. sherardii* (also 5x) had 24%, whereas offspring obtained from crossing these two species had only 2-9% (Werlemark and Nybom, 2001). Obviously, the aberrant canina meiosis results in comparatively low pollen viability in the ‘pure’ species. Presumably the low (or non-existant) pollen viability found in hybrids is due to breaking up of the strict bivalent formation between homologous genomes. Interestingly, seed set in the hybrid plants is still almost the same as in the parental species (Werlemark, 2000) suggesting that embryo sac formation is less sensitive than pollen grain formation.

**‘SEXUAL PREFERENCES’**

Matters pertaining to the reproduction of plant species, e.g. breeding system, appear to be most important for predicting amount and distribution of genetic variability in wild plants (Nybom, 2004). Choice of plant material for, e.g. ex situ preservation and/or utilization in plant breeding programs, should therefore always take mode of reproduction into account.

**Selfing vs. Outcrossing**

With their often large and spectacular flowers, most roses give the impression of being designated for crosspollination. Nevertheless, there are some species where pollination can take place within the still closed flower (Spethmann and Feuerhahn, 2003). In most species, pollination does, however, not occur until the flower has opened. Depending on the genetics of the species, pollination can then take place within the same
flower (strict selfing, autogamy), between flowers on the same plant (genetic selfing, geitonogamy) and/or between flowers on plants with different genotypes (outcrossing, allogamy). Ueda and Akimoto (2001) performed selfings and crosspollinations on numerous rose taxa. Apparently all diploid species are either completely self-sterile or have, at least, a seriously reduced seed set after selfing. By contrast, polyploid species appear to be, on the whole, fully self-fertile. The highest self-fertility (>75% seed set) was found in a hexaploid in section *Rosa*; *R. moyesii*, and in two pentaploids in section *Caninae*; *R. coriifolia* (synonym *R. caesia*) and *R. eglanteria* (synonym *R. rubiginosa*). A high fruit set (>75%) but somewhat lower seed set was noted for *R. spinosissima* (synonym *R. pimpinellifolia*, section *Pimpinellifoliae*) and *R. virginiana* (section *Carolinae*). Possibly, the function of the self-incompatibility system decreases in duplicated alleles of the higher ploidy levels in the genus *Rosa* (Ueda and Akimoto, 2001).

**Sexuality vs. Apomixis**

Most rose species appear to be fully sexual. However, there is evidence from morphological characterization and DNA markers that about 5-10% of the offspring obtained from interspecific crosses in dogroses (sect. *Caninae*) are formed by apomixis, i.e. seed set without prior fertilization (Nybom et al., 2004a; Werlemark et al., 1999; Werlemark, 2000; Werlemark and Nybom, 2001). Pollination is, however, necessary for triggering embryo development in these species, which are therefore termed pseudogamous. To what extent apomixis also occurs when pollination is taking place within the same taxon is not known, and considerably more difficult to determine, even with DNA markers. However, studies in the related genus *Rubus* have shown that the origin of the pollen can play a major role in determining whether there will be a regular sexual seed set or a parthenogenetic development of an unreduced eggcell (Werlemark and Nybom, 2003).

**Hybridization**

Spontaneous interspecific *Rosa* hybrids have often been reported and their parents have been identified from observations in the field and/or the herbarium. In reality, however, even diploid plant species, with equal gametic contributions from the two parents, do not produce perfectly intermediate offspring. When polyploid species are involved, the situation becomes even more complex. In dogroses, with their matroclinal inheritance, designation of a (recent) hybridogenous status for a particular dogrose plant, and identification of the parental species, must certainly be regarded as very tentative, at best.

Undoubtedly, however, hybridization can and does occur between many rose taxa, and interspecific hybridization is frequently used in plant breeding of commercial rose cultivars. This is especially true for entities at the tetraploid level where the resulting hybrids are easy to produce and have good vigour and fertility (Spethmann and Feuerhahn, 2003), although some multivalent formation often takes place in meiosis (Ma et al., 2000). Crosses between diploid species can also produce hybrids with regular bivalent formation (Ma et al., 2000) but these hybrids are often highly sterile, presumably due to crossing-over between incompletely homologous chromosomes during meiosis (Lewis and Basye, 1961). Crosses between different ploidy levels have been less successful due to developmental disorders between embryo and endosperm. This problem has seriously hampered the introduction of germplasm from the usually diploid wild species into the cultivated gene pool, which is almost exclusively tetraploid.

In rose breeding, the direction of crosses is important for several reasons. Diploid and pentaploid species generally are the best seed parents, also when crossed with species of different ploidy levels (Spethmann and Feuerhahn, 2003). In addition, hybrids between species at different ploidy levels are often more similar to the parent that has contributed the highest number of chromosomes. For crosses between dogrose species, the direction of the cross is also important when the species are at the same ploidy level, since

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matroclinal inheritance has been reported for many characters (Werlemark, 2000; Werlemark and Nybom, 2001). Directed crosses between dogroses on the one hand, and species in other sections on the other hand, are best conducted using the dogrose species as a seed parent and the other species (with higher pollen viability) as a pollen parent.

CONCLUDING REMARKS
Most rose species are diploid but there are several species with higher ploidy levels as well, especially tetraploids and, in section Caninae, also pentaploids. Species that belong to any of the other sections, have, in general, a regular meiosis, high seed and pollen fertility and they are cross-compatible with other taxa. In addition, diploid species are usually self-sterile whereas the polyploids are self-compatible. The deviating section Caninae contains mostly 5x species and is characterized by the canina meiosis with an uneven chromosomal contribution from the two parents. In addition, these species are self-fertile, sometimes apomictic, and have reduced pollen viability but a high seed set. When collecting germplasm and designing plant breeding programs, the ‘sexual preferences’ of the species should be considered to ensure that material with the desired amount of genetic variability is obtained and that crosses result in sufficient numbers of viable and fertile offspring.

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