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Studies of Intersectional Crosses between Pentaploid Dogrose Species (*Rosa* sect. *Caninae* L.) as Seed Parents and Tetraploid Garden Roses as Pollen Donors

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ABSTRACT

Intersectional crosses were performed between pentaploid dogrose species (*Rosa* sect. *Caninae*) as seed parents and different tetraploid garden roses as pollen parents. From 2810 crossings and with approximately 10% germination, 298 seedlings were obtained. Of these, more than 75% came from crosses with 10 different plants of *R. rubiginosa* as seed parents and either double flowering *Rosa hybrida* 'André Brichet' (cross 1) or the single flowering Canadian germplasm L83 (cross 2) as pollen parents. Selected seedlings from these two cross combinations together with their parents were used for a study with microsatellite markers. All studied seedlings obtained all alleles from their seed parents with two minor exceptions. In cross 1, 49% of the seedlings were hexaploid, and 51% were pentaploid, whereas in cross 2, 92% were hexaploid and only 8% were pentaploid. The pentaploid seedlings had not received any alleles from the pollen donor and were regarded as being of apomictic origin. The hexaploid seedlings had received two alleles from their pollen donor and were regarded as true hybrids. The alleles of the pollen donors appeared to segregate randomly to the seedlings, both the specific and the ones in common with the seed parents. As the 10 *R. rubiginosa* plants used as seed parents were extremely homogenous, the differences in rate of true, hexaploid hybrids versus pentaploid apomicts appears to be governed mainly by the pollen donor. Approximately half of the cross 1 hybrid seedlings had flowers with more than 5 petals, consistent with 'André Brichet' being simplex for the gene governing double flowers, but none of the plants from any cross combination showed recurrent flowering. All seedlings were more susceptible to leafspot caused by the fungi *Sphaceloma rosarum* and/or *Septoria rosae* compared to their seed parents, but there was no increase in infection of blackspot caused by *Diplocarpon rosae* and even a slight decrease in powdery mildew in cross 1. There were three times more rosehip fly larvae in rosehips from cross 2 vs. cross 1, probably due to differences in chemical composition of hips.

Keywords: leafspot, meiosis, microsatellites, morphology, rosehip fly

INTRODUCTION

Dogroses, i.e. rose species belonging to sect. *Caninae*, are sometimes grown as ornamentals in parks and semi-wilderness areas. The rosehips have been used and appreciated for their medicinal compounds since the Middle ages. Today, we know that these rosehips are extremely high in antioxidant content (Halvorsen *et al*. 2002), have the same level of lycopene as tomatoes (Böhm *et al*. 2003) and have anti-inflammatory properties (Winther *et al*. 2005). Plants from sect. *Caninae* have been used as rootstocks for ornamental roses since the 19th century and are still routinely used as rootstocks even though it has become increasingly common to grow rose cultivars on their own roots (De Vries 2003). The dogroses are generally regarded as being more vigorous and hardy than garden roses, and have sometimes been utilized in breeding programmes for ornamental purposes. Lord Penzance made several hybrids between *R. rubiginosa* L. and Bourbon roses at the end of the 19th century and developed a series of robust cultivars with single flowers and apple-scented leaves (a trait of *R. rubiginosa*), e.g. 'Anne of Geierstein' and 'Lady Penzance'. In the 1940s, Kordes, a German rose breeder, developed another series based on *R. rubiginosa* but this time crossed this species with different hybrid tea roses. The resulting plants had large, double flowers and the apple scent of the leaves had been removed. Well-known cultivars from this series are 'Fritz Nobis' and 'Goldbusch' (Gustavsson 1998).

A recently revived interest in the utilization of dogrose germplasm has been prompted by the search for improved tolerance to various fungal diseases. In dogroses, both blackspot and powdery mildew appear to be under polygenic control (Carlson-Nilsson and Uggla 2005; Uggla and Carlson-Nilsson 2005; Schwer *et al*. 2007), and the symptoms are often less serious when compared to roses in other sections (Carlson-Nilsson and Davidson 2006; Schwer *et al*. 2007). Another detrimental leafspot, caused by *Septoria rosae* Desmaz, occurs in some dogrose species like *R. canina* L., *R. tomentosa* Sm., *R. villosa* Herrm. and *R. rubiginosa*, whereas other dogroses lack symptoms altogether (Boerema 1963). In a Swedish study, *R. rubiginosa* had no symptoms although other species in the same field were heavily infested, suggesting a genetically controlled, perhaps monogenic resistance (Schwer *et al*. 2007).

Crosses have also been undertaken between dogroses and especially some section *Rosa* (=*Cinnamomeae*) cultivars with the aim to produce high-yielding plants for fruit production. The most well-known offspring from such crosses is the Pillnitzer Vitamin-Rose 'PiRo 3' which was developed at Pillnitz in Germany in the 1960s from a cross between *R. dumalis* Bechst. (sect. *Caninae*) and *R. pendulina* var. *salaevensis* Rapin (sect. *Rosa*). Major assets are good yielding capacity together with a high content of acids and soluble solids (Graf and Kreß 1996; Bundessortenamt 1999).

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Original Research Paper

Dogrose cytology

Before embarking on a breeding program involving dogrose species, there are some cytological peculiarities that must be taken into consideration. In contrast to most garden roses, dogroses are usually pentaploid although there are some taxa (or genotypes within taxa) that are tetraploid, hexaploid or even octaploid (Täckholm 1922; Wissemann 2003). Even more important is the fact that all dogroses are characterized by the peculiar *canina* meiosis described over 80 years ago (Täckholm 1922) and verified in several, more recent publications (e.g. Lim *et al*. 2005). Regardless of ploidy level, only 7 bivalents are formed in the first meiotic division while the remaining chromosomes occur as univalents. These univalents are not included in viable pollen grains, which instead contain only 7 chromosomes obtained from the bivalents. In contrast, all the univalents are transmitted to one of the daughter cells in the female meiosis, and are finally included in the viable egg cells.

Seven dogrose plants representing five taxa, together with numerous seedlings derived from interspecific hybridizations, were recently analysed for microsatellite DNA variability at 12-20 loci (Nybom *et al*. 2004, 2006). In the four pentaploid species, *R. caesia* Sm., *R. dumalis*, *R. sherardii* Davis and *R. rubiginosa*, these microsatellite DNA loci were found to contain a maximum of four simultaneously appearing alleles, but never five. Similarly, up to three but never four alleles were found in the tetraploid *R. mollis* Sm. By contrast, the maximum number of alleles for the ploidy level was found in many of the interspecific Caninae hybrid seedlings. These results suggest that bivalent formation in pure species takes place mainly between chromosomes that carry identical alleles. The pentaploid species can therefore be regarded as having two genomes which are identical and three genomes which are dissimilar. Bivalent formation takes place primarily between the two members of the diploid genome, whereas the three monoploid genomes are transmitted only maternally. Correspondingly, tetraploid species appear to have one diploid and two monoploid genomes. Alleles residing on the bivalent-forming genome relatively seldom occur on the other genomes, suggesting pronounced differentiation between biparentally and uniparentally inherited chromosomes.

Dogrose genetics

Dogroses behave as typical diploids if used as pollen parents since they produce monoploid pollen with one chromosome from each of the 7 bivalents (n=x=7). Unfortunately, pollen viability is generally quite low in dogroses, typically around 20-25% (Werlemark 2000). When used as seed parents, dogroses behave as polyploids since the egg cells have 21, 28 (most common) or 35 chromosomes (total chromosome number of sporophyte minus 7), and fertility is generally quite good. Directed crosses between dogroses and species or cultivars in other sections are therefore usually conducted with the dogrose species as a seed parent. Since all dogroses are highly self-fertile, flowers on designnated seed parents must be emasculated and bagged (Jicinska 1976a; Ueda and Akimota 2001).

Direction of the cross is very important since character inheritance is strongly affected by the unequal contributions of the two parents (Werlemark *et al*. 1999; Werlemark 2000; Werlemark and Nybom 2001). Observations of single traits have demonstrated matroclinal inheritance for, e.g., some cuticular wax characters (Wissemann *et al*. 2007), whereas intermediate or even paternal inheritance has been reported for other traits (Wissemann and Ritz 2007). Many of these results however need to be interpreted with great caution since they are based on only a few cross combinations, and often also on only a few offspring in each combination.

All crosses with section *Caninae* species, however, do not exclusively yield hybrid offspring. Both morphological characterization and DNA markers suggest that about 5-10% of the offspring obtained from interspecific crosses in dogroses are formed by apomixis, i.e. seed set without fertilization, although pollination still appears to be required for triggering embryo development (Werlemark *et al*. 1999; Werlemark 2000; Werlemark and Nybom 2001; Nybom *et al*. 2004, 2006).

Recently a set of crosses were conducted at Balsgård, Sweden, using dogroses as seed parents and garden roses as pollen parents in order to further study the effects of the canina meiosis on transmittal of genetic markers to the resulting offspring. This article presents germination rates and resulting ploidy levels of the first generation seedlings between dogroses (*R*. sect. *Caninae*) and ornamental garden roses, as well as their morphology, fungal and rosehip fly resistance.

MATERIALS AND METHODS

Plant material and experimental crosses

Dogrose plants originating from seed collections in Scandinavia and grown in an experimental field at Balsgård, southern Sweden, were used as seed parents. Since these plants started to bloom at the beginning of June 2003 before many garden roses, pollen from assumed tetraploid garden roses designated as pollen parents had to be obtained from abroad; Institute for Agricultural and Fisheries Research (ILVO) in Belgium ('André Brichet', 'Kanegem', 'Melglory', 'Melrose' and 'Marie-Louise Velge') and Meilland International in France ('La Sevillana'and 'Meidomonac'). By the end of the dogrose flowering period in 2003 (i.e. the beginning of July), the Canadian germplasm L83 (Svejda 1988) had started to flower at Balsgård and was used as one of the pollen donors for the late-flowering dogrose species *R. rubiginosa*.

In total, 2810 pollinations were performed; 1328 pollinations with 10 different *R. rubiginosa* plants as seed parents and 1428 pollinations with other dogrose species (*R. caesia*, *R. canina*, *R.*

Table 1 Cross combinations between pentaploid dogroses and tetraploid garden roses with number of hips and seeds, germination and resulting plants.

Seed parent	Pollen parent	No. crosses	No. hips	No. seeds	% germination	No. resulting plants
Rosa caesia	'André Brichet'	15	4	0	0	0
R. canina	'La Sevillana'	337	71	90	13	12
R. canina	'Melrose'	68	4	13	38	5
R. dumalis	'Kanegem'	153	4	9	11	1
R. dumalis	'Melrose'	63	8	8	13	1
R. mollis	'Melglory'	37	2	26	4	1
R. rubiginosa	'André Brichet'	838	166	493	26	128
R. rubiginosa	L83	490	212	1103	12	137
R. sherardii	'Kanegem'	105	1	0	0	0
R. sherardii	'Melrose'	155	6	21	0	0
R. sherardii	'Melglory'	112	9	59	7	4
R. sherardii	'Bonica'	249	49	141	4	5
R. sherardii	'Marie-Louise Velge'	132	8	10	0	0
R. subcollina	'Kanegem'	56	5	22	18	4
Total		2810	549	1995	10	298

dumalis, *R. mollis*, *R. sherardii* and *R. subcollina*) as seed parents (**Table 1**). The resulting seeds were collected in the autumn and sown in pots. Seeds were stratified for 12 weeks at +20°C and then 12 weeks at +4°C (Werlemark *et al*. 1995). In the spring of 2004, seeds began to germinate and the resulting seedlings were planted in an experimental field in a randomized design in the autumn of 2004. More than 75% of these seedlings were the result of crosses with *R. rubiginosa* as seed parent and either 'André Brichet' or L83 as the pollen parent.

Resulting seedlings

Observations of morphology, especially size and colour of flowers, were conducted in the seedling field in 2006 and 2007. All seedlings were also screened for symptoms of the fungal diseases blackspot (*Diplocarpon rosae* Wolf), powdery mildew (*Podosphaera pannosa* Wallr.:Fr.; syn. *Sphaerotheca pannosa* (Wall.:Fr.) Lév), rust (*Phragmidium* spp.), and leafspot (*Sphaceloma rosarum* Pass. Jenk. and/or *Septoria rosae*; both occur at Balsgård but cannot be unambiguously distinguished in the field) (Schwer *et al*. 2007) in the beginning of October, 2006. Each plant was divided in two levels; lower level from the ground to the middle of the plant and higher level from the middle to the top of the plant. Since all plants were young (planted in 2004) they had good foliage with leaves from the ground level and up. Both levels were rated as follows: A = free of disease, B = low occurrence (up to 20% infected foliage), C = moderate occurrence (21-50%), and D = severe occurrence (more than 51%). A plant could then have a low occurrence of infection on the lower part, i.e. B and a moderate occurrence on the upper part i.e. C, thus having a final rating of B/C. All the resulting scores were then put together in all possible combinations and transferred into a single numeric value between 0 and 9 according to a key (Schwer *et al*. 2007) where 0 denotes a plant completely free of symptoms, and 9 a highly infected plant. In 2007, the amount of rosehips on each seedling was estimated, where 0 denotes no rosehips at all on the plant, 1 denotes 1-10 rosehips and 2 more than 10 rosehips.

Forty-nine and 50 plants, from each of the two most productive cross combinations, i.e. *R. rubiginosa* as seed parent and either 'André Brichet' or L83 as a pollen parent, respectively, were selected randomly for genetic studies (see Microsatellite analysis below). In 2007, when the origination of these seedlings (true hybrids or apomictically derived) had been determined, seeds obtained from open pollination were collected by harvesting all rosehips from each of those seedlings that had been shown to be true hybrids. When the seeds were extracted from the rosehips, the number of rosehip fly (*Rhagoletis alternata* Fallén) larvae was counted. The extracted seeds were then sown and stratified (Werlemark *et al*. 1995), and germination was evaluated in the spring of 2008.

Microsatellite DNA analysis

The 10 *R. rubiginosa* plants (originating from Sweden, Denmark and Norway) used as seed parents, the pollen parents 'André Brichet' and L83, and 49 and 50 seedlings, respectively, from the two cross combinations (*R. rubiginosa* X 'André Brichet' and *R. rubiginosa* X L83) were analysed with microsatellite DNA primers. *Rosa rubiginosa* in all previous studies has been found to have very low intra-specific variation, and therefore these two cross combinations (*R. rubiginosa* X 'André Brichet' and *R. rubiginosa* X L83) were regarded as only two crosses, even though 10 different *R. rubiginosa* plants were used as seed parents. DNA was extracted from the leaves with a QIAGEN Dneasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions and kept in +4°C until amplifications. A set of 10 microsatellite loci were chosen from Esselink *et al*. (2003) representing different linkage groups on the genetic rose map (**Table 2**). These markers have previously been shown to be highly polymorphic in rose species belonging to section *Caninae* as well as in garden roses. The forward primers were fluorescently labelled with FAM, HEX and NED (Applied Biosystems, Darmstadt, Germany) and used in three multiplexes of 3, 3 and 4 primer pairs. Sequence tagged microsatellite (STMS) amplifications were performed in 20 µl reactions containing 1X buffer solution IV (Advanced Biotechnologies; Columbia, Maryland), 1.5 mM $MgCl_2$, 0.1 mM dNTP (Roche), 1-4 pmol of each primer, approximately 15 ng of genomic DNA and 0.2 U of *Taq* polymerase (Advanced Biotechnologies). The PCR program was run on a Thermo Electron PX2 thermal cycler and consisted of an initial denaturation step at 94°C for 3 min, followed by 30 cycles of 94°C for 30 sec, 50°C for 30 sec and 72°C for 2 min with a ramp time of 1°C/sec, and a final extension at 72°C for 3 min. The PCR products were separated and analysed on a 3730 DNA analyzer (Applied Biosystems). Sizes of the amplification products were calculated based on the internal standard ROX500 (Applied Biosystems) with GeneMapper Software ver. 3.0 (Applied Biosystems).

Table 2 Linkage groups for the 10 microsatellite markers used and number of alleles per parent.

Locus	Linkage group[a]	No. alleles			
		Rosa rubiginosa	Other *Caninae* spp.	'André Brichet'	L83
RhB303	n.d.[b]	3	5	4	3
RhO517	1	2	3	4	3
RhP518	5	2	4	3	1
RhAB73	7	2	4	3	3
RhD221	4	3	4	2	3
RhEO506	2	4	7	3	3
RhAB40	4	2	4	3	2
RhD201	n.d.	4	6	1	2
RhE2b	6	4	5	3	4
RhP50	3	5	11	2	2
Total		31	53	28	26

[a] Debener *et al*. 2001; Esselink *et al*. 2003)
[b] n.d.= not determined

The Microsatellite DNA Allele Counting – Peak Ratios (MAC-PR) approach (Esselink *et al*. 2004; Nybom *et al*. 2004) was applied to determine the number of copies of each allele in each individual seedling. Basically, all alleles in each locus are analysed in pairwise combinations, and peak area ratios are calculated in all samples where alleles occur together. The peak ratios are then compared with the expected relationships between alleles in hypothetical configurations of the analysed locus. In this study, MAC-PR allowed us to, e.g., distinguish between pentaploid and hexaploid seedlings even in those cases where the hexaploid seedlings did not have more than five different alleles at any locus. Three attempts were made to determine ploidy level in the seedlings by flow cytometry, however without any conclusive data (results not reported).

RESULTS AND DISCUSSION

Seed set and germination in experimental crosses

The crosses were divided into three groups: 1). *R. rubiginosa* X 'André Brichet', 2). *R. rubiginosa* X L83 and 3). Crosses of other dogrose species than *R. rubiginosa* as seed parents with mostly other pollen parents than 'André Brichet' or L83 (**Table 1**). In group 1, 166 rosehips were produced from the pollination of 838 flowers resulting in 493 seeds, of which 26% germinated the following year. In group 2, 490 pollinated flowers produced 212 rosehips with a total of 1103 seeds and 12% germination. In group 3, 1467 pollinated flowers produced 167 rosehips with 418 seeds but only 8%, i.e. 33 seeds, germinated. Unfortunately, none of the dogrose species used as seed parents in this group were pollinated with either 'André Brichet' (except for 15 *R. caesia* flowers) or L83, so it is not possible to ascertain whether the pollen used of the other cultivars had poor viability or whether the combinations were mostly unsuccessful for other reasons.

Microsatellite DNA analyses of *R. rubiginosa* seed parents

The 10 *R. rubiginosa* plants used as seed parents in crosses with 'André Brichet' and L83 as pollen parents were analysed with 10 microsatellite loci (**Table 2**). The number of

different alleles could theoretically reach five for each locus and individual. Instead, however, we found only two to four alleles, as reported previously for *R. rubiginosa* and other pentaploid dogrose species (Nybom *et al*. 2004, 2006). Most likely, this is due to the fact that the bivalent-forming chromosomes are very similar, carrying the same microsatellite alleles.

In the present study, 8 of the 10 *R. rubiginosa* plants used as seed parents were identical in all 29 alleles obtained with the 10 primer pairs. Two plants deviated by having a 6 basepairs (bp) longer allele in locus RhP50, in addition to which one of the plants had lost two bp in one allele in locus RhB303. Both of these plants belonged to a Norwegian population, which had also been noted to deviate slightly in a study using leaflet shape and RAPD markers (Olsson 1999). Extremely low intraspecific genetic variation has been reported for Scandinavian *R. rubiginosa* samples in studies based on morphology and RAPD markers (Nybom *et al*. 1997; Olsson *et al*. 2000; Olsson and Prentice 2001).

The ratio between number of alleles found and the theoretically possible number of alleles was 0.62. This is comparable with Nybom *et al*. (2004) where 15 polymorphic loci in Nordic dogrose species were evaluated with microsatellite markers, yielding a ratio of 0.56 in *R. rubiginosa*, 0.64 in *R. dumalis*, 0.63 in *R. sherardii* and 0.60 in *R. mollis*.

Microsatellite DNA analyses of pollen parents

Two tetraploid pollen parents ('André Brichet' and L83) were analysed with 10 microsatellite loci (**Table 2**). The theoretical maximum, four alleles, was found for two loci in 'André Brichet'. In addition, one locus was completely homozygous (four copies of the same allele), two loci had two different alleles and five loci had three different alleles. In total, 28 different alleles were found in 'André Brichet'. The ratio between number of alleles found and the theoretically possible number of alleles was 0.70.

L83 similarly had one completely homozygous locus, three loci with two different alleles, five loci with three different alleles and one locus with four different alleles. The total number of alleles was 26, and the ratio between found and theoretically possible number of alleles was 0.65. A comparison of these ratios suggests that 'André Brichet' was the most heterozygous, followed by L83 and finally *R. rubiginosa*.

'André Brichet' and L83 shared 11 alleles with one another. Both of them also had 9 alleles in common with *R. rubiginosa*, but only four of these were the same between each other. These results suggest that genetic distances were approximately the same between all three parents.

Microsatellite DNA analyses of the seedlings

Forty-nine and 50 seedlings, respectively, obtained in crosses with *R. rubiginosa* as a seed parent and 'André Brichet' and L83 as pollen parents, were analysed for ploidy using 10 microsatellite loci. All alleles found in the seed parents appeared in each of the seedlings except in two cases; one seedling in the L83 cross had lost one allele in locus RhE2b, while one seedling in the 'André Brichet' cross had lost one allele in locus RhAB73. The (almost) complete transmittal of maternal alleles to all offspring plants has been noted also in other studies of dogrose species. In a RAPD-based study on a pair of reciprocal crosses between *R. dumalis* and *R. rubiginosa*, all markers found in the seed parent occurred in all the seedlings, with a single exception (Werlemark *et al*. 1999). In two microsatellite-based studies involving several different dogrose species, all markers found in the maternal parent were again present in all of the seedlings (Nybom *et al*. 2004, 2006). Of the five genomes in a pentaploid dogrose individual, the two genomes that form the bivalent formation appear to share the same set of microsatellite DNA alleles and are thus highly homozygous (Nybom *et al*. 2004, 2006). Consequently, all egg cells have essentially the same set of alleles, and differ from the chromosome constitution of somatic cells only by having one copy less of these homozygous genomes. Comparison of the microsatellite allele peak ratios (MAC-PR), suggests that pentaploid as well as hexaploid seedlings had been obtained in both cross combinations. All the suspected pentaploid seedlings had the exact same alleles and allele ratios as the seed parent and had not received any alleles from the pollen parent. There is a slight possibility of achieving tetraploid seedlings, i.e. plants originating from an unfertilized egg, but as the allele ratios did not differ from the ratios of the seed parents, this does not seem to be a likely event. It was therefore assumed that these seedlings had originated by apomixis as previously reported also in crosses between different dogrose species (Nybom *et al*. 2004, 2006). In the suspected hexaploid seedlings, some pollen parent-specific alleles were always present; apparently these seedlings had been produced by fertilization of a reduced egg cell ($4x$) with a diploid generative nucleus ($2x$) from the pollen. Sometimes it was possible to find 6 different alleles at one locus (especially in loci RhEO506, RhP50 and RhE2b).

In the present study, pollen parent-specific alleles as well as alleles in common with the seed parent had been transmitted to the hexaploid seedlings in various amounts and apparently randomly, demonstrating that the diploid pollen of 'André Brichet' and L83 is variable and heterozygous. This is in stark contrast to previous studies on interspecific crosses within the *Caninae* section, where only approximately half of the pollen-specific alleles were transmitted from the pollen donors to the seedlings, but these were, on the other hand, transmitted to virtually all seedlings (Werlemark *et al*. 1999; Nybom *et al*. 2004, 2006). Obviously, the monoploid pollen of dogrose species is quite genetically uniform.

In the progeny of *R. rubiginosa* with L83 as pollen parent, 46 of the 50 investigated seedlings (92%) received some alleles from L83 and were apparently hexaploid. The remaining 4 seedlings were likely pentaploid and of apomictic origin. By contrast, only 24 seedlings of 49 (49%) investigated in the 'André Brichet' progeny had pollen parent-specific alleles and were apparently hexaploid, whereas the remaining 25 plants were likely pentaploid and of apomictic origin. Since the *R. rubiginosa* plants were very similar according to the studied microsatellite loci, the vast difference in the production of apomictically derived offspring vs. sexually derived offspring must have been governed mainly by the pollen donor. 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 regular sexual seed set or parthenogenetic development of an unreduced egg cell (Werlemark and Nybom 2003).

Fertility of the hybridogenous seedlings

Some of the seedlings flowered in 2005, and the majority flowered in 2006 and 2007. Among the true hybrids, especially those sired by 'André Brichet', some plants never flowered and some that flowered did not develop rosehips (**Table 3**). Number of seeds in the rosehips harvested in 2007 was somewhat higher (1.9) in the hybrids sired by 'André Brichet' compared to the hybrids sired by L83 (1.4), but seed germination was very similar (6.5 and 6.2%, respectively). These values are very low compared to *R. rubiginosa* itself, which has previously been reported to have about 15 seeds/hip (Werlemark 2000) and 19% seed germination (Werlemark *et al*. 1995) when open-pollinated. The values are also lower than the results obtained for the first generation crosses in the present study when *R. rubiginosa* was used as a seed parent in crosses with garden roses and produced 3.0-5.2 seeds/hip and had 12-26% seed germination (**Table 1**).

Two, very similar genomes form bivalents in dogrose species. The loss of one of these genomes, and the addition of two very dissimilar genomes from the tetraploid pollen

Table 3 Disease score data (2006) and fertility (2007) for three rose progeny groups. For the disease score, the plants were divided approximately at the middle at the plant and the upper and lower parts were evaluated separately for infection from A=no infection to D=severe infection. The resulting scores were then transferred into numeric values according to Schwer *et al*. 2007

Progeny	2006						2007			
	No. plants	Blackspot	Mildew	Rust	Leafspot	Hips (0-2)[a]	No. plants	No. hips	Seed/hip	% germination
Rosa rubiginosa X 'André Brichet'	16	0.1	0.1	1.2	4.6	0.1	24	9.7	1.9	6.5
R. rubiginosa X L83	26	0.1	1.2	0	5	1	21	22.9	1.4	6.2
R. rubiginosa apomicts	24	0	1.3	0.2	2.2	1.6	–	–	–	–

[a] Amount of rosehips scored from 0 (no hips) to 2 (>10 hips).

parent, is expected to produce serious disruptions of the meiosis in hybridogenous seedlings. Some previous studies involving crosses between dogrose species and rose species from other sections have reported considerable reductions in seed set compared to in the parental plants (Hálásova 1988; Gustafsson 1944), while other studies have reported of no reduction (Jicinska 1976b). Possibly, pollen formation is more sensitive than seed formation to meiotic disruptions. Thus, Werlemark (2000) reported reduced pollen stainability in seedlings resulting from interspecific hybridizations within the *Caninae* section, whereas seed set remained the same as in seedlings derived by selfing of the parental plants. Pollen viability has not been checked in the hybrid seedlings of the present study but is expected to be very low.

An example of a possible outcome of a combination between a species from *R.* sect. *Caninae* and a more ornamental tetraploid rose is *Rosa* x *alba* L. (syn. Alba rose or *R. alba*). It is a hexaploid species, which is assumed to be a spontaneous cross between a *Caninae* spp. and *R. gallica* (Hurst 1941, Darlington 1963). Its hybridogenous heritage is shown by its very low pollen viability as it is said to show " a very large percentage of abnormal grains" (Cole 1917). In a more recent study by Ritz *et al*. (2005) of nuclear ribosomal DNA, the internal transcribed spacer ITS-1, which is located at each single nucleolus organizer region (NOR) per chromosome set in *Rosa* (Ma *et al*. 1997), was sequenced and compared with species from other sections of wild roses. As the dogroses are pentaploids, five NOR sites and subsequently five ITS-1 sequences are expected. The study showed that four of the five sequences were shared with roses from other sections, but one ITS sequence, what the authors call "the canina type" was unique for the dogroses and it also occurred in the Alba roses. The Alba roses are also placed in clusters adjacent with species from sect. *Caninae* and *Gallicanae* in several phylogenetic studies (Millan *et al*. 1996; Leus *et al*. 2004; Scariot *et al.* 2006; Bruneau *et al*. 2007).

Morphology of the seedlings

Rosa rubiginosa X 'André Brichet'

'André Brichet´ ('Melrose' X 'Ausmary', introduced in 2001) is a recent cultivar from Belgium with double, white to pinkish-white flowers. *Rosa rubiginosa* instead has single, dark pink flowers. Flower type, i.e. how many petals the flower has, is governed by a single gene with single flowers (4-8 petals) being recessive (Debener 2003). Twenty-eight of the 128 seedlings (22%) obtained from *R. rubiginosa* X 'André Brichet' had more than five petals. Half of these seedlings were, in all likelihood, derived by apomixes based on the microsatellite data of the 49 sampled plants of this population, thus raising the frequency of filled flowers (> 4-8 petals) to almost 50% for the hybridogenous seedlings. The majority of these plants had 10 petals, only one had more than 20 petals. This would imply that 'André Brichet' is heterozygous (in simplex form) for the gene that controls the number of petals. Several of the seedlings, both those with single flowers and those with more filled flowers, also had a lighter flower colour than *R. rubiginosa* (**Fig. 1**). Some seedlings showed considerable affinity to cultivated roses in several other traits, like fewer prickles and glandules, more waxy leaves without any scent, and a more compact growth habit than *R. rubiginosa*.

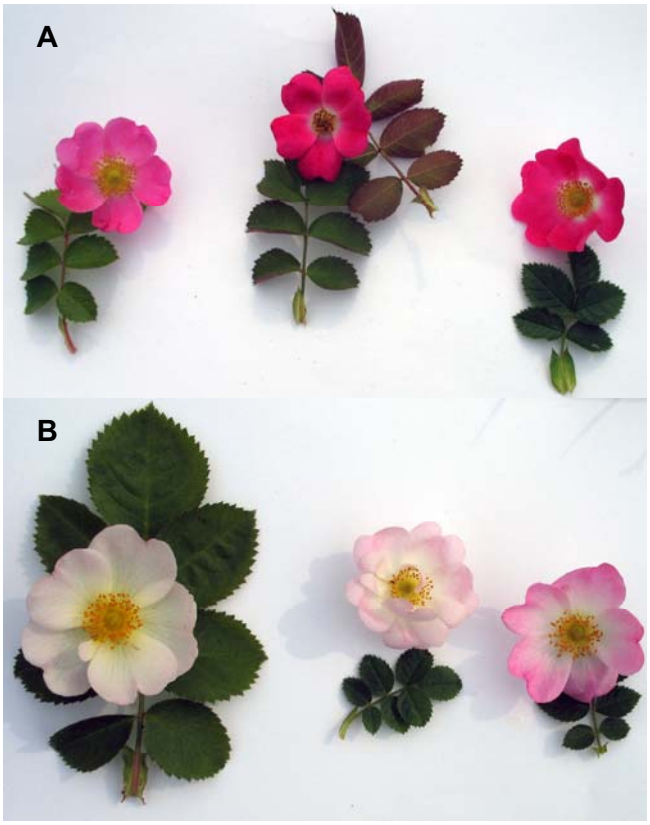

Fig. 1 (**A**) Flowers and leaves from representative seedlings of the *R. rubiginosa* X L83 population. (**B**) Flowers and leaves from representative seedlings of the *R. rubiginosa* X 'André Brichet' population.

All seedlings flowered at the same time, concurring with the flowering time of wild-growing dogroses. Some seedlings produced occasional flowers later in the summer, but this cannot be regarded as recurrent flowering since wild dogroses also produce a few flowers from time to time during summer. Since the highly desirable recurrent flowering is governed by a single recessive gene (Debener 2003), it will probably take at least one backcross and probably more to garden roses before it can be transferred into the dogrose hybrids.

Rosa rubiginosa X L83

L83 is a Canadian germplasm release, which has been used several times as a pollen parent at Balsgård with very good results. It is a large plant (2-3 m) with single, dark pink flowers, rather similar to those of *R. rubiginosa* but larger and with a deeper pink colour. One of the grandparents of L83, occurring on both sides of the pedigree, is 'Max Graf' which originated from a cross between *R. rugosa* Thunb. ex. Murray and *R. wichurana* Crépin (Svejda 1988).

In the cross combination with L83, none of the resulting seedlings had more than five petals, but sometimes the flowers had a darker colour than that of their seed parent (**Fig. 1**). It was however, difficult to distinguish the hybrid flowers from regular *R. rubiginosa* flowers. Also in this group, some seedlings were more similar to cultivated roses,

Fig. 2 Flowers from two hybrid plants of *R. sherardii* X 'Meidomonac' left and right, and a flower from *R. sherardii* for comparison in the middle.

with fewer prickles and more waxy leaves, while others instead resembled the parental *R. rubiginosa*. Just as in the previous cross combination, these seedlings flowered primarily once, but occasional flowers appeared throughout the summer.

Remaining dogrose X tetraploid garden rose combinations

Although almost 1500 pollinations were carried out in this group, only 33 plants were ultimately obtained. Two of these plants had more than five petals. The two most horticulturally valuable seedlings originated from the combination *R. sherardii* X 'Meidomonac', one with light pink flowers – lighter than both parental plants – and the other with dark pink flowers, and both having bright red filaments (**Fig. 2**).

Disease scorings in the seedlings

Average disease scores were calculated for three progeny groups: 1) *R. rubiginosa* X 'André Brichet', 2) *R. rubiginosa* X L83 and 3) apomictically derived offspring of *R. rubiginosa* (**Table 3**). Blackspot occurred very sparsely in all three groups, as did powdery mildew in the group sired by 'André Brichet', and rust in the group sired by L83 and the apomictic offspring of *R. rubiginosa*. Dogroses are usually superior to garden roses in tolerance to blackspot (Carlson-Nilsson and Davidson 2006). L83 was released as germplasm due to its high resistance to blackspot (Svejda 1988) and 'André Brichet' is marketed as having good resistance towards both blackspot and powdery mildew (www.bestselect.be/teksten_en/andrebrichet.html).

The only serious damage in our seedling field was caused by leafspot, and more so in the hybridogenous seedlings compared to those arisen by apomixis. *Rosa rubiginosa* had the highest leafspot tolerance in a study of five dogrose species at Balsgård (Schwer *et al*. 2007); obviously both pollen parents have had a negative influence on the hybridogenous seedlings for this trait. In addition, 'André Brichet' seems to have had a positive influence on mildew tolerance and a slightly negative influence on rust tolerance (**Table 3**).

Rosehip fly larvae in the seedlings

The rosehip fly deposits one egg per hip in immature rosehips and subsequently releases a pheromone that prevents other flies from laying eggs in the same hip (Bauer 1986). Inside the hip the larva develops, using all the material inside the hip as food. In October, the larva leaves its protective home for pupation and hibernation in the soil. These larvae can cause much damage and serious economical loss in commercial rosehip plantations.

Number of rosehip fly larvae was counted in all hips harvested from the hybridogenous seedlings obtained in two cross combinations: *R. rubiginosa* X L83 and *R. rubiginosa* X 'André Brichet'. Surprisingly, rosehips harvested on plants in the first progeny group were three times more seriously infested (0.43 larvae per hip) than rosehips harvested on plants in the second progeny group (0.15 larvae per hip) (**Table 4**). Explanation for this could be that 'André Brichet' contributed some sort of deterrent, or that L83 contributed some sort of attraction for the rosehip flies. Possibly, a study of the chemical composition in rosehips from these two cross combinations might help to identify compounds that are critical for the rosehip fly.

Table 4 Infection of rosehip fly larvae in rosehips of two cross combinations between pentaploid dogroses and tetraploid garden roses.

Cross	No. rosehip		
	No. hips	fly larvae	% with larvae
Rosa rubiginosa X 'André Brichet'	232	35	15
R. rubiginosa X L83	481	207	43

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