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ISSR (Inter Simple Sequence Repeat) markers reveal natural intersectional hybridization in wild roses [*Rosa* L., sect. Caninae (DC.) Ser. and sect. Cinnamomeae (DC.) Ser.]

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Summary: The genus *Rosa* comprises about 200–300 species worldwide with many critical taxa, especially among European members of the section Caninae. Available data on breeding systems and frequency of spontaneous interspecific hybridization are insufficient and contradictory. Our results obtained from field crossing experiments and analyses of morphological and molecular (ISSR) marker distributions in a natural rose population in Volgograd province of Russia reveal spontaneous introgressive hybridization between *R. canina* (sect. Caninae) and *R. majalis* (sect. Cinnamomeae). *R. canina* acts most probably as the ovule parent and *R. majalis* as the pollen parent. The resulting putative F1 hybrids are, however, morphologically identical to *R. caesia*, yet another species of the Caninae section. The putative backcrosses are morphologically similar to *R. canina*, deviating from it in leaf pubescence only. *R. donetzica*, a critical taxon of the Cinnamomeae section, probably results from this hybridization as well, though our data are insufficient to make any firm conclusions.

Keywords: Rosaceae, *Rosa canina*, *Rosa majalis*, *Rosa caesia*, *Rosa donetzica*, genetics, ISSR, hybridization, crossing experiments

The genus *Rosa* L. is one of the taxonomically most complicated groups of vascular plants. Total number of rose species is assessed by modern taxonomists between 200 and 300 (Wissemann 2003), while the total number of described infrageneric taxa of various rank excesses 4000 (the International Plant Names Index, http://www.ipni.org). Even regional treatments, especially those for the Eastern Europe, differ in assessments in tens of species (Dubovik et al. 1987; Buzunova 2001; Schanzer 2001). Several new rose species from the South of European Russia have been described recently (Buzunova & Grigorjevskaya 1994; Mironova 1994, 2002). Many of the morphotypes, including those described as species level taxa, are often treated as interspecific hybrids or notospecies or variously synonymized with each other (Klášterský 1968; Henker 2000; Buzunova 2001). To much extent this is due to the views of individual specialists on presence of apomixis and selfing in wild roses and frequency of interspecific hybridization within the genus.

Most of the European wild roses belong to the section Caninae (DC.) Ser. or dogroses, the taxonomically most complicated group in the genus. A whole range of investigations demonstrated the hybrid nature of species in this section (Blackburn & Harrison 1921; Fagerlind 1940; Zieliński 1986; Wissemann 2000, 2002; Ritz et al. 2005). All of the dogroses are polyploids, mostly odd, with x=7, 2n=35, and possess – unique among angiosperms – a breeding system. It can be generally characterized by an unequal meiosis in micro- and megaspore mother cells first revealed by Blackburn & Harrison (1921) and Täckholm (1920, 1922), and studied in more detail by Klášterská & Natarajan (1974a, 1974b), and Roberts (1975). In the case of pentaploids the unequal meiosis leads to formation of haploid (n=7) pollen and tetraploid

(4x=28) ovules and results in strongly matroclinal inheritance of many morphological characters (Gustafsson 1944; Werlemark et al. 1999; Werlemark & Nybom 2001; Ritz & Wissemann 2003). Such a breeding system, called 'balanced heterogamy' by Fagerlind (1940), lead many researchers to the opinion that apomixis (Täckholm 1922) or autogamy (Fagerlind 1951; Jičinská 1976) predominate among the members of the section Caninae. This, at least partly, explains the difficulties in species delimitation among dogroses, which are also characteristic for other apomicts and selfers.

However, numerous crossing experiments demonstrated the ability of many roses, including dogroses, to hybridize and giving rise to more or less fertile offsprings (Gustafsson 1944; Fagerlind 1951; Wissemann & Hellwig 1997; Ritz & Wissemann 2003). Recent experimental results show that autogamy and probably apomixis, though certainly present, play only a restricted role in dogrose propagation (Wissemann & Hellwig 1997; Werlemark et al. 1999; Werlemark 2000; Werlemark & Nybom 2001), while cross pollination predominates among species of this section (Wissemann & Hellwig 1997). Most of the studied species from other sections, including members of the section Cinnamomeae (DC.) Ser., proved to be self-incompatible and hence obligatory outcrossing (Jičinská 1975, 1976).

All of the above mentioned papers dealt with the species distributed in Western and Central Europe, and the entire crossing experiments were conducted in controlled environments of rose nurseries. However, the structure of natural wild rose populations and actual frequency of interspecific hybridization in nature remain poorly known so far, especially in Eastern Europe.

Hence the goals of our investigation include:

1) an assessment of breeding systems in a natural wild rose population;

2) an experimental proof of ability of different species to hybridization;

3) an attempt to reveal spontaneous interspecific hybridization in nature using morphological and molecular markers.

Material and methods

Location of the investigated population and plant sampling: As a model population we chose one in a gully nearby Strelnoshirokoye village about 90 km N of Volgograd (Volgograd province, Russia). The gully is about 5 km long and 0,8 km wide in the widest part (fig. 1). It covers about 4 km^2 and falls into Volga River. Slopes and bottom of the gully are covered with steppe vegetation and scrub, in places overgrazed by cattle. In the bottom, along a summer drying-up stream, there are small clumps of poplars. Wild rose bushes cover all slopes and the bottom of the gully. The gully is surrounded with steppe patches, wood plantations and abandoned fields, where wild roses occur only haphazardly.

We determined the sampled plants by using the 'Flora of East Europe' according to the taxonomic treatment of the genus suggested there (Buzunova 2001). Most of the specimens appeared to belong to the Caninae section: *Rosa canina* L. and *R. caesia* Smith. Some of the *R. canina* plants deviated in leaf pubescence towards *R. caesia*, so we marked them as putative hybrids *R. canina* × *caesia* (designated in tables as *R. hybr.*). Differential characters of the taxa are given in table 1. In the middle and upper parts of the gully we met 295 bushes of dogroses along a route of about

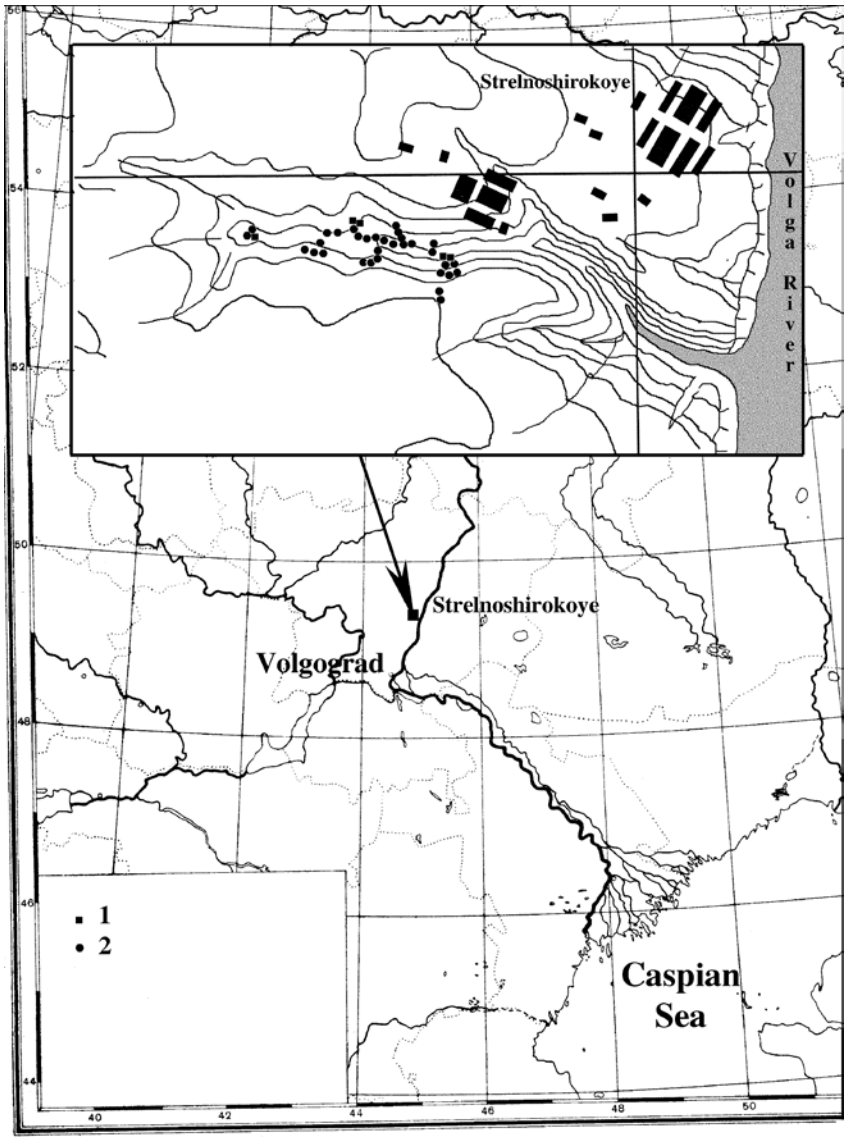

Figure 1: Geographic location of the wild rose population under investigation. 1) plants of the section Cinnamomeae; 2) plants of the section Caninae.

3 km long. Besides them there occur species of the section Cinnamomeae: *R. majalis* Herrm. and *R. donetzica* Dubovik. In contrast to dogroses, growing as compact bushes, these plants are capable of active vegetative propagation with underground xylorhizomes and usually form more or less diffuse thickets. We met 12 thickets of these roses, each covering 1–15 m^2, along the same route. The thickets are probably represented by separate clones or mixed groups of clones. All of the rose species in this locality are close to their geographic area limits.

38 plants were altogether selected for crossing experiments: 13 *R. canina*, 13 *R. caesia*, 6 *R. canina* × *caesia*, 5 *R. majalis*, and 1 *R. donetzica* (the only plant found in the population). The very same plants were studied in matter of their morphological characters and presence / absence of ISSR markers. From each of the plants used in crossing experiments two herbarium specimens were

Table 1: Differential morphological characters of the taxa under investigation. *R. hybr.* corresponds to putative hybrids *R. canina* × *R. caesia*.

character	*R. canina*	*R. hybr.*	*R. caesia*	*R. majalis*	*R. donetzica*
habit	compact bush	compact bush	compact bush	diffuse bush or thicket	diffuse bush or thicket
stem base armature	strong curved prickles	strong curved prickles	strong curved prickles	thin straight acicles	thin to strong straight acicles
petal colour	pale pink	pale pink	bright pink	bright pink to magenta	bright pink to magenta
sepals	pinnate	pinnate	pinnate	entire	entire
sepals at fruit	reflexed, deciduous	reflexed, deciduous	spreading to upwards directed, persistent to partly deciduous	upwards directed, persistent	upwards directed, persistent
glandular acicles on hip	absent	absent	absent	absent	present
leaf pubescence underneath	sparse along nerves	dense along nerves, loose on surface	dense along nerves and on surface	dense along nerves and on surface	dense along nerves and on surface

collected in flower and in fruit, in order to study the morphological characters. The voucher specimens are kept in MHA. 12 morphological characters studied are listed in table 2.

Experimental crossings were made in early June 2005 and 2006 prior to the start of rose mass blooming, right in the field, according to Wissemann & Hellwig (1997). Depending on the number of mature buds available on each plant, experiments were repeated one to three times as follows: 1) apomixis – isolation of an emasculated flower without pollination (64 flowers); 2) autogamy – isolation of a flower with sepals and petals removed (60 flowers); 3) geitonogamy – pollination of an emasculated flower with pollen from other flowers of the same plant (51

Table 2: Morphological characters used in the study.

Nr.	character	unit or states of qualitative characters
1	length of the terminal leaflet of a compound leaf	mm
2	terminal leaflet length/width ratio	
3	hip length	mm
4	hip length/width ratio	
5	pedicel length	mm
6	hip length/pedicel length ratio	
7	leaflet pubescence with simple hairs (above)	1 – glabrous; 2 – sparse; 3 – dense
8	leaflet pubescence with simple hairs (underneath)	1 – glabrous; 2 – sparse along nerves; 3 – sparse on surface; 4 – dense on surface
9	glandulous hairs on pedicels	1 – absent; 2 – present
10	glandulous acicles on hip	1 – absent; 2 – present
11	sepals of mature hip	1 – deciduous; 2 – persistent
12	petal colour	1 – pale pink; 2 – bright pink; 3 – magenta

flowers); 4) xenogamy – pollination of an emasculated flower with pollen from flowers of another plant of the same species (36 flowers); 5) interspecific crossing – pollination of an emasculated flower with pollen from flowers of another species plant (86 flowers). Total 297 flowers of 38 plants were used in crossing experiments. Close to maturation hips were collected in August 2005 and 2006. As a control we collected also 3–4 hips resulted from free pollination from each plant (164 fruits). In laboratory hips were opened and achenes and undeveloped ovaries counted.

DNA isolation and PCR conditions: DNA was extracted from fresh leaves stored in refrigerator using CTAB method (Doyle & Doyle 1987). ISSR (Inter Simple Sequence Repeat) markers were used to study DNA polymorphisms within and between species and to detect putative interspecific hybrids. These markers proved to be adequate for this purpose in other studies (e.g. Wolfe et al. 1998; Archibald et al. 2004; Sica et al. 2005) being less sensitive to PCR conditions than RAPDs (Bornet & Branchard 2001; Crawford & Mort 2004). Primers used for PCR were synthesized and purified in PAAG by Syntol Ltd. (Moscow, Russia). PCR was conducted in 20 µl aliquotes containing 4 µl of the Ready-to-Use PCR MasterMIX based on 'hot-start' SmarTaq DNA Polymerase (Dialat Ltd., Moscow, Russia), 14 µl of deionized water, 10 pmol of a primer, and about 10 ng of template DNA in Tercik thermocycler (DNA Technology Ltd., Moscow, Russia) under the following conditions:

94°C - 3 min., 46°C - 1 min., 72°C - 1 min. (2 cycles);
94°C - 30 sec., annealing temperature - 30 sec., 72°C - 1 min. (40 cycles);
94°C - 40 sec.; annealing temperature - 30 sec., 72°C - 3 min. (1 cycle).

Primer formulas and annealing temperatures are listed in table 3. Primers M4 and M10 were taken in higher (30 pmol) concentration to ensure better quality of the PCR. To assess the repeatability of the results we conducted PCR with each primer twice.

Amplification products were separated in 1.7% agarose (Amresco) gel in 1xTBE with ethidium bromide staining. Bands were counted by eye on digital photographs of the gels and afterwards analyzed in MS Excel. The results were converted into a binary matrix of band presence / absence.

Data analyses: Both morphological and ISSR band matrices were analyzed by using the non-metric multidimensional scaling (MDS) procedure as implemented in the PAST-programme (Hammer et al. 2001). The Gower distance measure (a range-normalized Manhattan distance) was used with the morphological data, the binary data of presence / absence of ISSR bands were analyzed by using Kulczynski-similarity-index (Hammer et al. 2007). The results of crossing experiments were analyzed in Statistica for Windows 6.0 (StatSoft, Inc. 2001).

Table 3: ISSR primers used for PCR.

primer		annealing t, °C
UBC881	GGG TGG GGT GGG GTG	54
UBC868	GAA GAA GAA GAA GAA GAA	48
UBC855	ACA CAC ACA CAC ACA CCY T	50
UBC840	GAG AGA GAG AGA GAG AAY T	50
M4	AGA GAG AGA GAG AGA G(C/T)C	58
M10	CAC ACA CAC ACA (A/G)G	48

Results

The results of crossing experiments are shown in table 4. As one can see from the 'Min' column of the table, in many cases (totally 114) no achenes developed at all. This includes all the experiments for detection of apomixis in all of the species, self pollination (both auto- and geitonogamy) in *R. majalis* and *R. donetzica*, and *R. majalis* × *R. majalis* intraspecific crosses. In those cases when achenes were set after artificial (totally 97 cases) or free (totally 164 cases) pollination the following trends could be observed:

1) The results of free pollination in *R. canina*, *R. caesia* and their putative hybrids yield on average more achenes than either autogamous, geitonogamous or strictly xenogamous pollination.

2) Xenogamous pollination in *R. canina* yields on average more achenes than geitonogamous or autogamous. Geitonogamous pollination yields more achenes than autogamous. However, the difference in all the cases is not statistically significant due to much variation.

3) The results of free pollination in *R. canina*, *R. caesia* and their putative hybrids vary significantly in achenes yielding from 0 to more than 90%.

4) Free pollination in *R. majalis* and *R. donetzica* yields a much smaller per cent of achenes (up to 26.9% maximum) than among Canina roses (up to 93.3%).

Table 4: Achene set (%) under auto-, geitono-, xenogamy and free pollination conditions.

species	cross	N	Mean % set	Std. Err.	Min % set	Max % set
R. canina	apo	27	0.00000		0.00000	0.00000
R. canina	auto	24	12.12013	3.88690	0.00000	56.52174
R. canina	geit	20	17.23959	4.24242	0.00000	61.53846
R. canina	× *canina*	10	23.51981	8.31025	0.00000	66.66667
R. canina	free pol	108	63.53220	2.02212	0.00000	93.33333
R. hybr.	apo	5	0.00000		0.00000	0.00000
R. hybr.	auto	5	2.04604	1.27621	0.00000	5.88235
R. hybr.	geit	3	27.01754	17.57541	0.00000	60.00000
R. hybr.	free pol	13	76.95947	1.98428	64.28571	90.62500
R. caesia	apo	21	0.00000		0.00000	0.00000
R. caesia	auto	19	1.36452	1.36452	0.00000	25.92593
R. caesia	geit	17	10.57875	5.73689	0.00000	66.66667
R. caesia	× *caesia*	17	7.20856	4.96896	0.00000	68.00000
R. caesia	free pol	33	46.63378	4.88120	2.63158	83.33333
R. donetzica	apo	2	0.00000		0.00000	0.00000
R. donetzica	auto	3	0.00000		0.00000	0.00000
R. donetzica	geit	3	0.00000		0.00000	0.00000
R. donetzica	free pol	5	15.54324	2.86043	12.00000	26.92308
R. majalis	apo	9	0.00000		0.00000	0.00000
R. majalis	auto	9	0.00000		0.00000	0.00000
R. majalis	geit	8	0.00000		0.00000	0.00000
R. majalis	× *majalis*	9	0.00000		0.00000	0.00000
R. majalis	free pol	5	11.01139	2.44058	3.22581	16.66667
All Groups		375				

Table 5: Achene set (%) in interspecific crosses.

pollen receiver	pollen donor	N	Mean % set	Std. Err.	Min % set	Max % set
R. canina	*R. caesia*	8	7.52926	4.91941	0.00000	40.00000
R. canina	*R. hybr.*	3	0.00000		0.00000	0.00000
R. canina	*R. majalis*	8	18.13596	11.89831	0.00000	76.66667
R. hybr.	*R. canina*	5	23.36996	14.71342	0.00000	69.23077
R. hybr.	*R. caesia*	3	0.00000		0.00000	0.00000
R. hybr.	*R. majalis*	3	0.00000		0.00000	0.00000
R. caesia	*R. canina*	15	11.69444	5.66195	0.00000	66.66667
R. caesia	*R. hybr.*	6	0.64103	0.64103	0.00000	3.84615
R. caesia	*R. majalis*	16	7.08862	4.02468	0.00000	52.38095
R. donetzica	*R. canina*	1	3.57143		3.57143	3.57143
R. majalis	*R. canina*	7	0.00000		0.00000	0.00000
R. majalis	*R. caesia*	9	4.51389	4.13763	0.00000	37.50000
R. majalis	*R. hybr.*	2	0.00000		0.00000	0.00000
All Groups		86				

The results of interspecific crosses are listed in table 5. Interspecific crosses yield some seed set, which, however, varies considerably from 0 to 76.7% in some *R. canina* × *R. majalis* crosses. This enables us to conclude that spontaneous interspecific hybridization is at least possible. No seeds were set in any of the 7 *R. majalis* × *R. canina* crosses. However, this may also be due to flower damage during emasculation or the physiological condition of the plants (see discussion below). So we cannot completely exclude the possibility of successful pollination of *R. majalis* with *R. canina* pollen since the only cross *R. donetzica* × *R. canina* still yielded a few achenes.

The result of the MDS analysis of morphological characters shown in fig. 2 demonstrates that *R. canina* and *R. majalis* form two distinct groups in the plot. *R. caesia* and putative hybrids *R. canina* × *R. caesia* specimens tend to have an intermediate position, *R. caesia* grouping mostly closer to *R. majalis*, while the putative hybrids are grouping around *R. canina*. The specimen of *R. donetzica* occupies an intermediate position, too.

The result of the MDS of the binary matrix of presence / absence of ISSR bands is shown in fig. 3. *R. caesia* and putative hybrids *R. canina* × *R. caesia* are loosely clustered around *R. canina* specimens here, while *R. majalis* forms a separate group of its own. *R. donetzica* and several specimens of *R. caesia* are not intermediate between these groups as in the morphological character analysis, but strongly deviate from all of them.

The results of direct count of bands specific for each of the groups are shown in table 6. *R. canina* possesses totally 61 bands, including 12 specific bands absent in *R. majalis*. *R. majalis* on its part possesses totally 59 bands, including 10 specific bands absent in *R. canina*. *R. caesia*, however, possesses all the bands specific to both of the species mentioned above whilst having only one band either specific or shared with *R. donetzica* or *R. canina* × *R. caesia* putative hybrids. These specific or nearly specific bands are found only in three *R. caesia* specimens. All the other *R. caesia* specimens are completely lacking any specific bands. *R. donetzica* and *R. canina* × *R. caesia* putative hybrids are also completely devoid of specific bands. Apart of the uninformative bands common to all of the specimens, *R. donetzica* shares 3 specific bands with *R. majalis* and one specific band with *R. canina*. *R. canina* × *R. caesia* putative hybrids possess all of the 12 bands

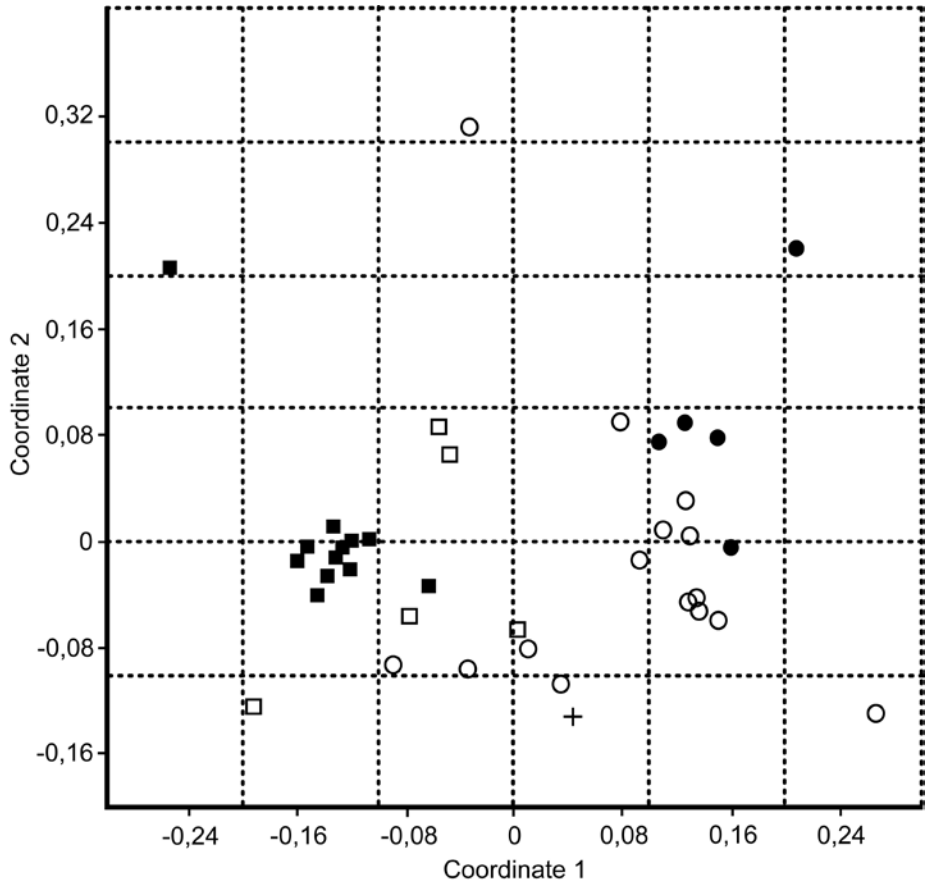

Figure 2: Non-metric multidimensional scaling (MDS) of morphological data (Gower distance). Filled squares – *R. canina*; filled circles – *R. majalis*; squares – *R. canina* × *R. caesia* putative hybrids; circles – *R. caesia*; cross – *R. donetzica*.

specific to *R. canina* and 4 bands specific to *R. majalis*. About half of the bands detected in *R. canina* (30 of 61) and *R. majalis* (27 of 59) appear to be polymorphic. In *R. caesia*, however, most of the bands (64 of 72) are polymorphic. Putative hybrids *R. canina* × *R. caesia* are intermediate in this sense having slightly more than a half (37 of 63) of the bands polymorphic.

Table 6: ISSR bands, found in *R. majalis*, *R. donetzica*, *R. caesia, R. canina* × *caesia* (*R. hybr.*), and *R. canina*. Specific markers are given in bold print. Figures in parentheses correspond to markers found in only a few plants (see in the text).

ISSR bands presence	***R. majalis***	***R. donetzica***	***R. caesia***	***R. hybr.***	***R. canina***
total bands	59	27	72	63	61
polymorphic	27	-	64	37	30
common	49	22	47	46	49
canina-specific	**0 (5)**	**1**	**12**	**12**	**12**
majalis-specific	**10**	**3**	**10**	**4**	**0 (6)**
specific	**10**	**0**	**1**	**0**	**12**
shared *hybr.*/*caesia*	-	-	**1**	**1**	-
shared *donetzica*/*caesia*	-	**1**	**1**	-	-

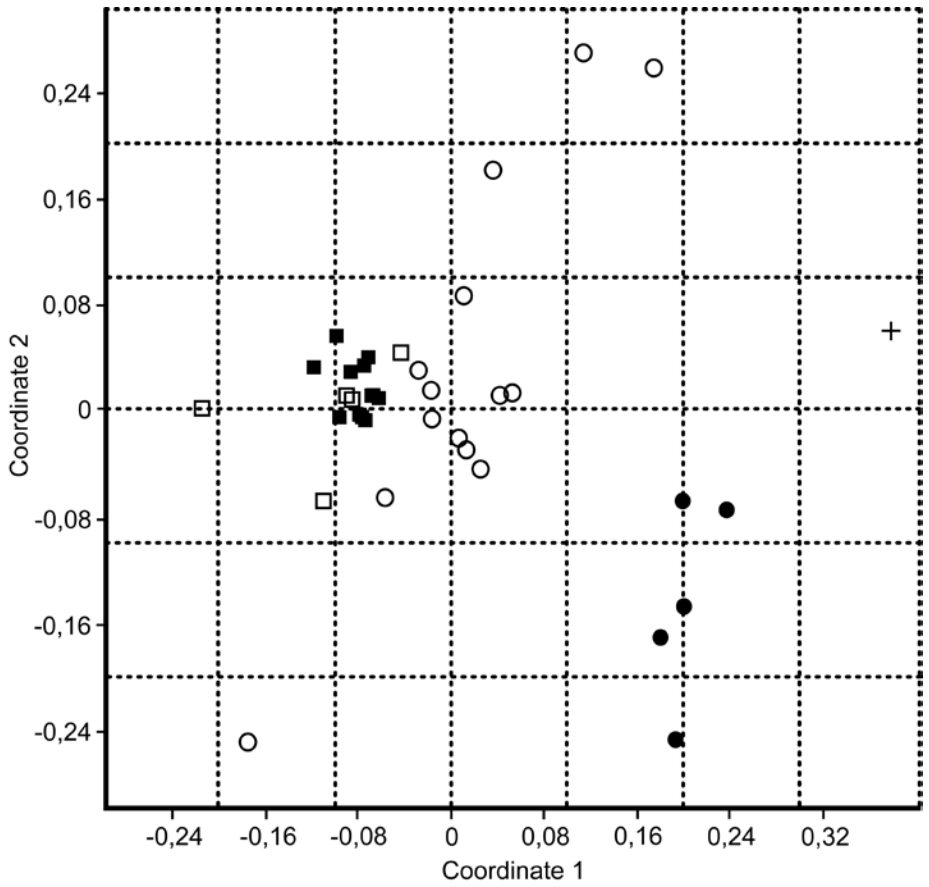

Figure 3: Non-metric multidimensional scaling (MDS) of ISSR bands absence / presence data (Kulczynski similarity). Filled squares – *R. canina*; filled circles – *R. majalis*; squares – *R. canina* × *R. caesia* putative hybrids; circles – *R. caesia*; cross – *R. donetzica*.

Discussion

Breeding systems in roses of the investigated population: The results of crossing experiments are not completely convincing due to many pollinations which failed to produce any seed. We suppose that this may be partly the result of the damage caused to flowers during emasculation. However, these failures may be due to the induced incompatibility (Fagerlind 1951) as well, since the experimental plants were growing in unequal environmental conditions and could be subjected to various physiological stresses leading to the 'newly set fruit drop'. Nevertheless, even qualitatively, some deductions can be drawn from the crossing experiments with a fair degree of confidence:

1) Apomictic set of achenes was not detected in the species of the population studied. In a number of other studies (Wissemann & Hellwig 1997; Werlemark et al. 1999; Werlemark 2000; Werlemark & Nybom 2001) achenes did set apomictically in species of the Caninae section, though always in a very low percentage. In any case, we may conclude that apomixis, even if sometimes present, is not the main mode of propagation in plants of our population.

2) According to our field observations, the studied plants of dogroses can hardly be specialized selfers because anthers were never detected open before anthesis. This corresponds to similar observations by Wissemann & Hellwig (1997) on blooming of Central European Canina roses. Autogamy can probably occur in already open flowers together with cross pollination or in the absence of the latter.

3) Auto- and geitonogamy usually result in a lesser number of achenes than cross pollination (xenogamy) within the same species in dogroses and their putative hybrids. Though not statistically proved, this result fully confirms Wissemann & Hellwig (1997). In normal roses (*R. majalis* and *R. donetzica*) autogamy and geitonogamy haven't lead to any seed set at all what confirms previous results on *R. majalis* being nearly obligatory outcrossing (Jičinská 1976; Schanzer 2006).

4) Free pollinations give consistently better results than artificial cross pollinations in the Caninae roses. We suppose this may be attributed to partial autogamy always present among dogroses and adding its share to the joint achene set. The results of free pollination in the self-incompatible Cinnamomeae roses always give worse results leading to a lesser achene set than in dogroses.

5) Interspecific crosses cause some seed set comparable to or lower than in crosses within the same species (tables 4 and 5). Similar results (seed set of more than 70%) were obtained by Fagerlind (1951) after pollination of *R. canina* with *R. majalis* pollen.

We may conclude from the crossing experiments that the population under investigation comprises plants capable of cross-pollination within the same species and between different species in different combinations. Autogamy (and probably apomixis) is only of restricted value among these plants and occurs among dogroses only. So there seems to be no serious objections to spontaneous interspecific hybridization between different species of *Rosa* in the given population.

Spontaneous interspecific hybridization: The analysis of ISSR bands distribution among the studied plants shows (see table 6) that only plants of *R. canina* and *R. majalis* consistently possess species specific bands (12 and 10 respectively). Plants morphologically identical to *R. caesia*, however, do not possess any species specific bands (with the exception of 3 specimens having one specific or nearly specific band shared with other hybrids only), but share all the 22 bands specific to both of the above species. We suppose that this may be only interpreted as they are *R. canina* × *R. majalis* interspecific hybrids, probably just F1 hybrids. It is worth mentioning that these plants also possess twice as more polymorphic ISSR bands comparing to their putative parents (see table 6), and demonstrate an average lower fertility than *R. canina* (table 4), what may be an additional proof of their hybrid status. The fact that the hybrids share all of the *R. majalis* specific bands may be indicative of *R. majalis* being the pollen parent. According to numerous chromosome counts available (e.g. Klášterská 1969; Małecka & Popek 1982, 1984) *R. majalis* is a diploid species (2n=14) with normal meiosis, and both of its genomes are transferred with pollen. *R. canina* is pentaploid (2n=35) with unequal Canina-meiosis, leading to only two of the five of its genomes being transferred with haploid pollen. If *R. canina* was the pollen parent, we could hardly expect finding all the *canina*-specific bands being present in the hybrids. The results of the crossing experiments (table 5) are consistent with this conclusion, too.

However, the presence of additional genomes in *R. canina* leads to a skewed distribution of molecular markers in hybrids. Indeed, as it can be seen in figure 4, the distribution of hybrid specimens in the MDS plot is strongly shifted towards *R. canina*. The distribution of morphological characters is different, however: the *R. caesia* plants are mostly more or less intermediate between the parents or even closer to *R. majalis* in reference to the characters included in the analysis, though they definitely belong to dogroses in their growth form and stem armature. In experimental crosses between the members of the Caninae section (Werlemark & Nybom 2001; Ritz & Wissemann 2003) matroclinal inheritance was much more pronounced, the F1 plants being identical to the maternal parents in such vegetative characters as leaf shape and pubescence. In the intersectional crosses with diploid *R. majalis* this is evidently not the case. Similarly, the F1 progeny of *R. canina* × *R. rugosa* L. crosses in Gustaffson's (1944) experiments demonstrated intermediate rather than matroclinal inheritance of morphological characters, too.

In matters of the plants initially supposed to be *R. canina* × *R. caesia* hybrids, they share all the 12 specific bands from *R. canina*, and only 4 specific bands from *R. majalis*, being mostly indistinguishable from *R. canina* in the MDS plot (fig. 3). We suppose they may truly represent the backcrosses. This means that the hybridization between *R. canina* and *R. majalis* in the investigated population does not stop at F1 but leads to an introgression between the two species. More thorough look at the specific ISSR band distributions (table 6) gives some additional evidence for the existence of such an introgression. Of the 49 bands common to both of the species, five bands (shown in parentheses) occur in all of the *R. canina* plants and not more than in one plant of *R. majalis*; and vice versa, six bands occur in all of the *R. majalis* plants and not more than in one or two plants of *R. canina*. From this evidence we conclude that *R. canina* and *R. majalis* introgressively hybridize in Volgograd province. The most interesting fact arising from this discovery is that some of the hybrid plants (probably the F1 plants) are morphologically indistinguishable from another dogrose species: *R. caesia*. Of course, we cannot state that *R. caesia*, a species described from England, is represented by such *R. canina* × *R. majalis* hybrids in the other parts of its geographic area.

The nature of *R. donetzica* represented in the study by the only plant cannot be established with any degree of certainty. Probably it is a product of hybridization between *R. majalis* and *R. canina* as well, since it shares a few specific ISSR bands with both of them and their putative F1 hybrid.

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