

# Self- and Cross-compatibility Relationships among Genotypes and between Ploidy of the Rose

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**Abstract.** The breeding of hybrid rose cultivars is part of the ornamental rose industry, but there have been few studies concerned with the factors limiting reproduction in the genus *Rosa* L. In this study, 28 species of *Rosa* were examined under field conditions for self- and cross-compatibility relationships. Twenty-five specimens of 25 species were tested for self-compatibility. All of the 23 diploid plants tested exhibited more than 96%. Twelve of the 16 polyploid plants preserved the 96% self-incompatibility reaction. A distinct separation existed between self-compatible and self-incompatible polyploid plants. Four of the 5 diploid cross-fertilizations produced unexpected reciprocal differences. Significant reciprocal differences were not obtained from the polyploid level cross-fertilizations. Some parthenocarpy occurred in the *R. stellata* species. Pollen viability and abundance, daily maximum and minimum temperature, and maximum and minimum relative humidity were not correlated with the compatibility reactions.

It has been assumed that species of the genus *Rosa* are self-compatible (4). Among the volumes of literature concerning the rose genus, only a few reports have been concerned with self-incompatibility (5, 9, 10, 12, 13, 15), but supportive data were largely absent and statistical analyses were not reported. Using no statistical analysis, Jicinska (12, 13) concluded that self-compatibility was present in some species and not in others. Inexplicably, Jicinska did not integrate levels of ploidy with this statement.

The genus *Rosa* is not dependent upon seed production for survival, because the species can increase vegetatively by numerous underground runners. Individual plants suspected of being extremely long-lived have been reported in the literature (20) and, although there has been no research on clonal age in the genus, it is not unreasonable to assume the age reached for a genotype could far exceed that of individuals within a clone. The combination of long life and vegetative reproduction places a high value on reproductive systems that assure genetic recombination (18).

Cross- and self-compatibility relationships are important to commercial rose hybridizing, because commercial cultivars are highly inbred. An increasing degree of relationship has been demonstrated to decrease seed-set in naturally outcrossing species (11). Therefore, the objectives of this study were to determine if a self-incompatibility system exists in the genus, and how ploidy level and genotype are related to such a system.

## Materials and Methods

The specimens examined in this study belong to the subgenera *Hesperhodos* and *Eurosa*. The ditypic *Hesperhodos* subgenera contributed 1 species while the large *Eurosa* subgenera contributed 27 species spread over 7 sections. Twenty-three diploids, 7 tetraploids, 3 pentaploids (all 3 belonging to the *Caninae* section) and 6 hexaploids plants were used. The *Caninae* have a well-documented system of heterogametic meiosis and have, with disagreement among researchers, been suspected of apomictic behavior (1, 8). Thirty-nine unopened flowers were emasculated on *R. eglanteria* as a test for apomixis. Seed-set on all

flowers not emasculated was assumed to be caused by sexual self-compatibility. Only self-pollinations could be evaluated for the single specimens of 24 species, but 4 other species were available in more than 1 genotype, which made cross-pollinations as well as self-pollinations possible among plants of those species. Specimens that were cross-pollinated originated from native sites in Colorado and New Mexico. Specimens either were dug or grown from seed collected at these sites. The closeness of their relationship is not known, although each is of a separate genotype.

All material was pollinated out-of-doors from March to July of 1983. Unopened, individual flowers were allowed to self-pollinate under a cloth bag secured by a wire. Unopened flowers

Table 1. Pollen abundance, pollen germination, and seed set following self- and cross-pollinations of 4 species of the genus *Rosa*.

Species specimen	Pollen		Compatibility	
	Abundance (class) <sup>z</sup>	Germination (%)	Selfed (%)	Crossed (%)
<i>R. Woodsii</i> Lindl.				
W1	4.0	55	0.0 <sup>y</sup>	65 <sup>y</sup>
W2	3.5	75	0.0	14
W3	4.0	65	2.5	77
W4	3.0	94	0.3	75
<i>R. stellata</i> Wooton subsp. <i>mirifica</i> (Greene) Lewis				
S1	1.0	64	1.4	84
S2	—	48	0.5	62
S3	4.0	36	3.7	97
<i>R. arkansana</i> Porter				
A1	4.0	83	0.0	65
A2	4.0	51	3.3	72
<i>R. × engelmanni</i> S. Wats. <sup>x</sup>				
E1	4.0	80	46.0	73
E2	4.0	55	0.0	58
E3	4.0	66	1.0	51
E4	4.0	35	0.0	32
E5	4.0	34	0.0	61

<sup>z</sup>Visual pollen index class. Zero = no visible pollen to 4 = abundant pollen visible.

<sup>y</sup>The difference in the averages between these two columns are all significant with the LSD being 10.3 at  $P = 0.05$ .

<sup>x</sup>It has been found that *R. acicularis* Lindl. and *R. nutkana* Presl. have formed a large natural hybrid population in the Rocky Mts. Rather than try to classify these specimens as belonging more to one species than the other, it was decided to use the hybrid designation as used by Dr. Erlanson (7).

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were removed from the plants, and anthers were allowed to dehisce for 24 to 48 hr before use in cross-pollinations. If the flower was to be cross-pollinated, petals and anthers were removed and pollen of another genotype immediately was placed on the stigmas. Flowers so treated were bagged in the same manner as those that were to self-pollinate. The self-pollinations were completed on 18 to 47 flowers per plant and cross-pollinations on 32 to 55, depending on the flower production of the individual plant over the flowering season. Each flower per plant was considered a replication. Instruments used for pollination were rinsed in alcohol and allowed to dry before reuse. After the hips were ripe, seeds were removed and tallied. Seed that was obviously shriveled or undersized was not counted. One seed is produced per pistil in this genus. However, there is some intraspecific variation in the average number of pistils per flower, and, therefore, the potential amount of seed that could be produced from each mating also varies. Thus, it was necessary to establish an average number of pistils and, therefore, potential seed production for each specimen to compare accurately the amount of self- or cross-compatibility between genotypes and polyploid levels. The pistil counts for 40 flowers were taken and averaged for each specimen, except for plants E5 and S3, where lack of material limited pistil count to 25 and 23 flowers, respectively. The average number of pistils per individual plant was multiplied by the number of flowers pollinated on each plant to arrive at a potential seed set. This figure was divided into the actual number of seeds produced and the result multiplied by 100 to give the percentage of fertilizers realized (self-compatibility percentage).

The contribution of inviable pollen to potential seed set was estimated by in vitro pollen germination tests. Freshly shed pollen was brushed onto a medium (20 g sucrose, 2 g agar, 100 ml distilled water) in petri dishes and incubated for 24 hr in the dark at 23°C. Two replications of not less than 200 grains were counted for each specimen used. Germination was considered successful if the pollen tube length exceeded the diameter of the pollen grain.

Pollen abundance was estimated by removing unopened petals from a flower and suspending the flower over black paper. After 48 hr at 22° to 25°C, the amount of pollen shed was graded visually into 5 classes. The classes ranged from no visible pollen, which was placed in the 0 class, to abundant pollen, which was placed in class 4. The study involved 2 replications with a separate flower used for each replication for each specimen.

All data were subjected to analysis of variance procedures with mean separation by LSD. Arcsin transformation was found to be unnecessary for percentage data. Simple coefficients were calculated to show relationships between self-compatibility and the following: pollen germination, pollen abundance, maximum and minimum temperature, and maximum and minimum humidity.

## Results and Discussion

*Species that were self- and cross-pollinated.* The diploid group of species had a collective potential fertility of 13,082 seeds when self-pollinated, but only 194 seeds were realized, for an overall 1.48% self-compatibility. Cross-pollinated potential diploid seed production was 23,991 seeds and 17,266 seeds were realized for 71.97% cross-compatibility. Therefore, the diploid group collectively was 50 times more cross-compatible than self-compatible. The potential seed production of polyploids was 9576 seeds when self-pollinated, and 594 seeds were obtained for a self-compatibility of 6.20%. When cross-pollinated, the

Table 2. Pollen abundance, germination, and self-compatibility for single specimens of the genus *Rosa* grouped by chromosome number.

Chromosome number species	Pollen abundance (class) <sup>z</sup>	Pollen germination (%)	Self-seed set (%)
Diploid (2n = 14)			
<i>R. bracteata</i> Wendl.	1.5	38	0.0
<i>R. naupaulensis</i> Andr.	4.0	26	0.0
<i>R. ecea</i> Ait.	2.0	24	0.0
<i>R. foliolosa</i> Nutt.	4.0	85	0.0
<i>R. forrestiana</i> Boulenger	---	58	0.0
<i>R. hugonis</i> Hemsl.	3.0	22	0.0
<i>R. laevigata</i> Michx. <sup>y</sup>	---	2	0.0
<i>R. moschata</i> Christ var. <i>nastarana</i> Rehd.	3.0	51	0.0
<i>R. nitida</i> Willd.	4.0	77	0.0
<i>R. primula</i> Boulenger	4.0	64	0.0
<i>R. rugosa</i> Thunb. <sup>x</sup>	4.0	75	0.0
<i>R. setigera</i> Michx.	3.0	1	0.0
<i>R. soulieana</i> Crep.	1.5	70	0.0
<i>R. wichuriana</i> Crep.	4.0	71	0.0
<i>R. wichuriana</i> Crep. 'hybrid' <sup>w</sup>	4.0	10	0.0
<i>R. xanthina</i> Lindl.	4.0	40	0.0
Tetraploids (2n = 28)			
<i>R. fedtschenkoana</i> Regel.	4.0	65	0.0
<i>R. foetida</i> Herm. var. <i>bicolor</i> (Jacq.) Willm <sup>z</sup>	---	2	0.0
<i>R. setipoda</i> Hemsl. & Wils.	---	86	0.0
<i>R. spinosissima</i> L. var. <i>altaica</i> (Willd.) Rehd.	4.0	58	0.0
<i>R. virginiana</i> Mill.	4.0	33	16.0
Pentaploids (2n = 35)			
<i>R. eglanteria</i> L.	---	6	16.6
<i>R. horrida</i> Fisch.	1.5	2	0.0
<i>R. laxa</i> Froeb.	---	10	45.0
Hexaploid (2n = 42)			
<i>R. xenglemannii</i> S. Wats. cultivar 'E6'	4.0	49	0.1

<sup>z</sup>Visual pollen index class. Zero = no visible pollen to 4 = abundant pollen visible.

<sup>y</sup>*R. setigera* is functionally dioecious (6). A comparison of the pollen grains of *R. setigera* with those of *R. laevigata* revealed that both exhibited the same kind of shriveling of the contents sometime after they have become morphologically mature.

<sup>x</sup>This is the *rugosa* cultivar 'Frau Dagmar Hastrup'. It is suspected that this rose is a selection of *R. rugosa* Thunb. var. *rosea* Rehd. rather than a hybrid.

<sup>w</sup>This rose was purchased as *R. leschenaultii* — a species of the *Synstylae* section, but it does not have pistils united in a column and therefore cannot be a member of that section. It is possibly a hybrid with *R. wichuriana*.

polyploid potential seed production was 10,085 seeds, and 5954 seeds were produced for a cross-compatibility of 59.04%. The polyploid group thus was 9 times more cross-compatible than self-compatible. The existence of a few self-compatible polyploid genotypes caused the polyploid class to be approximately 5 times more self-compatible than the diploid class.

Within each species, some variability among genotypes occurred in percentage of self-compatibility. The seed set ranged from 0% to 2.5% in the diploid *R. woodsii*, 0.5% to 3.7% in the diploid *R. stellata*, 0% to 3.3% in the tetraploid *R. arkansana*, and 0% to 46.0% in the hexaploid *R. × engelmannii* (Table 1). The high average self-compatibility found in *R. ×*

*engelmannii* resulted from the presence of specimen E1 which was the most self-compatible plant of all those tested and which was distinctly different in compatibility reaction than the other *R. × engelmannii* genotypes studied. Seed set in E1 following cross-pollination was only 1.6 times as great as seed set following self-pollination.

Pollen abundance among specimens that were selfed and crossed did not correlate with self-, female cross-, or male cross-fertility ( $r = 0.12$ ,  $r = -0.22$ , and  $r = -0.38$ , respectively). Pollen germination also did not correlate with self-, female cross-, or male cross-fertility ( $r = 0.27$ ,  $r = -0.01$ , and  $r = 0.12$ , respectively).

The relationships between daily average maximum and minimum temperature, and maximum and minimum relative humidity with self-compatibility were evaluated using 2 specimens, genotypes S1 and E1. S1 was chosen because of the extended time period pollinations were performed on that plant. E1 was chosen because it was the only polyploid plant tested that set seed over an extended time period. The self-pollinations of S1 were done during a 90-day period from 9 May 1983 to 5 July 1983. During this time, daily maximum temperatures ranged from 25° to 39°C, minimum temperatures ranged from 5° to 20°, maximum relative humidity ranged from 51% to 99%, and minimum relative humidity ranged from 10% to 32%. Maximum and minimum temperature and maximum and minimum relative humidity were not correlated with the seed set of specimen S1 ( $r = 0.18$ , 0.19, 0.01 and 0.13, respectively). Plants of E1 were self-pollinated over a 10-day period from 16 Apr. 1983 to 26 Apr. 1983. The range of maximum daily temperature during that period was from 20° to 28°, while maximum temperature ranged from 2° to 9°. Maximum relative humidity ranged from 57% to 96%, and minimum relative humidity ranged from 12% to 34%. Correlations between seed set and maximum and minimum temperature, and maximum and minimum relative humidity, were not significant ( $r = -0.11$ , 0.16, 0.02 and 0.17, respectively). In comparison, the correlation of maximum and minimum temperature, and maximum and minimum relative humidity, to cross seed set for E1 × E2 (done on the same day as the self-pollinations) were increased slightly but still not significant ( $r = 0.29$ , 0.30, 0.04, and 0.24, respectively). Thus, it was assumed that both self- and cross-compatibility were relatively stable across the environments that occurred during this study.

*Species that were self-pollinated only.* Additional species and specimens, available in only single genotypes, were evaluated for the same indices of fertility (excepting cross-compatibility), with results similar to those mentioned previously. *R. bracteata*, *R. ecea*, *R. soulieana*, and *R. horrida* were low in pollen production. All of the diploid single plants were totally self-incompatible (Table 2). One tetraploid (*R. virginiana*), 2 pentaploids (*R. eglanteria* and *R. laxa*) and 1 hexaploid (*R. × engelmannii* 'E6') exhibited some self-fertility. *R. laxa* was almost as self-compatible (45%) as the specimen E1 (46%) mentioned previously. Pollen abundance did not correlate with self-compatibility ( $r = 0.16$ ). Pollen germination varied from 1% to 86%, but there was no correlation of germination to self-compatibility ( $r = -0.11$ ).

*Higher ploidy levels.* Generally, the effectiveness of self-incompatibility systems decreases in the higher ploidy levels (16). Although the most self-compatible genotype (E1) was from the hexaploid group, it would be misleading to say a general decrease in self-incompatibility was evident at higher ploidy level because 5 of the 6 hexaploid plants exhibited 1% or less self-

Table 3. Reciprocal differences in cross-fertility among genotypes of the genus *Rosa*.

Species	Reciprocal cross <sup>z</sup>	Comptability percent
<i>R. woodsii</i> (diploid)	W1 x W2	76 <sup>y</sup>
	W2 x W1	14
	W1 x W3	53 <sup>y</sup>
	W3 x W1	76
	W1 x W4	64
	W4 x W1	76
	W2 x W3	14 <sup>y</sup>
	W3 x W2	79
	LSD (0.05)	12
<i>R. stellata</i> (diploid)	S1 x S2	86 <sup>y</sup>
	S2 x S1	62
	S1 x S3	82 <sup>y</sup>
	S3 x S1	97
	LSD (0.05)	13
<i>R. arkansana</i> (tetraploid)	A1 x A2	65
	A2 x A1	72
	LSD (0.05)	8
<i>R. × englemanni</i> (hexaploid)	E1 x E2	73
	E2 x E1	58
	E3 x E4	48
	E4 x E3	32
	E3 x E5	53
	E5 x E3	60
	LSD (0.05)	17

<sup>z</sup>Female parent always listed first.

<sup>y</sup>Reciprocal difference significant at  $P = 0.05$ ; others nonsignificant.

compatibility and only (*R. virginiana*) of the tetraploid specimens exhibited more than 4% self-compatibility. The preservation of self-compatibility by most of the polyploid genotypes is contrary to the studies of Jicinska (12) where the polyploids appeared to be more self-compatible than was found in this study. The Jicinska studies, however, counted only hip formation as an indication of self-compatibility, and those studies used many (18 out of 42 specimens) of the anomalous members of the *Caninae* section. The specimens of the *Caninae* section included in this study also produced a high percentage of what is probably sexually produced self-set seed. *R. horrida*, of the *Caninae* section, showed no self-compatibility, but it must be pointed out that this species shed little pollen, and the pollen germinated poorly. None of the 39 emasculated flowers of *R. eglanteria* produced any apomictic seed in this study.

*Genotype differences.* Two of the 7 diploid specimens that were both self- and cross-pollinated were completely self-incompatible while the other 5 exhibited less than 3.8% self-compatibility (Table 1). None of the 16 diploid plants that were single specimens (and therefore only self-pollinated) exhibited any self-compatibility (Table 2). Two of the 7 tetraploid genotypes exhibited self-compatibility but, as mentioned previously, only 1 exhibited more self-compatibility than was produced in the diploid group (Tables 1 and 2). It should be noted that *R. foetida bicolor* was one of the 5 self-incompatible tetraploids,

and this rose is known to have low pollen production (20). Microscopic examination of the pollen of *R. foetida bicolor* revealed many shrunken and angular grains. Three of the 6 hexaploid genotypes of *R. × engelmanni* specimens exhibited a degree of self-compatibility (Tables 1 and 2). The highly self-compatible E1 was mentioned previously, but the other 2 hexaploid (E3 and E6) self-fertilizations resulted in a small percentage (1% or less) of self-compatibility. Because of the low level of self-compatibility in the diploids, the self-compatibility differences of the genotypes were not as marked as were the self-compatibility genotype differences of the higher ploidy groups.

Reciprocal differences existed between genotypes, but only at the diploid level (Table 3). Reports concerning the loss of reciprocal differences at the polyploid level were not found in the literature. Because the diploid species *R. setigera* is functionally dioecious, there may be a relationship between a dioecious tendency and the reciprocal differences found in the diploid groups of *Rosa*. The unusually high percentage of male plants in a functionally dioecious species, as found in *R. setigera* [72.2% males, 19.4% females and 8.4% hermaphrodites (5)], may be the result of the inheritance of sex forms as found in *Silene otites*. *Silene otites* exhibits a high percentage of males in the population because of the function of the subandroecious plants (19). A system such as this might explain the difficulty of finding plants that are female fertile, which is a problem limiting cross combinations in commercial rose breeding.

Almost half the *R. stellata* S1 self-pollinations and an occasional self-pollination of the other 2 *stellata* genotypes resulted in the development of seedless hips. This seedlessness may have been the result of an ovarian reaction site with the incompatibility reaction occurring postzygotically. The genus *Rosa* carries hollow styles and wet stigmas, both characteristic of species with ovarian incompatibility sites (2). No seedless hips were found in the other rose species. *R. stellata* has been described as a primitive rose species, which could explain an ovarian reaction site for this species, in a reactive site phylogeny from postzygotic to stigmatic inhibition reaction (3, 14). Comments concerning the dubious distinction between self-incompatibility and inbreeding depression have been found in recent literature (17).

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