

The Inheritance of Juvenile Recurrence in *Rosa* Species Hybrids

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ABSTRACT

The current market for roses demands that new cultivars bloom throughout the growing season. The form of recurrence found in most modern roses can be termed juvenile recurrence, because bloom begins only months after germination. Previous studies have observed that juvenile recurrence is conferred by a recessive allele at a single locus. Most species in the genus *Rosa* have not yet been used to produce commercialized hybrids, in part because they carry the dominant allele for non-recurrence. Furthermore, only a few species have been tested for their effect on recurrence when used for hybridization. This study investigated the inheritance of juvenile recurrence by crossing several hardy tetraploid species and near-species hybrids with modern roses, and then backcrossing the resulting hybrids with modern roses to recover recurrence. For all species, and all but one first-generation hybrid, juvenile recurrence was recovered. This suggests that using non-recurrent species in a rose breeding program is feasible. The numbers of recurrent and non-recurrent second-generation backcross offspring produced by each first-generation hybrid varied. Some progeny groups did not differ significantly from the theoretically predicted 1 recurrent: 5 non-recurrent ratio, while the number of recurrent offspring was lower than predicted in the rest. The ability of some species and near-species hybrids to produce some late blooms did not affect the frequency of juvenile recurrence in the second generation.

Keywords: modern roses, *Rosa altaica*, *Rosa arkansana*, *Rosa pendulina*, *Rosa spinosissima*, *Rosa virginiana*

Abbreviations: F₁, first generation hybrid; M₁, modern rose crossed with F₁; M_p, modern rose parent; OP, open pollinated; R₂, second-generation backcross hybrid; S_p, species (or near-species hybrid) parent

INTRODUCTION

Juvenile recurrence in *Rosa*

When non-recurrent rose species and cultivars are grown in climates where they receive adequate vernalization, they produce a single flush of bloom in the late spring or early summer, giving them adequate time for ripening of fruit. In the strictest sense, any production of additional flowers after this first cycle constitutes recurrence. Most modern roses (for example, hybrid teas, floribundas, and miniatures) generally initiate their first flower bud within two months of germination (de Vries 1976; Zlesak 2006). These roses then continue to bloom whenever conditions are suitable for growth, either continuously or in repeated cycles. Although the juvenile period technically ends when a plant is capable of responding to flower-inducing stimuli, it is useful to call this bloom pattern juvenile recurrence. Today's market demands this kind of heavy recurrence in cut-flower, potted florist, and garden roses (Gudin 1999; Zlesak 2006). Additionally, juvenile recurrence can help rose breeders by allowing early selection of young seedlings for flower characteristics (Hess 2005).

The established model of juvenile recurrence

The investigation of inheritance in roses has generally proven difficult. Problems of seed-set and germination can make it a challenge to produce enough offspring to allow statistically valid conclusions, and this is exacerbated by polyploidy (Crespel *et al.* 2002). Nonetheless, the importance of juvenile recurrence has attracted the attention of rose geneticists. Rose breeders have long noted a tendency for crosses between recurrent and non-recurrent roses to produce only non-recurrent offspring (Shepherd 1954). Semeniuk (1971a, 1971b) was the first to investigate recurrence rigorously, using *Rosa wichurana* Crépin lines to stu-

dy diploid crosses and adding a tetraploid modern rose cultivar to examine a polyploid series. His data were consistent with the hypothesis that recurrence is controlled by a single locus, with recurrence (*r*) recessive to non-recurrence (*R*). Even the pentaploid genotype *Rrrrr* was non-recurrent.

Subsequent studies were able to confirm this result in diploid crosses of *R. wichurana* and a diploid derived as a haploid from a tetraploid modern rose (Crespel *et al.* 2002), diploid crosses involving *R. multiflora* Thunb. and modern roses (Debener 1999), tetraploid crosses between modern roses and *R. foetida* Hermann (de Vries and Dubois 1978), and tetraploid crosses between modern roses and moss roses (de Vries and Dubois 1984). In contrast, other studies observed ratios of recurrent to non-recurrent offspring that were significantly lower than predicted by the single-locus, *r/R* theory. For example, in diploid crosses of *R. wichurana* and a hybrid China rose ('Old Blush'), Shupert and Byrne (2007) saw greatly reduced ratios of recurrent to non-recurrent offspring. Svejda (1976) also saw low percentages of recurrence in diploid crosses of recurrent hybrid China rose cultivars with selected forms of the recurrent species *R. rugosa* Thunb. ex. Murray. In this case, only 28% of the offspring were recurrent, much lower than the 100% predicted by the single-locus theory. Taken collectively, these studies indicate that the single-locus, *r/R* theory, while generally accurate, can be complicated by other factors.

The case for the single-locus, *r/R* theory of juvenile recurrence was strengthened when Crespel *et al.* (2002) mapped the *r/R* locus to a genetic linkage group, utilizing AFLP markers. Roberts *et al.* (1999) also reinforced this theory by illuminating the physiology behind the regulation of blooming in roses. They found that gibberellin levels were much lower in a recurrent sport than the non-recurrent original cultivar. They hypothesized that gibberellins normally inhibit bloom in most roses, while the *r* allele reduces gibberellin levels, allowing recurrence.

Juvenile recurrence appears to be the form of recurrence

involved in the studies reviewed above. The most common source that the various investigators used for recurrence was modern roses, which possess juvenile recurrence. Two exceptions (Svejda 1976; Shupert and Byrne 2007) used hybrid China roses, which were one of the original sources of juvenile recurrence in modern roses (Zlesak 2001). The other exceptions (Semeniuk 1971a, 1971b) used a *R. wichurana* line as a source of recurrence. The appearance of juvenile recurrence in *R. wichurana* lines and hybrids is well documented (Quest-Ritson 2003).

Species rose breeding and implications for juvenile recurrence

The importance of learning more about the inheritance of juvenile recurrence lies in the many species of the genus *Rosa*, of which only a tiny fraction have contributed to modern rose cultivars (Cairns 2000). Many rose researchers and breeders have advocated the incorporation of additional species as sources of disease resistance, cold hardiness, and other traits (de Vries and Dubois 1996; Gudin 1999; Zlesak 2006). Substantial progress has been made with the introgression of traits from species (Buck 1979; Svejda 1988; Collicutt 1992), but the majority of species have still not been used.

Generally, the reluctance to incorporate species into breeding programs is caused by concern that species will introduce undesirable traits that will require significant effort to eliminate. Without a doubt, the importance of recurrence to the market, coupled with the non-recurrence of most species, is an important example. Since rigorous data regarding the inheritance of recurrence in the hybrids of species are limited to those studies listed above, it is not surprising that some breeders are reluctant to use species in breeding.

Variable forms and expression of recurrence

The issue of recurrence is further complicated by the apparent existence of more than one form of expression. The work of Svejda (1976) suggests that forms and hybrids of *R. rugosa* gain recurrence from the same *r* allele as modern roses. Despite this, the expression of recurrence is different. *Rosa rugosa* does not usually begin to bloom until plants have grown to nearly mature size (Zlesak 2001). Late-season bloom has also been described in a number of other wild species roses, including *R. laxa* Retzius (Buck 1962) and *R. arkansana* (Erlanson 1938; Collicutt 1992). Much work remains to be done to determine which, if any, of these species are genuinely recurrent, whether they share the same mechanism of recurrence as modern roses, and whether their recurrence can be utilized to simplify the task of breeding species hybrids possessing juvenile recurrence.

A rose breeding program in Big Rapids, Michigan, United States

In 1998, a rose breeding program was initiated by the author in Big Rapids, Michigan, United States (typical expected minimum temperature: -29°C). The program's long-term goal was to produce recurrent hybrids that were more cane-hardy to both winter cold and late spring freezes than typical modern roses. The initial work focused on producing first-generation hybrids of hardy species (and near-species hybrids) with modern roses, and then back-crossing to modern roses to produce recurrent offspring in the second generation. Planning this program was difficult because studies of how recurrence is inherited were not available for any of the hardy species parents chosen. In some cases, it was not even known whether these parents could produce fertile hybrids with modern roses. Once a consistent method for growing seedlings had been adopted, records of recurrence were kept. This allowed an analysis of the inheritance of recurrence in crosses between several different species and modern roses.

MATERIALS AND METHODS

Plant material

Recurrent modern roses were selected to represent a wide diversity of commercial classes: hybrid teas, grandifloras, floribundas, mini-floras, miniature roses, large-flowered climbers, climbing miniatures, and shrubs. They were obtained from garden centers or mail-order suppliers in the United States and Canada.

For species and near-species hybrids, the main criteria for selection were tetraploidy (to simplify crosses with typical tetraploid modern roses), ready commercial availability, and extreme winter hardiness in Minnesota as reported by Zuzek *et al.* (1995). An exception was the hybrid gallica, 'Tuscany', which does not demonstrate the same degree of cold hardiness, but was selected for use due to its striking dark purple petal color and the availability of published information regarding its breeding behavior (Austin 2005).

The hybrid 1-1 was produced early in the Big Rapids breeding program and was a seedling of *R. altaica* Willd. The other parent of 1-1 was apparently a form of *R. spinosissima* L. The remaining species and cultivars were obtained from commercial sources, and matched published descriptions (Shepherd 1954; Thomas 1994). *Rosa altaica* is sometimes classified as *R. spinosissima altaica* or 'Altaica' (Cairns 2000). The hybrid Gallica 'Alike' is also called *R. gallica grandiflora* Hansen and may be the old rose 'Rose Pavot' (Verrier 1995). The sources of species that did not have clone names were: *R. arkansana* 1 (White Rabbit Roses; Elk, California), *R. arkansana* 2 and *R. virginiana* 4 (Forest Farm; Williams, Oregon), *R. carolina* 1 and *R. pendulina* 1 (Hortico; Waterdown, Ontario), and *R. virginiana* 2 (Heirloom Roses; St. Paul, Oregon). Numbers were assigned to species without clone names as a part of clonal documentation in the Big Rapids breeding program.

The near-species hybrids 'Suzanne' (Skinner 1967) and 'William Baffin' (Richer and Davidson 2004) were selected because they were recurrent and also showed exceptional winter-hardiness in Minnesota. 'Kakwa', 'William III', and 'Commander Gillette' showed complete winter hardiness in Big Rapids, although they were not included in the Minnesota study (Zuzek *et al.* 1995). 'Kakwa' and 'William III' are both hybrids of *R. spinosissima*, although 'Kakwa' is formally classified as a shrub rose (Cairns 2000). The shrub rose 'Commander Gillette' is also known by the breeding code number 65-626, (Basye 1985) and has apparently been circulated under the synonyms 'Basye's Thornless' and 'Basye's Legacy' (Kim Rupert, pers. comm.). 'Commander Gillette' has the parentage [(Hugh Dickson' X *R. carolina*) OP] OP. It was used in this program in a role similar to that of a first-generation hybrid.

Crosses

Crosses were made, seeds were germinated, and seedlings were grown as previously described (Mitchell 2001, 2007). The convenient shorthand F₁ was used for the first-generation hybrids, even though neither parent was likely to have been true-breeding for traits other than recurrence or non-recurrence. Both *Rosa* species (Joly *et al.* 2006) and cultivars (Debener *et al.* 1996) tend to be highly heterozygous. The F₁ were produced by using greenhouse-grown, recurrent modern roses (M_p, modern parent) as seed parents, and applying pollen from species roses (S_p, species parent).

In the second generation, the designation R₂ was chosen to emphasize that these were the result of a backcross to a recurrent parent. The R₂ was produced by two methods. For most R₂ crosses, greenhouse-grown modern roses (M₁, modern rose crossed with F₁) were used as seed parents, with field-grown F₁ parents serving as pollen donors. In a minority of cases, the reciprocal cross was used; field-grown F₁ plants produced seed after pollination with M₁ pollen. In summary, R₂ = [M₁ X F₁ (M_p X S_p)] or [F₁ (M_p X S_p) X M₁].

Seed (achene) germination

Seeds were placed on wet sand in Petri plates and exposed to 1 month at room temperature, followed by cool, moist stratification at 4°C. Seeds of these crosses tended to germinate slowly, and

were kept at 4°C indefinitely. Seedlings were removed from the Petri plates at germination, and transferred into small individual cells (3 cm square, 5 cm tall, 1206 standard bedding pack insert; ITML Horticultural, Brantford, Ontario). Juvenile-recurrent seedlings were retained and transplanted for further evaluation in the breeding program. Seedlings classified as non-recurrent were discarded.

Data collection and analysis

This study was based on R₂ crosses made during the 2005 and 2006 breeding seasons. Seedlings were scored as recurrent if flowering was initiated within four months of germination, as evidenced by any visible stage of flower development. Although this was a longer time than usually seen in juvenile-recurrent roses, it was chosen to provide a margin of error against inconsistent environmental conditions, and in case any of the species used led to a modest delay in first bloom. Seedlings that had not produced a visible flower bud within four months of germination were scored as non-recurrent.

Seedlings were not scored if they died before reaching a size at which a recurrent seedling would have been expected to bloom. Most non-blooming seedlings either grew quickly to the limits imposed by the small cells (scored as non-recurrent), grew into dwarfed plants under 1 cm and failed to grow further (not scored), or died when very small, well before the 5-leaf stage (also not scored). Early death and stunted growth may have been partially due to hybrid incongruity, which has been observed previously in hybrids between rose species (Zlesak 2006).

Seedlings obtained from crosses utilizing the roses ‘Commander Gillette’, ‘Lynnie’, ‘MEImonblan’ (Marmalade Sky™), ‘WEKstephitsu’ (Outta the Blue™), and ‘MORcarlet’ (Scarlet Moss™) as female parents were all excluded due to evidence that these cultivars frequently self-pollinated, perhaps due to pollen release before blooms opened and emasculation was performed. Crosses utilizing ‘J. P. Connell’ and the Agriculture and Agri-Food Canada germplasm release L83 (Svejda 1988) as either male or female parents were also excluded from analysis. Crosses of typical modern roses with these two roses often produced non-recurrent offspring (data not shown). ‘William Booth’ was also excluded as both a male and female parent, as it produced evidence of both self-pollination and the suppression of recurrence in its offspring. An exception was made for the F₁ hybrid 1636-1 (‘William

Booth’ X ‘Alika’). This F₁ was confirmed to be a true hybrid because the traits of flower form (double), leaf morphology (rough), and plant habit (upright) all match those of ‘Alika’. ‘William Booth’ has single flowers, smooth and shiny leaves, and a spreading habit. Double flowers are genetically dominant to single (Debener 1999; Shupert and Byrne 2007) and were the clearest evidence of the hybrid origin of 1636-1.

To analyze the data, the R₂ seedlings were grouped according to common parentage, and the totals from each group were subjected to the chi-square test to evaluate goodness of fit. Expected outcomes were based on an expected ratio of 1 recurrent: 5 non-recurrent (de Vries and Dubois 1978). All groups with less than 30 scored seedlings were omitted (30 X 1/6 = 5, the minimum size for an expected population in the chi-square test). Chi-square (χ^2) and probability (*P*, degrees of freedom = 1) values were calculated using Microsoft Excel 2008 for Mac, version 12.0 (Microsoft Corporation 2007).

RESULTS AND DISCUSSION

Data used to investigate the inheritance of juvenile recurrence

The total number of R₂ cross combinations that produced at least 1 scored plant was 596, and the total number of scored plants was 3410. Of these, 311 (9.12%) were recurrent. These numbers represent the final data analysis. Some cultivars were excluded from the preliminary data analysis due to evidence that they self-pollinate despite emasculation or suppress juvenile recurrence in their offspring (see Materials and Methods). After the preliminary data analysis, two additional cultivars were identified as problematic (see the next subsection), and were also excluded from the final analysis. Offspring of the hybrid 1750-1 and ‘Commander Gillette’ also were not included in the totals above, because neither is a true F₁ rose as defined here. The parentage of 1750-1 was ‘Indigo’ X *R. virginiana* ‘Harvest Song’, and ‘Indigo’ is of the Portland commercial class and is not a modern rose (Cairns 2000). The complete pedigree of ‘Commander Gillette’ is not known (Basye 1985).

When the R₂ data were grouped according to the F₁ parent, all but one group with 30 or more scored seedlings segregated for recurrence (Table 1). Those F₁ that produced

Table 1 Frequency of juvenile recurrence in the R₂ backcross generation, grouped by F₁ parent.

F ₁	parents of F ₁		N _{scored}	% rec	N _{rec obs}	N _{rec exp} ^b	χ^2	<i>P</i>
	M _P ^a	S _P						
1192-1	‘BUCbi’ (Carefree Beauty™)	1-1 <i>R. altaica</i> hybrid	253	7.1	18	42.2	16.6	4.6 × 10 ⁻⁵
1245-1	‘WEKcalroc’ (George Burns™)	‘Alika’	50	22.0	11	8.3	1.0	0.31
1407-1	‘SAVacent’ (Center Gold™)	‘Alika’	120	20.8	25	20.0	1.5	0.22
1474-1	‘JACjem’ (Sun Flare)	‘Alika’	345	13.6	47	57.5	2.3	0.13
1474-2	‘JACjem’ (Sun Flare)	‘Alika’	98	10.2	10	16.3	2.9	0.086
1517-1	‘KORresia’ (Sunsprite)	‘Alika’	124	18.5	23	20.7	0.3	0.57
1517-2	‘KORresia’ (Sunsprite)	‘Alika’	99	7.1	7	16.5	6.6	0.010
1632-1	‘William Booth’	‘Alika’	60	21.7	13	10.0	1.1	0.30
1827-5	‘MORsegold’ (Sequoia Gold™)	<i>R. arkansana</i> 1	287	7.0	20	47.8	19.4	1.0 × 10 ⁻⁵
1431-1	‘MORsegold’ (Sequoia Gold™)	<i>R. carolina</i> 1	38	5.3	2	6.3	3.6	0.059
1343-1	‘SAVajerry’ (Jerry-O™)	<i>R. pendulina</i> 1	139	7.2	10	23.2	9.0	0.0027
1173-1	‘BUCbi’ (Carefree Beauty™)	<i>R. virginiana</i> ‘Harvest Song’	52	0.0	0	8.7	10.4	0.0013
1411-1	‘SAVacent’ (Center Gold™)	<i>R. virginiana</i> ‘Harvest Song’	295	7.5	22	49.2	18.0	2.2 × 10 ⁻⁵
1906-1	‘Yellow Jewel’	<i>R. virginiana</i> ‘Harvest Song’	79	5.1	4	13.2	7.7	0.0057
1946-1	‘MORgogard’ (Golden Gardens)	<i>R. virginiana</i> ‘Harvest Song’	181	4.4	8	30.2	19.5	9.8 × 10 ⁻⁶
1435-1	‘MORsegold’ (Sequoia Gold™)	<i>R. virginiana</i> 2	159	3.1	5	26.5	20.9	4.8 × 10 ⁻⁶
1467-1	‘Arthur Bell’	<i>R. virginiana</i> 2	475	4.4	21	79.2	51.3	8.0 × 10 ⁻¹³
1479-1	‘JACjem’ (Sun Flare)	<i>R. virginiana</i> 4	42	4.8	2	7.0	4.3	0.038
1465-1	‘Arthur Bell’	‘Suzanne’	227	18.5	42	37.8	0.6	0.46
1425-1	‘SAVaralph’ (Ralph Moore)	‘Tuscany’	41	12.2	5	6.8	0.6	0.44
1396-1	‘MEIzeli’ (The McCartney Rose™)	‘William III’	45	4.4	2	7.5	4.8	0.028
‘Commander Gillette’ ^c			318	17.9	57	53.0	0.4	0.55
1750-1 ^c		<i>R. virginiana</i> ‘Harvest Song’	421	0.5	2	70.2	79.5	4.9 × 10 ⁻¹⁹

Abbreviations: S_P, species parent; M_P, modern parent; rec, recurrent; obs, observed; exp, expected.

^a Cultivar name followed by trademark or exhibition name, if different, in parenthesis.

^b Expected based on 1 recurrent: 5 non-recurrent.

^c ‘Commander Gillette’ and 1750-1 are not F₁ by the definition used in this study.

Table 2 Frequency of juvenile recurrence in the R₂ backcross generation, grouped by S_p grandparent.

S _p	N _o scored	% rec	N _o rec obs	N _o rec exp ^a	χ ²	P
1-1 <i>R. altaica</i> hybrid	253	7.1	18	42.2	16.6	4.6 × 10 ⁻⁵
'Alika'	912	15.2	139	152.0	1.3	0.25
<i>R. arkansana</i> 1	299	6.7	20	49.8	21.4	3.7 × 10 ⁻⁶
<i>R. carolina</i> 1	41	4.9	2	6.8	4.1	0.043
<i>R. pendulina</i> 1	159	8.2	13	26.5	8.3	0.0041
<i>R. virginiana</i> 'Harvest Song'	655	5.3	35	109.2	60.5	7.5 × 10 ⁻¹⁵
<i>R. virginiana</i> 2	661	4.2	28	110.2	73.5	9.9 × 10 ⁻¹⁸
<i>R. virginiana</i> 4	42	4.8	2	7.0	4.3	0.038
'Suzanne'	227	18.5	42	37.8	0.6	0.46
'Tuscany'	43	11.6	5	7.2	0.8	0.38
'William III'	83	2.4	2	13.8	12.1	4.9 × 10 ⁻⁴

Abbreviations: S_p, species parent; rec, recurrent; obs, observed; exp, expected.

^a Expected based on 1 recurrent: 5 non-recurrent.

less than 30 R₂ offspring are not included in **Table 1**. The offspring of 'Commander Gillette' and 1750-1 are included in **Table 1**, for comparative purposes.

Some S_p were represented by more than one F₁ hybrid. When the data were grouped according to the original S_p parent, all groups with 30 or more scored seedlings segregated for recurrence (**Table 2**). More total scored individuals were included in **Table 2** than in **Table 1** (exclusive of 'Commander Gillette' and 1750-1 offspring), because some F₁ with less than 30 R₂ offspring shared their S_p parent with other F₁ hybrids.

One species, *R. altaica*, and two near-species hybrids, 'Kakwa' and 'William Baffin', were used as S_p but not included in **Table 2** because they produced fewer than the 30 scored R₂ plants required for chi-square analysis. These did produce F₁ seedlings, which went on to produce R₂ seedlings segregating for recurrence.

A detailed phenotypic description of traits other than juvenile recurrence in the F₁ and R₂ hybrids produced by this breeding program is beyond the scope of this article. Briefly, all of the F₁ hybrids showed clear signs of their species parents' traits. This was also true with the R₂ hybrids, although to a lesser extent.

The modern roses used were true-breeding for juvenile recurrence

Most modern roses belong to commercial classes that are generally regarded as true-breeding for juvenile recurrence, such as hybrid teas, gradifloras, floribundas, mini-floras, and miniatures (Shepherd 1954; Zlesak 2006). Climbing roses, such as large-flowered climbers and climbing miniatures, have a wider range of blooming behavior (Quest-Ritson 2003), which makes it less certain that cultivars from these commercial classes are true-breeding for juvenile recurrence. In the case of shrub roses, many have recent species ancestors (Buck 1979; Cairns 2000; Richer and Davidson 2004), which could potentially prove problematic.

All of the modern cultivars (both M_p and M₁) used for this study were screened for the recurrent phenotype when grown in Big Rapids for at least two years. All cultivars showed full recurrence, with substantial bloom in early summer, mid-summer, and fall.

M₁ cultivars were also evaluated for their production of recurrent individuals in their R₂ offspring. The full R₂ population (with the deletions described in Materials and Methods) was combined with the offspring obtained by crossing 'Commander Gillette' with modern roses. This combination of R₂ and similar plants totaled 670 cross combinations and 4114 scored individuals of which 402 (9.77%) were recurrent. A total of 150 modern rose cultivars served as the M₁ parents of this expanded R₂. The expanded R₂ was divided up into family groups, based upon common M₁ parents.

For one analysis, the family groups were divided into two categories, those with at least one juvenile-recurrent member and those with none. Even if all M₁ are true-

breeding for recurrence, some family groups will lack recurrent members by chance, with the probability increasing for groups with fewer members. The distribution of family groups without juvenile-recurrent members was a good fit for what would be theoretically predicted based on the overall recurrence frequency (results not shown). The two largest R₂ family groups with no juvenile-recurrent members each contained only 22 individual R₂ hybrids. The M₁ parents of both of these groups did produce recurrent R₂ offspring in the preliminary phase of this breeding program, before data on non-recurrent offspring was recorded (results not shown). The presence of a substantial number of M₁ cultivars that were not true-breeding for juvenile recurrence should have led to more and larger R₂ groups without juvenile recurrence.

Those R₂ family groups large enough to be subjected to the chi square test (greater than 50 members) were also examined for anomalous breeding behavior. Nineteen M₁ produced R₂ family groups at least this large, with 51 to 268 offspring each, for a total of 2530 R₂. Assuming M₁ that are true-breeding for juvenile recurrence are at least in the majority, any M₁ that are not true-breeding should produce a number of recurrent individuals in their R₂ family group that is smaller than average. Based on this assumption, the chi-squared test was set up to test for any departure from the average results obtained from all of the M₁ collectively. Because the M₁ were not all crossed with the same F₁ partners, the expected numbers were calculated based on a weighted average of the actual F₁ partners used to produce each R₂ family group. Two cultivars, both from the shrub commercial class, showed significant deviations from average. 'RADsun' (Carefree Sunshine™), produced 50% less juvenile-recurrent offspring than expected ($P = 0.026$). 'BAIface' (Funny Face™) produced 60% more ($P = 0.013$). 'RADsun' has recent species rose ancestors (Radler 2001) from which it may have inherited factors suppressing juvenile recurrence. The offspring of 'BAIface' often appeared to be the result of self-pollination, which could explain the above average number of juvenile-recurrent individuals in its R₂ family group. Both of these cultivars were dropped from the data set before calculating the numbers in **Table 1** and **Table 2**, and presented elsewhere in Results and Discussion. On the other hand, neither departure was significant at the $P < 0.01$ level, again suggesting that departures from the true-breeding condition amongst the modern roses used as M₁ were small or absent.

As an additional confirmation that the cultivars used as M₁ were true-breeding, the performance of different commercial classes was compared. Specifically, the shrub, large-flowered climber, and climbing miniature commercial class members among the M₁ were grouped for analysis. They comprised 105 M₁ cultivars with 3673 R₂ offspring. The chi-square test described above was used to detect any departure from the average breeding behavior of the whole of the extended R₂. The observed and expected frequencies were nearly identical ($P = 0.99$). This analysis suggests that the shrub and climbing roses, the groups of the greatest

theoretical concern, did not differ significantly from the remaining types in their influence on juvenile recurrence. This reinforces the conclusion that the M_1 cultivars were entirely or largely true-breeding for recurrence.

All but two of the M_P modern parents used to produce the F_1 hybrids belonged to commercial classes for which the true-breeding condition for juvenile recurrence is expected (specifically, hybrid teas, floribundas, and miniatures). The two exceptions were the shrub roses 'BUCbi' (Carefree Beauty™) and 'William Booth'. 'BUCbi' was also used as an M_1 parent, and the number of juvenile-recurrent R_2 offspring it produced was not significantly different than the average R_2 ($P = 0.70$). The true-breeding nature of 'BUCbi' was also established by the observation of self-pollinated offspring by Zlesak (1998). 'William Booth' does sometimes suppress juvenile recurrence in its offspring (results not shown), but its F_1 hybrid 1632-1 showed no evidence of this in its own R_2 offspring (Table 1).

Implications for the r/R theory of juvenile recurrence

The observed production of juvenile-recurrent R_2 hybrids was compared to expected numbers based on the single-locus, r/R theory. Modern roses were assumed to be true-breeding for juvenile recurrence (genotype $rrrr$). The species and near-species hybrids of the S_P were assumed to be true-breeding for non-recurrence ($RRRR$). This would produce F_1 hybrids with the genotype $RRrr$. Only R_2 offspring with the genotype $rrrr$ would exhibit juvenile recurrence.

Since the roses used in this study were all tetraploids or presumed tetraploids, it was necessary to make assumptions regarding how the four homologous copies of each chromosome synapse at prophase I of meiosis. Although the actual behavior of rose chromosomes is complex (Erlanson 1933; Ma *et al.* 2000), previous studies (de Vries and Dubois 1978, 1984; Yan 2005) have found good correspondence with the results predicted by assuming that the four homologues randomly synapse into two pairs (random chromosome assortment). Given this assumption, a ratio of 1 juvenile recurrent: 5 non-recurrent seedlings was predicted for the R_2 .

The breeding behavior of some F_1 hybrids directly supported the single-locus, r/R theory of juvenile recurrence. Nine F_1 hybrids produced an R_2 population that did not differ significantly from the predicted ratio at the $P < 0.05$ level (Table 1). These included F_1 hybrids that were derived from 'Alika', *R. carolina* 1, 'Suzanne', and 'Tuscany'. These results were consistent with the results of Semeniuk (1971a, 1971b), Crespel *et al.* (2002), Debener (1999), and de Vries and Dubois (1978, 1984). 'Commander Gillette' also produced the R_2 ratio expected for an F_1 hybrid with the genotype $RRrr$, although its full pedigree is not known.

On the other hand, the remaining twelve F_1 hybrids produced R_2 results that were not consistent with the simplest form of the single-locus, r/R theory (Table 1). These F_1 hybrids showed a juvenile recurrence ratio in their R_2 offspring that was significantly less than 1:5. Although all of these F_1 progenies were associated with a P value less than 0.05, the range was wide: $P = 0.038$ down to $P = 8.0 \times 10^{-13}$. This variability reflected both a wide variation in sample sizes of scored R_2 progeny for each F_1 and in the actual ratio of recurrent to non-recurrent individuals. The S_P that generated F_1 progeny with suppression of juvenile recurrence in their R_2 were 1-1, 'Alika', *R. arkansana* 1, *R. pendulina* 1, *R. virginiana* 'Harvest Song', *R. virginiana* 2, *R. virginiana* 4, and 'William III'. Suppression of juvenile recurrence was consistent with the results of Shupert and Byrne (2007) and Svejda (1976), although they saw a much greater suppression of recurrence than most of the F_1 in this study exhibited.

Suppression of juvenile recurrence

There are a number of possible explanations for the suppression of juvenile recurrence in the offspring of some F_1 hybrids. Such suppression could be the result of negative selection, preferential chromosome pairing, or secondary loci. The evidence from this study cannot conclusively indicate the presence or extent of any of these mechanisms. It is important to note that this study tested only for juvenile recurrence. Any R_2 seedlings that began recurrent bloom after four months would not have been scored as recurrent.

One explanation for the suppression of juvenile recurrence is that the r allele (or an allele at a closely linked locus) might be subject to negative selection at either the pre-zygotic or post-zygotic stage (Shupert and Byrne 2007), reducing the number of seedlings with juvenile recurrence. The greatest weakness of this explanation is that any model of distorted recurrence ratios must account for the variability observed in this and earlier studies. In particular, the S_P 'Alika' produced one F_1 hybrid that showed suppression of juvenile recurrence in its offspring and six F_1 hybrids that did not.

It is also possible that the pairing of chromosomes at meiosis is not fully random in some F_1 hybrids. If the two S_P -derived chromosomes, each carrying the R allele, show a preference to pair with each other, recurrence will be suppressed. This hypothesis is supported by the observation by Ma *et al.* (2000) of partial preferential chromosome pairing in tetraploid hybrids involving various combinations of species and modern roses. On the other hand, diploid backgrounds have also been described where juvenile recurrence is suppressed (Svejda 1976; Shupert and Byrne 2007). This suggests that other mechanisms must operate, at least in some instances.

The literature gives support to the idea that secondary loci can suppress juvenile recurrence. The example of *R. rugosa* is particularly instructive. Zlesak (2001, 2006) has proposed a theoretical framework for understanding recurrence in this diploid species, based on his own work and that of Svejda (1976). This species is naturally recurrent, but rarely blooms at all until it has attained most of its mature size after a year or more. Svejda crossed *R. rugosa* with recurrent, diploid hybrid China roses, and only 28% of the offspring were recurrent. The fact that some were recurrent is taken to show that *R. rugosa* is recurrent due to the same r allele as modern roses. This conclusion is also supported by the observation that *R. rugosa* seedlings will produce juvenile blooms on rare occasion (Zlesak 2001), suggesting *R. rugosa* has juvenile recurrence that is simply suppressed in most cases.

On the other hand, the fact that most of Svejda's *R. rugosa* hybrids were non-recurrent suggests that secondary loci, independent of the r/R locus, are also at work. These secondary loci could potentially control a system that suppresses what would otherwise be juvenile recurrence. Breeders have successfully selected *R. rugosa* cultivars with stronger recurrent bloom than the wild species (Svejda 1976). This response to selection implies that *R. rugosa* can be heterozygous at some of the secondary loci suppressing juvenile recurrence. This could then explain how Svejda was able to recover the occasional recurrent hybrid. A system to prevent juvenile recurrence in a naturally recurrent species makes evolutionary sense, as it would prevent plants from blooming when they should be allocating all of their resources to vegetative growth, gaining the size necessary for successful reproduction (Zlesak 2001).

Even non-recurrent species might harbor additional loci to suppress juvenile bloom, or late-season bloom that cannot produce seeds. Such extra loci could reinforce the action of the R allele, aiding in the prevention of bloom at inappropriate times. Species with such secondary non-recurrence mechanisms might occasionally pass on the relevant alleles to their F_1 hybrids, suppressing the appearance of juvenile recurrence in the R_2 .

The breeding behavior of species and F₁ with late-season bloom

The data gathered for this study partially addresses the issue of *Rosa* species that exhibit some type of late-season bloom. *Rosa arkansana* has widely been reported to be recurrent (Erlanson 1938; Collicutt 1992), and the clone used for this study was highly recurrent (all observations not attributed to other authors were made in Big Rapids, Michigan). The *R. arkansana* 1 F₁ hybrid 1827-5 never showed any late bloom, but two of its full siblings did produce a few fall blooms in some years (results not shown). *Rosa laxa* is another species often said to be recurrent, although the trait is variable among individuals (Buck 1962). The difficulty of obtaining *R. laxa* seedlings prevented direct F₁ hybrids from being studied, but the highly recurrent *R. laxa* hybrid 'Suzanne' (Skinner 1967) was useful. The 'Suzanne' F₁ hybrid 1465-1 was itself fully recurrent once mature. The recurrent hybrid 'William Baffin' also has a background including *R. laxa*, although modern roses and *R. kordesii* are also involved (Richer and Davidson 2004). Turning to instances of more limited late bloom, *R. altaica* produced a few fall blooms during most years, and *R. pendulina* 1 did so rarely. The *R. altaica* F₁ hybrid 1192-1 showed moderate recurrence, always blooming lightly in fall, and sometimes in mid-summer as well. A minority of the *R. pendulina* 1 F₁ hybrids produced a few scattered blooms in the fall of some years, as did some 'Alika' F₁ hybrids (results not shown).

Despite this evidence of varying degrees of late-season bloom, none of the F₁ hybrids studied produced ratios of juvenile recurrence to non-recurrence in their R₂ offspring that were significantly higher than predicted (Table 1). The predicted ratio was based upon the assumption that all of the S_p parents, even the near-species hybrids, are homozygous non-recurrent (RRRR). It therefore follows that late bloom in the cases discussed above is not due to the *r* allele-mediated juvenile recurrence system of modern roses.

In a related case, the hybrid 1750-1 was similar to the F₁ hybrids produced for this study, but the recurrent Portland rose 'Indigo' was used instead of a modern rose (1750-1 = 'Indigo' X *R. virginiana* 'Harvest Song'). Unlike most F₁ hybrids, 1750-1 failed to produce a useful percentage of recurrent second-generation offspring (Table 1), indicating that the recurrence of 'Indigo' is also not the result of the *r* allele system.

Although this study suggests that the *r* allele juvenile recurrence mechanism is different than the mechanism for recurrence (or late bloom) in such species as *R. laxa*, it does not rule out the possibility that the different mechanisms of recurrence might interact. Zlesak (2001) has suggested that recurrence mechanisms other than the *r* allele system might also act by suppressing the levels of gibberellins. If that is the case, different recurrence mechanisms might function additively. This could possibly be the explanation for why some of the F₁ hybrids discussed above showed more late bloom than their S_p parents.

Non-recurrent species are a practical option for rose breeding

The results presented here clearly indicate that neither sterility nor non-recurrence present insurmountable obstacles for breeding with the rose species investigated (Tables 1, 2). The production of F₁ hybrids was straightforward, although these non-recurrent seedlings generally required 3 to 4 years to bloom. Most S_p pollen readily formed seeds when placed on modern roses, except for *R. laxa*, *R. arkansana* 1, *R. arkansana* 2, and *R. carolina* 1, which had poor fertility with modern roses. With persistence, all except *R. arkansana* 2 eventually produced a few F₁ seedlings (results not shown). Producing the large number of R₂ seedlings needed to recover recurrent individuals required more labor, but was accomplished by a single worker (full-time June through August, half-time otherwise) during two breeding seasons. The ability to discard non-recurrent seedlings while very

small greatly reduced the space and other resources required.

The best use of R₂ hybrids with juvenile recurrence will vary with the goals of individual breeding programs. Some breeders might choose to use successive generations of backcrosses to modern roses to minimize the overall impact of the species. This approach would work best to introgress single (or a few) valuable genes from species into highly elite breeding lines. An example would be transferring major disease resistance genes into cut-flower roses. It may be possible to use DNA marker-assisted selection to aid in such projects (Debener *et al.* 2004). On the other hand, some desirable species traits may be inherited in a quantitative manner. In such cases, breeders might implement a strategy to maximize the amount of species influence in later generations. An example might be the introduction of winter hardiness into landscape roses (Mitchell 2008).

Only one F₁ failed to produce any R₂ offspring with juvenile recurrence. The rest produced recurrence in their R₂ ranging in frequency from 3.1% up to 22.0%. It is hoped that these results will encourage other rose breeders to use *Rosa* species in their programs more frequently. Although many species have yet to be tested, it seems safe to conclude that a wide range of tetraploid species can be crossed with modern roses without requiring excessive effort to recover individuals with the critical trait of juvenile recurrence.

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