

# EVIDENCE OF A CROSSABILITY BARRIER IN DIPLOID $\times$ HEXAPLOID AND DIPLOID $\times$ OCTOPLOID CROSSES IN THE GENUS *FRAGARIA*

W. D. EVANS

Department of Horticultural Science, Ontario Agricultural College,  
University of Guelph, Guelph, Ontario, Canada

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## SUMMARY

A large number of diploid  $\times$  hexaploid and diploid  $\times$  octoploid crosses have been made by numerous workers with poor results. Crosses between synthetic tetraploids and species of higher levels of ploidy were successful. The success of these crosses and the high incidence of pentaploid progeny obtained by other workers from successful diploid  $\times$  hexaploid crosses indicates that chromosomal or genetic imbalance in the endosperm or between zygote and endosperm is most likely the cause of failure to produce viable plants from  $2x \times 6x$  and  $2x \times 8x$  crosses.

## INTRODUCTION

In *Fragaria* there is a wide range of species which may be used in further improvement of the octoploid cultivars. While numerous workers have made interspecific crosses, few have attempted to explain their results when crosses failed.

EVANS & JONES (1967) established that a gametophytic incompatibility system functions in diploid species with unilateral incompatibility occurring in interspecific crosses where the male parent is self-compatible and the female parent is self-incompatible. Such a system does not account for the poor results obtained in *F. vesca* L. ( $2n = 14$ )  $\times$  *F. moschata* DUCH. ( $2n = 42$ ) crosses by other workers (Table 1) as *F. vesca* is a self-compatible species and should function as a female parent regardless of the presence or absence of incompatibility alleles in *F. moschata*. Seed set is satisfactory in most of these crosses but the seed fails to germinate or germinates poorly. With few exceptions the surviving plants have an abnormal number of chromosomes.

The diploid  $\times$  octoploid crossing results of other workers (Table 2) are not as well documented as those for diploid  $\times$  hexaploid crosses but they are similar in that seed set is often good but germination generally poor.

This paper reports results of crosses similar to those made by other workers and results of crosses using diploid interspecific hybrids, amphidiploids and synthetic octoploids. An explanation of the results of such crosses should be of value to strawberry breeders.

## MATERIALS AND METHODS

The following species and species hybrids were used: *F. vesca* L. ( $2n = 14$ ), *F. viridis* DUCH. ( $2n = 14$ ), *F. nilgerrensis* SCHLECHT. ( $2n = 14$ ), *F. nubicola* LOS. ( $2n = 14$ ),

Table 1. Detail of results of other workers from crossing diploid species of *Fragaria* with the hexaploid species *F. moschata*, the hexaploid being used as male parent.

Female parent	Seed set	Seed sown	Nature of progeny	Reference
<i>F. vesca</i>	good	600	4 weak seedlings, all died	MANGELSDORF & EAST, 1927
	good	406	1 maternal plant	YARNELL, 1931
	good	5430	11 maternal diploids 2 tetraploids 2 pentaploids	FEDOROVA, 1934
	good	NR <sup>1</sup>	1 tetraploid 4 pentaploids 1 fifteen-ploid	SCHIEMANN, 1937, 1944
	none			MILLARDET, 1894; ICHIJIMA, 1930
<i>F. viridis</i>	good	NR	no germination	YARNELL, 1931
	good	NR	1 pentaploid	SCHIEMANN, 1937
<i>F. nilgerrensis</i>	none			ICHIJIMA, 1930; YARNELL, 1931
<i>F. nipponica</i>	good	273	1 pentaploid	LILIENFELD, 1936

<sup>1</sup> NR = not reported.Table 2. Detail of result of other workers from crossing diploid species of *Fragaria* with octoploid species, the octoploid being used as male parent.

Combination	Seed set	Germination (%)	Reference
<i>F. vesca</i> × <i>F. chiloensis</i>	good	0.5	MANGELSDORF & EAST, 1927
	good	poor	ICHIJIMA, 1930; YARNELL, 1931
<i>F. vesca</i> × <i>F. virginiana</i>	poor	2.2 and 4.4	ICHIJIMA, 1930
	good	NR <sup>1</sup>	MANGELSDORF & EAST, 1927
<i>F. vesca</i> × <i>F.</i> × <i>ananassa</i>	good	poor	DOGADKINA, 1941
<i>F. viridis</i> × <i>F. chiloensis</i>	none		ICHIJIMA, 1930; YARNELL, 1931
<i>F. nilgerrensis</i> × <i>F. virginiana</i>	none		YARNELL, 1931

<sup>1</sup> NR = not reported.

*F. vesca* × *viridis* F<sub>1</sub> (2n = 14), *F. moschata* DUCH. (2n = 42), *F. chiloensis* (L.) DUCH. (2n = 56), *F. virginiana* DUCH. (2n = 56), *F.* × *ananassa* DUCH. (2n = 56) and the amphidiploids *F.* (*vesca* × *viridis*) (2n = 28), *F.* (*nilgerrensis* × *viridis*) (2n = 28) and *F.* (*moschata* × *nubicola*) (2n = 56). With the exception of *F. nilgerrensis* a number of clones of each species or species hybrid were used. The crosses made were 2x × 6x, 4x × 6x, 2x × 8x, 4x × 8x.

Flowers to be pollinated were emasculated approximately 3 days before anthesis. Pollen was applied 3 days after emasculation and again 3 days later. In all cases emasculated flowers were bagged as were flowers to be used as a source of pollen.

At least three flowers (approximately 180 pistils) were pollinated in each combination. Three flowers were also self- or sib-pollinated and three emasculated flowers were not pollinated.

The number of seeds produced from each combination was recorded. The number of seeds which germinated following scarification in concentrated sulfuric acid for 15 min and stratification at 2°C for 30 days, and the survival of seedlings were also recorded.

Chromosome counts on surviving plants were made on root-tips using the Feulgen-acetocarmine squash technique following pre-fixation overnight in ice water.

RESULTS AND DISCUSSION

The results are reported in Tables 3, 4 and 5. There was no set on any emasculated flowers which were not pollinated. All species and species hybrids set seed and produced normal plants when they were selfed or sib-crossed.

The most striking results are the failure to establish plants from the 2x × 6x crosses and the relatively good success in producing hybrids from the 4x × 6x combinations (Table 3). The production of seeds from the *F. viridis* × *F. moschata* and 2x *F. vesca* × *viridis* F<sub>1</sub> × *F. moschata* crosses with subsequent failure in germination or death of the seedlings is similar to the results of other workers (Table 1). The success of the 4x × 6x crosses suggests that unilateral incompatibility is not the cause of failure in the 2x × 6x crosses. *F. viridis* is known to be a self-incompatible species and *F. moschata* could not effect fertilization in the 4x × 6x crosses unless it too possesses incompatibility alleles.

The high proportion of pentaploids in the surviving plants obtained by other workers (Table 1) is significant. Evidently unreduced gametes in the female parent have

Table 3. Results of crossing diploid species, species hybrids and amphidiploids of *Fragaria* with the hexaploid species, *F. moschata*, the hexaploid being used as male parent.

Female parent	Flowers pollinated	Seed produced	Seed germinated
<i>F. vesca</i>	6 (2) <sup>1</sup>	0	
<i>F. viridis</i>	7 (1)	17	2 <sup>2</sup>
<i>F. nubicola</i>	14 (1)	0	
<i>F. nilgerrensis</i>	6 (1)	0	
<i>F. (vesca × viridis) F<sub>1</sub></i>	10 (1)	39	0
<i>F. (vesca × viridis) 4x<sup>3</sup></i>	3 (1)	30	27 <sup>5</sup>
<i>F. (nilg. × viridis) 4x<sup>4</sup></i>	6 (1)	36	18 <sup>5</sup>
<i>F. (moschata × nubicola) F<sub>1</sub></i>	6 (2)	62	38 <sup>5</sup>

<sup>1</sup> Bracketed figures indicate the number of crosses in which different clones were used.

<sup>2</sup> Both seedlings died before second true leaf formed.

<sup>3</sup> *F. (vesca × viridis) 4x* = progeny of sib-crosses of colchipsoid plants of *F. vesca* × *F. viridis*, 2n = 28.

<sup>4</sup> *F. (nilgerrensis × viridis) 4x* = progeny of sib-crosses of colchipsoid plants of *F. nilgerrensis* × *F. viridis*, 2n = 28.

<sup>5</sup> All seedlings survived and were pentaploid.

been involved in the production of the zygote. Since unreduced gametes are probably rare, the high proportion of pentaploids indicates that such zygotes have a better chance of survival than tetraploid zygotes. The tetraploid zygote itself is not inviable as LILIENFELD (1933) and SCHIEMANN (1951) had considerable success in producing tetraploids from crosses of *F. moschata* × *F. nipponica* and *F. moschata* × *F. viridis*, respectively.

THOMPSON (1930) reported similar differences in reciprocal crosses of *Triticum vulgare* ( $2n = 42$ ) and *T. monococcum* ( $2n = 14$ ). He attributed the failure of the  $2x \times 6x$  crosses to inferior development of the endosperm owing to an imbalance in the chromosomal complement of the endosperm. MÜNTZING (1930), with similar results in *Galeopsis*, attributed poor hybrid seed development to disturbances of the normal chromosome complement of the embryo, endosperm and maternal tissues. STEPHENS (1942), in studying reciprocal differences in  $2x \times 4x$  crosses in *Gossypium*, suggested that the genome ratio between zygote and endosperm was responsible for the failure of certain combinations. ALLARD (1960) suggested that imbalance of gene dosage may be responsible for the abnormal development of endo-

Table 4. Results of crossing diploid species of *Fragaria* with octoploid species and synthetic octoploids, the octoploids being used as male parent.

Combination	Flowers pollinated	Seed produced	Seed germinated
<i>F. vesca</i> ×			
<i>F. chiloensis</i>	24 (7) <sup>1</sup>	56	11 <sup>2</sup>
<i>F. virginiana</i>	9 (3)	102	14 <sup>3</sup>
<i>F.</i> × <i>ananassa</i>	103 (21)	184	42 <sup>3</sup>
<i>F. (mo. × nu.) 8x</i> <sup>4</sup>	6 (2)	0	
<i>F. viridis</i> ×			
<i>F. chiloensis</i>	6 (1)	0	
<i>F. virginiana</i>	5 (1)	0	
<i>F.</i> × <i>ananassa</i>	15 (4)	0	
<i>F. (mo. × nu.) 8x</i>	6 (2)	0	
<i>F. nubicola</i> ×			
<i>F. chiloensis</i>	6 (2)	0	
<i>F. virginiana</i>	3 (1)	0	
<i>F.</i> × <i>ananassa</i>	27 (4)	35	0
<i>F. (mo. × nu.) 8x</i>	6 (2)	0	
<i>F. nilgerrensis</i> ×			
<i>F. chiloensis</i>	12 (2)	44	0
<i>F. virginiana</i>	3 (1)	0	
<i>F.</i> × <i>ananassa</i>	27 (4)	0	
<i>F. (mo. × nu.) 8x</i>	6 (2)	0	

<sup>1</sup> Bracketed figures indicate the number of crosses in which different clones were used.

<sup>2</sup> All plants matroclinous.

<sup>3</sup> All seedlings died before second true leaf formed.

<sup>4</sup> *F. (mo. × nu.) 8x* = colchipsoid plant of *F. moschata* × *F. nubicola*,  $2n = 56$ .

sperm rather than chromosomal imbalance between zygote and endosperm or chromosome imbalance in the endosperm.

In the  $2x \times 6x$  crosses in *Fragaria* the high proportion of pentaploids among surviving plants and the poor germination of seeds obtained indicates that some chromosomal or genic imbalance is responsible for poor success in crosses. In a  $2x \times 2x$  cross the zygote : endosperm chromosome ratio is 2:3(0.66) while in a  $2x \times 6x$  cross it is 4:5(0.80). Pentaploids arising from a  $2x \times 6x$  cross probably result from an unreduced female gamete uniting with a reduced male gamete. Assuming the fusion nuclei are also unreduced the zygote : endosperm ratio is 5:7(0.71) which is closer to the normal  $2x \times 2x$  ratio than is the  $2x \times 6x$  ratio.

If the ratio of maternal to paternal chromosomes within the endosperm only is considered the following ratios exist: in a  $2x \times 2x$  cross 2:1(2.0), in a  $2x \times 6x$  cross 2:3(0.66), in a  $2x \times 6x$  cross producing a pentaploid 4:3(1.33), the latter being much closer to the normal  $2x \times 2x$  ratio.

The ratios discussed above may be interpreted as ratios of gene dosage as suggested by ALLARD (1960) but on the evidence it is not possible to determine whether genic or chromosomal ratios are the cause of the barrier.

In crosses in which the female parent possesses incompatibility alleles and the incompatibility status of the male parent is not known failure must not be attributed to the genic or chromosomal imbalance barrier. Such results are those reported in

Table 5. Results of crossing diploid species hybrids and tetraploid amphidiploids of *Fragaria* with octoploid species and synthetic octoploids, the octoploid being used as male parent.

Combination	Flowers pollinated	Seed produced	Seed germinated
<i>F. (vesca × viridis) F<sub>1</sub> ×</i>			
<i>F. chiloensis</i>	3 (1) <sup>1</sup>	0	
<i>F. virginiana</i>	3 (1)	0	
<i>F. × ananassa</i>	14 (3)	8	0
<i>F. (mo. × nu.) 8x<sup>2</sup></i>	6 (2)	0	
<i>F. (vesca × viridis) 4x<sup>3</sup> ×</i>			
<i>F. chiloensis</i>	15 (5)	1	0
<i>F. virginiana</i>	3 (1)	0	
<i>F. × ananassa</i>	21 (6)	0	
<i>F. (mo. × nu.) 8x</i>	6 (2)	66	64 <sup>5</sup>
<i>F. (nilg. × viridis) 4x<sup>4</sup></i>			
<i>F. chiloensis</i>	12 (3)	0	
<i>F. virginiana</i>	6 (2)	0	
<i>F. × ananassa</i>	12 (3)	25	0
<i>F. (mo. × nu.) 8x</i>	6 (2)	27	24 <sup>5</sup>

<sup>1</sup> Bracketed figures indicate the number of crosses in which different clones were used.

<sup>2</sup> *F. (mo. × nu.) 8x* = colchiploid plants of *F. moschata* × *F. nubicola*,  $2n = 56$ .

<sup>3</sup> *F. (vesca × viridis) 4x* = progeny of sib-crosses of colchiploid plants of *F. vesca* × *F. viridis*.  $2n = 28$ .

<sup>4</sup> *F. (nilg. × viridis) 4x* = progeny of sib-crosses of colchiploid plants of *F. nilgerrensis* × *F. viridis*,  $2n = 28$ .

<sup>5</sup> All plants were hexaploid and evidently hybrids.

Tables 4 and 5 in which the self-incompatible species *F. viridis* and *F. nubicola* (EVANS & JONES, 1967) or their progeny are used as female parents and *F. chiloensis*, *F. virginiana* and *F. × ananassa*, species in which the incompatibility status is not known, are used as male parents. Although a large number of seeds was produced from *F. vesca* × 8x crosses no true hybrids were produced indicating that a barrier similar to that in 2x × 6x crosses exists. Success of the 4x × *F. (moschata* × *nubicola*) 8x crosses (Table 5) show that at the 4x × 8x level the chromosomal or genic balance is satisfactory.

In the genus *Fragaria* species of higher levels of ploidy should be used as female parents when possible. If other factors preclude making the cross in this direction attempts should be made to increase the chromosome complement of the species of lower level of ploidy or to use other means such as the culture of embryos soon after they develop.

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