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Cytogenetics of Vitis

III. Partially fertile F_1 diploid hybrids between V. vinifera L. × V. rotundifolia MICHX.[1]

by

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Introduction

Hybrids between *Vitis vinifera* L. (2n = 38) and *V. rotundifolia* MICHX. (2n = 40) are of both practical and theoretical interest. From the practical point of view, the commercial varieties of both species might be considerably improved by such hybridization. For example, the widely grown *V. vinifera* varieties are susceptible to most diseases and pests of grapes, while *V. rotundifolia* varieties are almost immune to them (OLMO, 1954). Transfer of desirable genetic factors for resistance into the *V. vinifera* varieties should be of great importance to practical viticulture. On the other hand, the *V. rotundifolia* varieties grown in the southeastern part of the United States lack fruit quality, and the fruit clusters are very small, characteristics in which *V. vinifera* varieties excel. Despite such a potential, relatively little work has been done on the hybridization of the two species. European countries early abandoned this approach because of sterility problems and a lack of winter hardiness in *V. rotundifolia*. Up to a decade ago, the work on hybridization in the United States was fragmentary and inconclusive.

The two species are only distantly related, and taxonomists have classified them into different subgenera: *V. vinifera* with other grape species (2n = 38) in the subgenus *Euvitis* and *V. rotundifolia* with *V. munsoniana* (2n = 40) in the subgenus *Muscadinia* (SMALL, 1903, considered the species to be in separate genera). From the theoretical point of view, cytological studies of hybrids obtained by such interspecific crosses may contribute to the knowledge of hybridization of remote plant species and its consequences. Moreover, the two species display a unique cross incompatibility system which may be of interest from the evolutionary standpoint.

Previous work has indicated that the two species hybridize successfully only when *V. vinifera* varieties are used as female parents and *V. rotundifolia* varieties as male parents (WYLIE, 1871; PATEL and OLMO, 1955). From such a cross a large number of seeds may be obtained. The hybrid seedlings are vigorous but highly sterile (DETJEN, 1917; PATEL and OLMO, 1955). In 1871, in his report to the American Pomological Society concerning hybridization of the two species, WYLIE mentioned that male sterile hybrids bear no fruits.

In the period following WYLIE's work, the emphasis was on establishing the fact of hybridity. This was mainly because of the claims of MILLARDET in France (1901) and MUNSON in Texas (1910), both of whom described "hybrids" of the two species

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which phenotypically were *V. rotundifolia*. DETJEN (1919), however, compared the morphological description of true hybrids that he produced with the "hybrids" of MILLARDET and MUNSON, and concluded that the latter were not hybrids at all, but simply seedlings of *V. rotundifolia*. The true hybrids were intermediate in morphology, and highly sterile, only two berries from 17 clusters reached maturity. DETJEN did not present data on the relative fertility of his hybrids.

PATEL and OLMO (1955), working on the hybridization of diploid clones of *V. vinifera* and *V. rotundifolia*, established that the two species hybridize readily only when *V. vinifera* is used as a female parent. By such crosses they were able to raise a population of more than 200 seedlings to maturity. Fertility tests by OLMO (unpublished) prove that the hybrids were completely sterile.

PATEL and OLMO (1955) reported fertility tests on some F_1 hybrids introduced from the North Carolina Agricultural Experiment Station. They found that some seedlings set fruit, but fertility was very low. Their studies of meiotic chromosomal behavior disclosed very poor chromosomal pairing in the introduced F_1 hybrids.

This paper presents the results of cytogenetic investigation of a diploid population of *V. vinifera* × *V. rotundifolia* hybrids (hereafter referred to as VR hybrids).

Materials and Methods

The hybrid population used in the present investigation was grown in the vineyard of the Department of Viticulture, University of California at Davis. Some vines were also transplanted to the greenhouse to supply early pollen.

The diploid hybrids of particular interest are those derived from crossing a *V. vinifera* male sterile selection, 'F2-35', with two male clones of *V. rotundifolia*, 'Trayshed' and 'Male'. Crosses made in 1958 with 'Trayshed' produced a population of 37 vines, designated as T6-11 to T6-47; those with 'Male' were T6-51 to T6-64.

In 1959, the above crosses with 'Trayshed' were repeated, and the diploid F_1 hybrids numbered b54-11 to b54-17 were obtained. In all, 57 F_1 hybrid seedlings were grown to maturity in the grape seedling block. The more fertile plants from these populations were saved and used in the present investigation.

The F_1 seedling Y14-56 was an exceptional *V. rotundifolia* × *V. vinifera* hybrid of the following origin. A population of *V. rotundifolia* seedlings, 'Thomas' × 'Trayshed', was planted in a block completely surrounded by *V. vinifera* types. The seeds were collected and planted. Among many seedlings of *V. rotundifolia* phenotype, Y14-56 was found to be a diploid hybrid with *V. vinifera*.

Standard varieties and some selections (Table 1) of both species have been used to test the pollen and ovule fertility of the diploid F_1 hybrids.

Pollination and related techniques

Whenever possible, male sterile vines were used in crossing to avoid tedious emasculation. The flower clusters were isolated in paper bags and the vine caged before bloom. Details of isolation and techniques of pollen collection and pollination were as described by PATEL and OLMO (1955). Pollen germination was tested by the hanging drop technique, using 20 percent sucrose. The flowers were pollinated only once unless stated otherwise. Harvesting of the fruits, extraction of the seeds from the berries, calculations of the fertility parameters, planting of the seeds, and growing of the seedlings were also as described by PATEL and OLMO (1955).

Table 1

Derivation of diploid forms used in the present investigation

Clone	Genomic formula[1])	Parentage	Functional flower type
		Parents of the F_1 Hybrids	
F2—35	VV	Carignane × Cabernet-Sauvignon	Female
Trayshed	RR	*V. rotundifolia*	Male
		F_1 Progeny	
T6—38	VR	F2—35 × Trayshed	Female
T6—42	VR	F2—35 × Trayshed	Female
T6—44	VR	F2—35 × Trayshed	Female
b54—17	VR	F2—35 × Trayshed	Male
Y14—56	RV	(Thomas × Trayshed) × O. P.[1])	Female
		Selections as Testers	
L12—80	VV	Emperor × Hunisa	Female
G22—24	VV	Zinfandel × Refosco	Hermaphrodite
79—25c	VV	L12—80 × K5—81	Hermaphrodite
91—60c	VV	H42—39 × I8—17	Hermaphrodite
S37—17	VV	Ribier × Beauty Seedless	Hermaphrodite

[1]) *V. vinifera* = V; *V. rotundifolia* = R.
K5—81 = Scolokertek kiralynoje × Black Kishmish
H42—39 = Olivette blanche × Black Kishmish
I8—17 = Emperor × Pirovano 75
Ribier = Alphonse Lavallée.

Cytological

The squash technique was used exclusively for determination of the somatic chromosome number in the hybrid seedlings and for studies of meiotic behavior in the pollen mother cells.

For somatic chromosome counts, the most actively growing shoot tips or bursting buds were collected and treated with paradichlorobenzene for 3—4 hours. The tips were then fixed in a mixture of chloroform, alcohol, and acetic acid (2 : 1 : 1) for 24 hours. Whenever necessary, the material was stored in the freezer at −5° C. Before the slides were prepared, the shoot tips were transferred into distilled water containing 6—8 percent pectinase 16-s (manufactured by Wallerstein Company) for at least 24 hours. Pectinase 16-s hydrolyzes the middle lamella of the cell wall. The young growing tissues were separated with needles, and a small piece of the tissue transferred to a slide in a drop of acetocarmine. A cover slip was gently pressed over the tissue and the slide lightly heated with a flame.

For studies of meiotic behavior, the flowers were collected through three seasons and fixed and stored in the same manner as were the shoot tips. The aceto-carmine staining procedure was used in preparing temporary slides.

The meiotic behavior of the chromosomes in the male sterile hybrids was investigated in the microspore mother cells.

Morphological

The scoring of trunk characters was done in the field during the dormant period in 1964. Canes were brought into the laboratory and examined visually or under a binocular microscope.

The s ecific gravity of the wood is different in the two species (WILLIAMS, 1923). *V. rotundifolia* has a specific gravity over 1.0, whereas that of *V. vinifera* is less than 1.0. Pieces about 1.5 to 2 cm in length were cut from different positions on the cane and immersed in distilled water. Type of wood in the hybrids was determined on the basis of sinking or floating of immersed pieces.

Phyllometric studies were carried out on the fully developed leaves collected from the sixth to eighth nodes on the primary shoots. The leaves were pressed and dried between blotters, and later examined.

Experimental Results

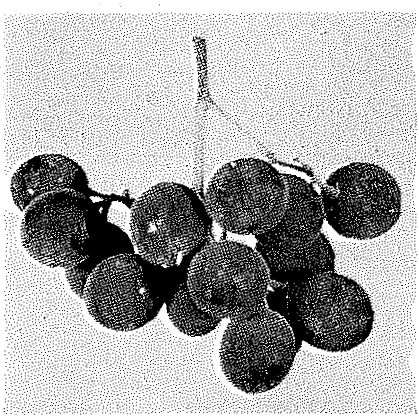
1. Fertility studies of F_1 diploid progeny

Ovule fertility of F_1 seedlings, V × R

Table 2

Ovule fertility of the F_1 VR hybrids pollinated with *V. vinifera* and *V. rotundifolia* varieties

Parents	Pollinated	Set (%)		Seed		
	Flowers (clusters)	Berry	Ovule	Av./berry	Total	Floaters (%)
VR × VV						
T6—42 × Alicante Bouschet	432 (12)	16.2	4.3	1.0	74	36.5
T6—42 × Early Muscat	163 (4)	8.6	2.1	1.0	14	71.4
T6—42 × Emperor	260 (7)	12.7	3.3	1.0	34	32.4
T6—42 × Muscat of Alexandria	195 (6)	23.1	5.8	1.0	45	60.0
T6—42 × Ruby Cabernet	480 (12)	10.0	2.5	1.0	51	15.7
T6—42 × Scolokertek	245 (7)	4.9	1.2	1.0	12	25.0
T6—42 × Flame Tokay	645 (17)	5.4	1.4	1.0	36	38.9
T6—42 × Thompson Seedless	290 (7)	2.4	0.6	1.0	7	28.6
T6—42 × White Riesling	270 (8)	13.7	3.6	1.0	39	20.5
T6—42 × 79—25c	330 (7)	9.1	2.3	1.0	30	40.0
T6—42 × 91—60c	380 (8)	9.4	2.4	1.0	36	22.2
T6—42 × S37—17	360 (8)	8.1	2.2	1.1	32	3.1
T6—42 × Red Malaga	161 (4)	5.0	1.2	1.0	8	25.0
T6—38 × Aramon	237 (6)	9.7	2.9	1.2	28	28.6
T6—38 × Carignane	285 (7)	15.4	4.4	1.1	50	24.0
T6—38 × Ruby Cabernet	184 (4)	14.7	4.1	1.1	30	16.7
T6—38 × Flame Tokay	205 (5)	15.6	4.6	1.2	38	26.3
T6—38 × White Riesling	266 (7)	19.2	4.9	1.0	52	44.2
T6—38 × Sylvaner	250	7.6	2.2	1.1	22	18.8
T6—44 × Grenache	432 (10)	22.4	5.9	1.0	102	5.9
VR × RR						
T6—42 × Burgaw	250 (6)	8.0	2.0	1.0	20	10.0
T6—42 × Tarheel	290 (6)	3.1	0.9	1.1	10	10.0
T6—42 × Trayshed	325 (8)	0.0	0.0	0.0	0	0.0
T6—42 × Male	280 (7)	2.1	0.5	1.0	6	33.3
T6—42 × Willard	700 (16)	1.1	0.3	1.0	8	37.5
T6—38 × Tarheel	190 (4)	12.1	3.1	1.0	24	25.0
T6—38 × Trayshed	198 (4)	0.0	0.0	0.0	0	0.0



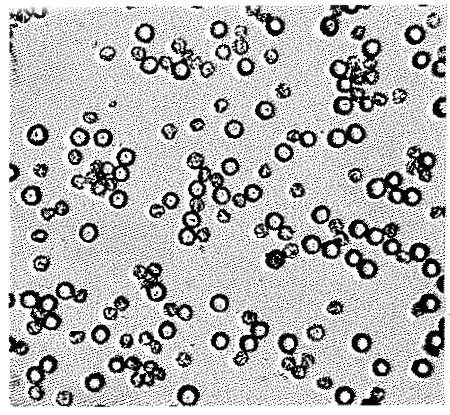


Fig. 1.: Fruit set of the diploid VR hybrid T6—42 when pollinated with *V. vinifera* Muscat of Alexandria (× 1.0).

Fig. 2: Pollen of the F_1 hybrid b54—17 ×(230).

The male sterile F_1 hybrid T6-42 was pollinated with 13 different *V. vinifera* and 5 *V. rotundifolia* clones (Table 2). The berry and ovule set in two additional male sterile F_1 hybrids, T6-38 and T6-44, were investigated. Berry and ovule set were, on the average, lower when pollen of *V. rotundifolia* clones was used.

The average number of seeds per berry had a narrow range, between 1.0 and 1.2. Most of the berries contained only a single seed. Using seeds that float in water as a criterion of viability, the variation was large.

Among the male-sterile F_1 seedlings there was no marked difference in ovule fertility. The seedling T6-42 had somewhat greater variability in the berry and ovule set, and percent of seeds that floated. These variations might have been due to the fact that pollinations in T6-42 were done from late June to mid-August, under changing physiological and environmental conditions. Most of the pollinations to test ovule fertility of T6-42 were done on the flower clusters of secondary shoots.

Table 3

Ovule fertility of the male sterile F_1 hybrid Y14—56

Pollen parent	Pollinated	Set (%)		Seed		
	Flowers (clusters)	Berry	Ovule	Av./berry	Total	Floaters (%)
VV						
Thompson Seedless	455 (9)	6.8	1.9	1.1	35	20.0
G22—24	436 (7)	18.6	6.4	1.4	111	20.7
Grenache	861 (10)	0.5	0.2	1.5	6	16.7
Cabernet-Sauvignon	875 (12)	4.7	1.2	1.0	43	18.6
Mission	120 (2)	5.0	1.4	1.2	7	28.6
Red Malaga	411 (7)	4.1	1.1	1.0	18	50.0
White Riesling	381 (7)	4.9	1.3	1.0	19	26.3
RR						
Trayshed	601 (11)	18.5	5.1	1.1	123	9.7
Tarheel	489 (11)	2.4	0.7	1.2	14	21.4

Table 4

Pollen fertility of the F_1 diploid VR hybrid b54—16

Female parent	Pollinated	Set (%)		Seed		
	Flowers (clusters)	Berry	Ovule	Av./berry	Total	Floaters (%)
VV						
F2—35	544 (2)	3.1	0.8	1.0	17	0.0
L12—80	536 (2)	0.4	0.1	1.0	2	0.0
Hunisa	276 (1)	2.5	0.6	1.0	7	28.6
RR						
Hunt	295 (9)	0.0	0.0	0.0	0	0.0
Dulcet	192 (5)	0.0	0.0	0.0	0	0.0
VR						
T6—42	327 (10)	0.6	0.1	1.0	2	0.0

The primary flower cluster sets very poorly with Thompson Seedless and Red Malaga and not at all with *V. rotundifolia* 'Trayshed'.

Fig. 1 illustrates the types of fruit clusters obtained by using pollen of *V. vinifera* varieties.

Ovule fertility test of the F_1 hybrid, R × V

The male sterile hybrid Y14-56, as explained earlier, originated from seed that resulted from open pollination of a *V. rotundifolia* seedling. Since the cytoplasm of this hybrid is *V. rotundifolia*, the results of pollination with the pollen of the varieties of both species can lead toward elucidation of the cross-incompatibility of the two species.

Pollen of seven *V. vinifera* varieties produced fruit set (Table 3) in all instances. Pollen of two varieties of *V. rotundifolia* also produced fruit set. Berry set with *V.*

Table 5

Chromosome associations at MI of diploid F_1 VR hybrids

Vine	PMC analyzed	Univalent I	Bivalent II	Trivalent III	Quadrivalent IV	Pentavalent V
T6—38	21	1.23[1])	15.57	1.38	0.19	0
		0—12[2])	12—18	0—2	0—1	0
T6—42	75	1.77	15.29	0.77	0.33	0.05
		0—11	12—19	0—3	0—2	0—1
T6—44	14	1.61	14.35	1.10	0.43	0
		0—8	11—17	0—2	0—1	0
b54—17	61	7.58	13.09	0.81	0.39	0.03
		0—19	7—18	0—3	0—3	0—1
Y14—56	18	2.22	13.88	1.61	1.22	0.05
		0—7	11—18	0—5	0—5	0—1

[1]) Mean; [2]) Range.

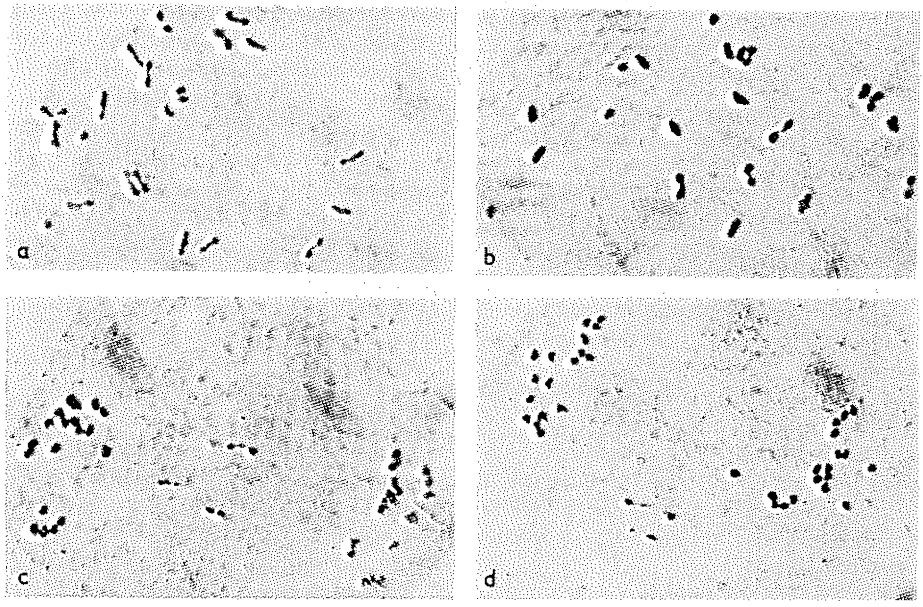


Fig. 3a: Metaphase I, Vine T6—38. $18_{II} + 3_{I}$. b: Metaphase I, Vine T6—42. $16_{II} + 1_{III} + 4_{I}$. c: Anaphase I, vine b54—17, showing three lagging bivalents. d: Anaphase I, vine T46—41, showing 18 + 17 chromosome distribution, with 2 lagging bivalents. $2_{n} = 39$. (× 1400).

vinifera 'Grenache' was 0.5 per cent and with *V. vinifera* selection G22-24, 18.6 per cent. Ovule set followed the same pattern. The highest average number of seeds per berry was 1.5. The variations in fertility among different pollination combinations were most likely due to time of pollination and position of the flower clusters. Thus, the first pollinations in 1964 were with 'Grenache' on primary flower clusters at the basal position of primary shoots. Later in the season, pollens of G22-24 and 'Trayshed, were used on secondary inflorescences.

Pollen fertility test

Pollen of the male F_1 hybrid b54-17 was used on male-sterile *V. vinifera* and *V. rotundifolia* varieties (Table 4). The pollen used in these crosses was collected in the latter part of the flowering season from primary shoots, and during summer from the secondary shoots. All crosses with *V. rotundifolia* varieties as female failed. Pollen of b54-17 resulted in a low berry and ovule set with *V. vinifera* varieties; the average seed number per berry never exceeded 1.0.

Germinability of the pollen *in vitro* approached 0.6 per cent. Most of the pollen grains (about 81 per cent) were unstained and shriveled (Fig. 2) after treatment with acetocarmine. Plant b54-17 blossomed for the first time in the spring of 1964, and only a small amount of pollen was available.

2. Chromosomal analysis of the diploid F_1 hybrids

All seedlings in this class have 39 somatic chromosomes, cytological proof of the hybridity of these plants. The data on the meiotic studies of the chromosomes are summarized in Table 5.

The chromosomes of the species under consideration do not stain well in the earlier stages of meiosis; therefore, studies of meiotic behavior were confined mostly

to MI and AI. Of the five hybrids, the first four originated from the same cross. The most frequent chromosomal association at MI are bivalents, with the lowest average frequency (13.09) in the seedling b54-17 and the highest (15.57) in the seedling T6-38.

The variation in average number of bivalents at MI per PMC in different seedlings was therefore relatively small. The largest range of bivalents per PMC (7 to 18) was found in the seedling b54-17. The bivalents at MI were mostly rod-shaped and loosely associated (Fig. 3 a, b). Because of the small size of the chromosomes, the exact number of chiasmata per bivalent at MI could not be determined; ring bivalents were observed only rarely. The seedling Y14-56, of different origin, had essentially the same bivalent formation.

The mean number of univalents varied from 1.23 in T6-38 to 7.58 in in b54-17. The largest range (0 to 19) occurred in b54-17 and the smallest (0 to 8) in T6-44. The average univalents per PMC in Y14-56 was slightly over 2, and the range of the univalents was relatively narrower than in other F_1 hybrids. The univalents usually were not oriented in the equatorial plane at metaphase, and no equational separation was observed at AI. Trivalents, quadrivalents, and pentavalents were observed. The mean number of trivalents among the hybrids varied from 77 in the seedling T6-42 to 1.61 in Y14-56. The mean number of quadrivalents was below 0.5 per PMC, except in Y14-56 where the mean value reached 1.2.

The frequency distribution of pollen mother cells with univalents and bivalents at metaphase are presented in Table 6. About 38 per cent of the PMC were without univalents in T6-38. In contrast, plant b54-17 had only 3.22 per cent without univalents. If we assume normal disjunction of the rest of the chromosomal complement at AI, then the cells with one and two univalents at MI should yield some functional microspores. On this basis, more than 70 per cent of the PMC studied could have contributed to the pool of viable gametes in each seedling, with the exception of b54-17 in which there were only about 6 per cent able to give viable gametes.

Bivalent formation recorded at first metaphase was highly variable among plants. Thus, PMC with 19 bivalents were found only in T6-42, where more than

Table 6

Frequency distribution of PMC in relation to bivalents and univalents at MI

Clone	Percentage of PMC with indicated number of univalents							
	0	1—2	3—4	5—6	7—8	9—10	11—12	Over 13
T6—38	38.1	52.3	0.0	0.0	4.8	0.0	4.8	0.0
T6—42	20.7	42.7	18.7	2.7	2.7	1.3	1.3	0.0
T6—44	28.6	42.8	14.3	7.1	14.3	0.0	0.0	0.0
b54—17	3.2	3.2	12.9	17.9	25.8	17.7	9.7	8.1
Y14—56	11.1	61.1	11.1	11.1	5.5	0.0	0.0	0.0

	Percentage of PMC with indicated number of bivalents								
	19	18	17	16	15	14	13	12	11
T6—38	0.0	14.3	23.8	4.8	38.1	9.5	0.0	9.5	0.0
T6—42	8.1	25.8	24.2	25.8	12.9	14.5	6.4	3.2	0.0
T6—44	0.0	0.0	28.6	35.7	7.1	0.0	14.3	7.1	7.1
b54—17	0.0	4.8	3.2	11.3	11.3	14.5	20.1	9.7	24.2[1])
Y14—56	0.0	5.5	0.0	11.1	22.2	27.8	5.5	11.1	16.6

[1]) This value is for eleven and less.

Table 7

Frequency distribution of the PMC in relation to laggards and chromatin bridges at AI and AII of diploid F_1 VR hybrids

Vine	PMC analyzed	Number of laggards and bridges													
		0		1		2		3		4		5		6	
		L[1]	B[2]	L	B	L	B	L	B	L	B	L	B	L	B
						AI									
T6—38	16	6		1	0	6	2	1	0	0	0	0	0	0	0
T6—42	27	18		2	1	3	2	1	0	0	0	0	0	0	0
b54—17	24	1		1	6	2	0	3	2	4	5	0	0		
Y14—56	12	4		4	0	4	0	0	0	0	0	0	0		
						AII									
T6—42	9	6	9	0	0	2	0	0	0	1	0	0	0	0	0
b54—17	3	2	1	0	0	0	0	0	2	0	0	0	0	1	0

[1]) L = laggards; [2]) B = bridges.

30 per cent had 18 or more bivalents. The clones T6-38 and T6-44 had relatively high percentages of PMC with 17 and 18 bivalents. In contrast, clone b54-17 had some 8 per cent of the PMC with 17 and 18 bivalents, with 13 bivalents in the majority. The pattern was similar in Y14-56, but the 14 bivalent class was most frequent.

F_1 vines had meiotic irregularities in the first anaphase (Fig. 3 c, d). The results are summarized in Table 7. In T6-42, more than 60 per cent of the cells were without laggards. At the opposite extreme was b54-17, in which almost all cells had some irregularity. Cells with four laggards were found, as well as cells with as many as four bridge-like irregularities. In T6-38 and Y14-56, about one third of the cells were without irregularities. Only a few cells were scored in AII. Some of them had the same irregularities as in AI. The irregularities of the chromosome disjunction in AI and AII must be considered as indicative rather than conclusive. The number of cells included in these studies was relatively small. Moreover, the chromatin bridges were hardly distinguishable from attenuated bivalents at AI. The same applies for fragments expected to accompany bridge formation. Thus, errors in classifying irregularities were likely.

3. Morphology of diploid F_1 hybrids

The two species possess contrasting morphological characters that may be used as genetical markers in studies of hybrid populations. These distinctive phenotypical traits are listed by PATEL and OLMO (1955).

The F_1 hybrids were vigorous and morphologically similar. During the growing season they had more the aspect of *V. vinifera* varieties. The shiny leaf of *V. rotundifolia* is a good genetical marker and is dominant in all F_1 progeny. By this marker alone one can easily distinguish diploid F_1 VR hybrids from the *V. vinifera* varieties.

More detailed studies of trunks and canes revealed other *V. rotundifolia* characters dominant in the F_1 hybrids. In all seedlings the bark adhered tightly to the trunk, the specific gravity of the wood was greater than 1.0, and striation of the cane surface was completely absent. The diaphragm was absent at the node and the pith was green. Lenticels were present on the cane bark of all seedlings, but the number was much reduced on vine T6-44. Canes of F_1 progeny were thicker in diameter than those of the *V. rotundifolia* varieties. Only tendrils were intermediate between the parent species (bifid).

Flower clusters were relatively small in size, averaging about 35 flowers. Leaf size was intermediate. With regard to lobing, only upper sinuses were found, indicating incomplete dominance of the *V. vinifera* character. Entire leaves were present in clone T6-44. One parent was heterozygous for this character, probably the *V. vinifera* parent.

The berry characteristics were *V. rotundifolia* type. The skin was very thick, the pulp mucilaginous, and the flavor musky. All progeny had colored fruits, an indication that the *V. rotundifolia* parent was homozygous for black color. The berries detached easily at complete maturity. The seeds were *V. rotundifolia* type, with short beak, shallow fosette, and creases on the ventral and dorsal sides. Inheritance of phenotypic characteristics in the F_1 clone Y14-56 was similar to that in the T6-series.

Discussion

All plants of the diploid VR hybrids proved that hybrids in the present investigation had some degree of fertility. This is the first instance known in which consistent fertility was obtained in the F_1 generation of VR hybrids.

Pollen fertility was lower than fertility of the ovule in the F_1 hybrids, not an unexpected result. This can be attributed to greater chromosomal irregularities in b54-17 and to the fact that female gametes *in situ* can tolerate more irregularities than can male ones.

Fertility of the F_1 hybrids in the T6-series was not far below that of standard commercial varieties of *V. vinifera*. RANDALL (1940) found wide variation in per cent berry and ovule set in reciprocal crosses of diploid *V. vinifera* varieties ('Muscat of Alexadria', 'Folle blanche', and 'Zinfandel'). The seedlings of the T6-series ranged in berry set between 2.4 and 22.4 per cent and ovule set between 0.6 and 5.9 ALLEY (1957) reported berry set between 10.3 and 33.0 and ovule set from 6.1 to 11.3 per cent in three diploid varieties of *V. vinifera* wine grapes. With regard to average seed number per berry the hybrids were well below standard varieties, ranging between 1.0 and 1.2. RANDALL's (1940) diploid varieties had an average of 1.9 seeds per berry.

Fertility of the F_1 hybrids of the T6-series was in sharp contrast to that reported by DETJEN (1919) and PATEL and OLMO (1955) who noted almost total sterility in the F_1 generation. OLMO (unpublished) observed more than 200 mature vines annually over a period of 4 years and found no seeds. Occasionally a few small berries developed, but these were parthenocarpic. Pollen viability tests disclosed complete sterility. The difference in fertility between these two populations (T6-series and OLMO's) reflects the influence of the maternal *(V. vinifera)* genotype on the outcome of hybridization. The male parents used in both series of crosses were the same clones and the *V. rotundifolia* varieties were genotypically rather uniform. DETJEN (1919) mentioned that success in hybridization was affected by varieties of both species used in the cross. PATEL and OLMO (1955) and DERMEN (1964) reported that seed setting was influenced by the *V. vinifera* genotype. It seems plausible to attribute this difference in hybrid behavior to the contribution of the *V. vinifera* genotype.

In crosses of distantly related species in other plant genera, cases have been reported wherein success may have been largely a result of using certain varietal genotypes (KARPECHENKO, 1927; SEARS, 1941 b; GREENLEAF, 1941; O'MARA, 1953; SHAVER, 1962). However, we are not aware of any case similar to the present one, whereby changing the genotype in one of the species altered so drastically the results of hybridization, i. e., from a state of complete sterility to one of almost complete fertility. Two explanations can be offered for such an unusual behavior of *V. vinifera* clones. First, cultivated *V. vinifera* varieties have been propagated vegetatively for a long

time. It is possible in this way to accumulate a large number of mutations in a single clone. Assuming that *Vitis* species have had a common origin, then the kind and amount of mutational load in each clone may affect hybridization with *V. rotundifolia*. Secondly, cultivated *V. vinifera* may have arisen from different sub-species having different genomic relations with ancestral *V. rotundifolia*.

In the partially fertile F_1 diploid hybrid (T6-series)there was a positive correlation between chromosomal pairing and fertility of the hybrids. For example, b54-17, with many univalents, had a very high degree of pollen sterility; whereas T6-38, with a very low average of univalents, had a high degree of ovule fertility. It is well established that chromosomal behavior is genetically controlled (REES, 1961 b). That failure of chromosomes to pair may be caused by lack of homology between the chromosomes is indicated by the reduction in the average chiasma number in the F_1 hybrids in comparison to that in the parents, and by the occasional observation of unpaired segments of chromosomes at pachytene.

In diploid hybrids of the T6-series, trivalents and quadrivalents were frequently found. Presence of the multivalent configurations in diploid hybrids can be explained either by structural changes of the chromosome complement in the parental species or by homeologous pairing. It has been suggested that *Vitis* species are ancient secondary polyploids (OLMO, unpublished). If this is the case, then it is possible that genetic factors which control pairing of the chromosomes are upset by bringing together two distantly related genomes, and multivalent formation occurs.

RILEY (1960) has reported that the presence of chromosome V in hexaploid wheat (*Triticum aestivum*) permits homeologous pairing, but, in its absence (nullosomic for both V's), multivalents are formed in most PMC's (homeologous pairing). Apparently diploidizing factors are located on this chromosome.

On the other hand, *Vitis* species behave cytologically and genetically as completely diploidized species. The diploid ratios obtained for genetic factors and the fact that so far no aneuploidy has been reported in *Vitis* are evidence for diploidy. Some of the seedlings had high frequencies of irregularities in AI and AII. Bridge-like configurations and laggards were noticed in seedlings of b54-17. Such chromosomal abnormality might be caused by structural heterozygosity (inversions and translocations), by breakage and reunion due to unbalanced genotypes, or by attenuation of the bivalents. We are therefore inclined to believe that multivalent formation is probably due to structural rearrangements of the chromosomes.

Regarding hybridization of the two species, it is well established that the species cross only when *V. vinifera* is used as a female parent (WYLIE, 1870; DETJEN, 1919; PATEL and OLMO, 1955). For such a unique pattern, the term "unilateral cross-incompatibility" may be used, although in the literature this term is restricted to the phenomenon wherein mating of self-fertile to self-sterile species fails (MARTIN, 1964). However, since only one of the reciprocal crossing combinations is unsuccessful, and the breakdown occurs prior to fertilization, (PATEL and OLMO, 1955) usage of this descriptive term is justified. The F_1 hybrids are crossable among themselves. With *V. vinifera*, they are reciprocally crossable. Pollinated with *V. rotundifolia*, they produce fruit sets sometimes comparable to those from pollination with *V. vinifera*. These findings confirm the results obtained by DUNSTAN (1962, 1964). The reciprocal cross, i. e., *V. rotundifolia* pollinated with the pollen of F_1 hybrids, resulted in failure. Although the number of pollinated flowers was relatively few (487), we can conclude that this backcross cannot be made as readily as with *V. vinifera*. Gametes having some *V. vinifera* chromosomes are inhibited just as are pure *V. vinifera* gametes. On

the probability of random chromosome assortment, gametes with 20 *V. rotundifolia* chromosomes are formed in a ratio $(\frac{1}{2})19 \times (\frac{1}{2})^1) = 1 : 1{,}572{,}864$. Randall (1940) estimated that in hand pollination of grapes more than 3,000 pollen grains are placed on the stigmatic surface. Even with such an amount of pollen, the chance for pure *V. rotundifolia* gametes to function is very low in a small sample of pollinated flowers. In addition, a high sterility (80 per cent) characterized the male F_1 hybrid used in pollination.

The difference in reciprocal crosses led Patel and Olmo (1955) to suggest that the causes of such rigid, one-sided cross-incompatibility might be cytoplasmic in nature. Critical evidence concerning this problem came in the present work from the crossing behavior of Y14-56. The fact that pollination with clones of both species resulted in berry set may be considered as conclusive evidence that the cause of this cross-incompatibility is not extranuclear in nature. Crossability between the two species evidently is controlled by nuclear factors.

Summary

1. An unusual, partially-fertile population of diploid *Vitis vinifera* × *V. rotundifolia* F_1 hybrids is described. These hybrids are partially fertile with varieties of both parental species.
2. It is suggested that the success of hybridization, as measured by the fertility of the F_1 hybrids, depends on the *V. vinifera* clone originally used as a female parent.
3. In meiosis of these F_1 plants, average bivalent formation varies from 13.1 to 15.6 per cell at MI. There is a correlation in the diploid VR hybrids between chromosomal pairing at MI and fertility of the vines.
4. Some *V. rotundifolia* characters are dominant in diploid F_1 hybrids.
5. In regard to crossability, the F_1 hybrids are reciprocally crossable with *V. vinifera* varieties, but can only serve as female parents with *V. rotundifolia*.
6. Evidence from breeding tests indicates that incompatibility between *V. rotundifolia* × *V. vinifera* is not due to cytoplasmic inheritance, but is caused by nuclear factors.

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[1]) Assuming probability of $\frac{1}{2}$ for odd chromosome to be included in the gametes.

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