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Conversion and determination of sex in Vitis vinifera L. (sylvestris)[1]

by

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Introduction

Occasional sex conversion from functionally male to functionally hermaphroditic flowers has been reported to occur in male vines of *Vitis vinifera* L. *(sylvestris)* (Baranov and Rajkova 1929, Negi and Olmo 1970) and other *Vitis* species (Bethmann 1939, Levadoux 1946). Negi and Olmo (1970) found that sex conversion in male *V. vinifera (sylvestris)* clone 030—44 was not due to somatic mutation.

Based on results obtained in crosses involving the three main flower sex types (♂, ♀ and ☿), different authors have proposed different schemes for sex determination in *Vitis* (Valleau 1916, Müller-Thurgau and Kobel 1924, Negrul 1936, Breider and Scheu 1938, Oberle 1938, Bethmann 1939, Levadoux 1946). Male heterogamety was assumed by several authors (Valleau 1916, Müller-Thurgau and Kobel 1924, Negrul 1936, Breider and Scheu 1938, Oberle 1938, Levadoux 1946). The only evidence of this was produced by Bethmann (1939) who obtained an approximate ratio of 3 males to 1 female in the progeny of self-pollinated "male" vines of the interspecific hybrids 'Couderc 1616' *(Solonis × V. riparia)* and 'Teleki C' *(V. berlandieri × V. riparia)*. This has, however, not been verified in any pure *Vitis* species. Furthermore, no one has so far reported the sex determining mechanism in wild *Vitis vinifera* L. *(sylvestris)*.

The purposes of the present investigation were to conduct further studies on the nature of sex conversion in male *V. vinifera (sylvestris)* clone 030—44 and its progeny and to establish a cytogenetic basis of sex determination in *Vitis vinifera* L. *(sylvestris)*.

Materials and Methods

A summary of crosses used for the present studies is presented in Table 1. The methods already described by Negi and Olmo (1970) were used for classification of flower types and for determining the percentages of seeded berry set and fruitful clusters on individual male vines.

Classification of male vines:

Two classes of male vines may be broadly differentiated: (1) Pure male, which consistently produces only functionally male flowers of types 1 to 3 and are unfruitful; and (2) hermaphroditic male, which besides producing function-

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Table 1

Summary of crosses used to study the nature of sex conversion and inheritance of flower types in the male *V. vinifera* clone 030—44 and its progeny

Population number	Female parent	Male parent	Total number of seedlings grown	Series
94—125	030—44	030—34	264	'a'
104	Z30—45	a26—27	79	'e'
105	Z30—45	a26—30	76	'e'
106	Z30—45	a26—35	19	'e'
107	Z30—45	a26—48	33	'e'
108	Z30—45	a26—63	23	'e'
109	Z30—45	a26—66	30	'e'
110	Z30—45	a26—83	12	'e'
111	Z30—45	a26—90	15	'e'
308	Hunisa	a24—93	209	—
309	Hunisa	a24—95	21	—
310	Hunisa	a25—7	24	—
312	Hunisa	a25—27	22	—
313	Hunisa	a25—35	14	—
314	Hunisa	a25—39	22	—
315	Hunisa	a25—45	21	—
316	Hunisa	a25—49	17	—
318	Hunisa	a25—54	17	—
319	Hunisa	a25—62	20	—
320	Hunisa	a25—65	20	—
321	Hunisa	a25—69	23	—
323	Hunisa	a25—75	24	—
324	Hunisa	a25—93	13	—
325	Hunisa	a25—104	19	—
326	Hunisa	a26—51	22	—
328	Hunisa	a26—91	21	—
330	Hunisa	M5:13	78	—
331	Hunisa	M5:25	50	—
341	033—60	M5:29	20	—
355	034—46	M5:33	23	—

ally male flowers of types 1 to 3, produces varying numbers of functionally hermaphroditic flowers of type 4 and thus is partially fruitful (Negi and Olmo 1970).

Crossing and related techniques:

The clusters of male and female vines to be used in crossing were bagged before anthesis to avoid contamination. Where possible the female vines were caged beforehand. Pollen was collected from the male clusters and dusted on the clusters of a receptive female which were then rebagged.

The viability of pollen grains was determined by the acetocarmine staining method and by germination in hanging drop cultures in a 20 % sucrose solution.

Seeds that sank in water were planted in flats containing sterilized soil, sand and peat moss (3:1:1). The flats were kept outside during winter for 3 months

of stratification and were then moved into the greenhouse at 80° F for germination. Some seedlings were transplanted in open beds in the greenhouse. In order to obtain early flowering, buds were taken from the seedlings in the greenhouse and were T-budded on mature vines in the vineyard. Early flowering was also obtained by cutting back the seedlings in the month of July and August, followed by removal of the axillary laterals, and thus forcing the fruiting buds to grow prematurely.

Cytology:

Buds for meiotic chromosome studies were collected at the proper stages from vines of the clones 030—44 (♂), 033—60 (♀), and 034—46 (♀), and from homozygous and heterozygous males as well as females in the 030—44 selfed progeny. These buds were killed and fixed immediately in a mixture of chloroform, acetic acid and absolute alcohol (2:1:1). To obtain rapid penetration of the solution, air was evacuated from the vials in an aspirator. After 48 hours of fixation, the buds were transferred to 70 % ethyl alcohol for storage at 15° F until used. The acetocarmine squash technique was used for preparing temporary slides.

Chi-square analysis was used as a statistical test of significance of the segregations.

Experimental Results

Breeding results on selfing the male clone 030—44:

A progeny of 264 vines were grown from the seeds collected from open-pollination of 7 hermaphroditic male vines of the clone 030—44 in 1960. Considering the structure of grape flowers, it is highly probable that the functionally hermaphroditic flowers on these vines were self-pollinated. This conclusion is further supported by the fact that no morphological characters typical of hybrids with other *V. vinifera* were observed in the progeny. The seedling vines were very uniform in growth and morphology. The flower sex types and fruitfulness of 242 of the progeny were studied in 1966, 1967 and 1968. The remaining 22 vines could not be classified as they did not blossom. The data presented in Table 2 show that 184 (76.0 %) were males and 58 (24.0 %) were females. No completely hermaphroditic vines were found in the progeny. The ratio of pure males to hermaphroditic males was not clear cut and it varied from year to year: 7:1 in 1966, 10:1 in 1967 and 11:1 in 1968. The ratio of males to females was distinct and constant, i. e., 3.2:1.0 in all 3 years of observations, which is very close to the expected ratio of 3:1.

These data suggest that (1) conversion of functionally male flowers (types 1-3) to functionally hermaphroditic flowers (type 4) in the clone 030—44 is not due to mutation of the major gene for sex; (2) in *Vitis vinifera* L. *(sylvestris)*, the male sex is heterogametic and is inherited as a simple Mendelian factor, maleness being dominant. Homozygous males are equally as viable as heterozygous males.

Table 3 lists those F_1 male seedlings in the 030—44 selfed progeny which had variable fruit set. The percentage of hermaphroditic males varied from year to year; being 13.6 in 1966, 10.2 in 1967 and 8.9 in 1968; in a total population of 184 male seedlings. Some seedlings classified as hermaphroditic males in one season became pure males in another season or seasons. However, 9 male seed-

Table 2

Sex ratios in the progeny of a self pollinated male *Vitis vinifera* clone 030—44. Total number of seedlings: 264

	Males			Females	Undetermined	Ratio of pure males to hermaphroditic males	Ratio of males to females	χ^2 analysis of sex ratio of males to females (1966—1968)		
	Pure males	Hermaphroditic males	Total males					χ^2	d.f.	P
1966	162	22	184	58	22	**7:1**	3.2:1:0			
1967	167	17	184	58	22	10:1	3.2:1.0	0.14	1	0.6—0.7
1968	169	15	184	58	22	11:1	3.2:1.0			

lings numbered a24—74, a24—95, a25—10, a25—63, a25—65, a25—75, a25—112, a26—18 and a26—63 were hermaphroditic males in all 3 years of observation. The percentages of fruitful clusters and seeded berry set fluctuated from season to season on the same male seedling and from seedling to seedling in the same season. Of 184 male seedlings, 152 were pure males in all 3 seasons. This is in contrast to the vines of the first and second vegetative propagation of 030—44 (Negi and Olmo 1970), where no vine remained pure male in 2 consecutive years. These results suggest that sex conversion is due to the influence of genetic modifiers, as well as to environmental conditions, both local and seasonal.

These data on frequency of natural sex conversion in male seedlings demonstrate that a particular male seedling must be classified for several years in order to determine whether or not it has the capacity to produce functionally hermaphroditic flowers and mature seeded berries.

Breeding results of some F_1 males of 030—44 crossed with unrelated *V. vinifera* females:

In 1964, from the 030—44 selfed progeny, 7 pure male seedlings numbered a26—27, a26—30, a26—35, a26—48, a26—66, a26—83, a26-90 and 1 hermaphroditic male a26—63 (all chosen at random) were crossed with a female *V. vinifera*, 'Z30—45', a selection known to be completely male sterile. Similarly, 14 other pure male seedlings numbered a24—93, a25—7, a25—11, a25—27, a25—35, a25—45, a25—49, a25—53, a25—54, a25—62, a25—72, a25—104, a26—51, a26—56, and 7 hermaphroditic males numbered a24—95, a25—39, a25—65, a25—69, a25—75, a25—93, and a26—91 were crossed with the female *V. vinifera* 'Hunisa'. Almost all the progeny of the former crosses blossomed in 1967 and thus were classified for flower sex types and fruitfulness in 1967 and 1968. The progeny of the latter crosses did not flower in 1967. However, except in 4 combinations involving pollen parents a25—11, a25—53, a25—72, and a26—56, enough seedlings blossomed to obtain flower sex classification in October, 1968. But the fruitfulness of male seedlings could not be determined because of removal of the planting. Data on sex ratios in the progeny of these crosses are presented in Table 4. Again the ratio of pure males to hermaphroditic males in every cross

Table 3

Fruitfulness and average number of normal seeds per berry of 32 F_1 male seedlings resulting from self pollination of the clone 030—44

Seedling number	Fruitful clusters, %			Seeded berry set, %			Normal seeds per berry, avg.		
	1966	1967	1968	1966	1967	1968	1966	1967	1968
a24—74	2.9	1.4	4.6	0.08	0.01	0.04	1.00	1.00	0.88
a24—78	1.7	0.0	0.0	0.02	0.00	0.00	1.00	—	—
a24—95	7.5	2.8	10.5	0.13	0.01	0.18	1.00	0.80	1.10
a24—111	3.3	0.0	1.7	0.03	0.00	0.08	0.92	—	0.93
a25—1	1.7	1.6	0.0	0.01	0.02	0.00	0.80	1.00	—
a25—10	5.9	3.8	1.7	0.06	0.14	0.13	0.96	1.01	1.00
a25—18	1.7	0.0	0.0	0.02	0.00	0.00	0.83	—	—
a25—39	0.0	0.0	9.2	0.00	0.00	0.08	—	—	0.94
a25—56	0.0	0.0	3.4	0.00	0.00	0.16	—	—	1.05
a25—63	8.7	5.0	9.3	0.18	0.07	0.23	1.00	1.00	1.00
a25—65	10.0	12.5	3.7	0.17	0.81	0.12	1.01	1.01	1.10
a25—69	1.7	0.0	0.0	0.01	0.00	0.00	1.00	—	—
a25—75	10.1	8.9	3.3	0.18	0.22	0.11	1.00	0.98	1.12
a25—93	1.7	1.5	0.0	0.03	0.002	0.00	0.92	1.00	—
a25—96	0.0	5.8	0.0	0.00	0.04	0.00	0.00	0.87	—
a25—100	1.8	0.0	0.0	0.02	0.00	0.00	0.85	—	—
a25—108	1.5	0.0	0.0	0.02	0.00	0.00	0.90	—	—
a25—112	5.9	6.2	1.7	0.10	0.11	0.05	1.02	1.07	0.95
a26—3	1.7	1.6	0.0	0.01	0.003	0.00	1.20	1.00	—
a26—18	10.1	7.1	9.3	0.17	0.08	0.11	1.02	1.03	0.94
a26—38	0.0	3.0	0.0	0.00	0.01	0.00	—	1.00	—
a26—54	0.0	3.1	0.0	0.00	0.04	0.00	—	0.94	—
a26—61	1.7	0.0	0.0	0.02	0.00	0.00	1.00	—	—
a26—63	10.0	3.3	7.7	0.17	0.01	0.08	1.00	1.00	1.38
a26—78	1.7	0.0	1.8	0.03	0.00	0.03	1.00	—	0.83
a26—86	0.0	3.1	3.3	0.00	0.02	0.01	—	0.90	1.00
a26—89	1.7	0.0	0.0	0.01	0.00	0.00	1.10	—	—
a26—91	0.0	0.0	3.3	0.00	0.00	0.11	—	—	1.09
a26—94	0.0	0.0	1.7	0.00	0.00	0.04	—	—	1.07
a26—97	0.0	2.0	0.0	0.00	0.06	0.00	—	1.00	—
a26—99	1.7	0.0	0.0	0.02	0.00	0.00	0.83	—	—
a26—109	1.6	0.0	0.0	0.02	0.00	0.00	0.89	—	—

studied was not clear cut, and it varied from year to year in populations e104, e105, e106, e107 and e111. The ratios of males to females of each population were in either one of two categories. In one, all males and no females; in the other approximately equal numbers of males and females. Nine of the test progenies were composed exclusively of males while 15 showed the ratio of the second category, i. e., they showed segregations of males and females in the ratio of 1:1 or not deviating from it significantly. In the progeny of Z30—45 × a26—48 (population e107), an abnormal segregation occurred, i. e., 31 males to 2 females. This anomalous segregation may have been due to mislabelling of seedlings at planting time, but can not be satisfactorily explained. With this exceptional

Table 4

Sex ratios in the progeny obtained by crossing *V. vinifera* females with some F_1 males from 030—44 selfed

Population number	Female parent	Male parent	Males						Females		Undetermined		Ratio of pure males to hermaphroditic males		Ratio of males to females		χ^2 analysis of sex ratios of males to females in the segregating families (1968)		
			Pure males		Hermaphroditic males		Total males												
			1967	1968	1967	1968	1967	1968	1967	1968	1967	1968	1967	1968	1967	1968	χ^2	d.f.	P
e104	Z30—45	a26—27	11	5	66	72	77	77	0	0	2	2	1:6	1:14	77.0:0.0	74.0:0.0			
e105	Z30—45	a26—30	20	12	54	62	74	74	0	0	2	2	1:3	1:5	74.0:0.0	77.0:0.0			
e106	Z30—45	a26—35	0	2	8	6	8	8	11	11	0	0	0:8	1:3	1.0:1.4	1.0:1.4	0.48	1	0.4—0.5
e107	Z30—45	a26—48	7	2	24	29	31	31	2	2	0	0	1:3	1:14	15.5:1.0	15.5:1.0	29	1	<0.0001
e108	Z30—45	a26—63	1	1	13	13	14	14	9	9	0	0	1:13	1:13	1.6:1.0	1.6:1.0	1.08	1	0.2—0.3
e109	Z30—45	a26—66	0	0	30	30	30	30	0	0	0	0	0:30	0:30	30.0:0.0	30.0:0.0			
e110	Z30—45	a26—83	0	0	12	12	12	12	0	0	0	0	0:12	0:12	12.0:0.0	12.0:0.0			
e111	Z30—45	a26—90	2	0	3	5	5	5	10	10	0	0	1:1	0:5	1.0:2.0	1.0:2.0	1.66	1	0.2
308	Hunisa	a24—93	—	—	—	—	—	62	—	50	—	94	—	—	—	1.3:1.0	1.96	1	0.1—0.2
309	Hunisa	a24—95	—	—	—	—	—	4	—	4	—	13	—	—	—	1.0:1.0			
310	Hunisa	a25—7	—	—	—	—	—	19	—	0	—	5	—	—	—	19.0:0.0			
312	Hunisa	a25—27	—	—	—	—	—	7	—	5	—	10	—	—	—	1.4:1.0	0.34	1	0.5—0.6
313	Hunisa	a25—35	—	—	—	—	—	2	—	2	—	10	—	—	—	1.0:1.0			
314	Hunisa	a25—39	—	—	—	—	—	5	—	5	—	12	—	—	—	1.0:1.0			
315	Hunisa	a25—45	—	—	—	—	—	13	—	0	—	8	—	—	—	13.0:0.0			
316	Hunisa	a25—49	—	—	—	—	—	11	—	0	—	6	—	—	—	11.0:0.0			
318	Hunisa	a25—54	—	—	—	—	—	10	—	5	—	2	—	—	—	2.0:1.0	1.66	1	0.2—0.3
319	Hunisa	a25—62	—	—	—	—	—	12	—	6	—	2	—	—	—	2.0:1.0	2.00	1	0.1—0.2
320	Hunisa	a25—65	—	—	—	—	—	9	—	5	—	6	—	—	—	1.8:1.0	1.14	1	0.2—0.3
321	Hunisa	a25—69	—	—	—	—	—	5	—	5	—	13	—	—	—	1.0:1.0			
323	Hunisa	a25—75	—	—	—	—	—	6	—	5	—	13	—	—	—	1.2:1.0	0.90	1	0.3—0.4
324	Hunisa	a25—93	—	—	—	—	—	5	—	4	—	4	—	—	—	1.2:1.0	1.12	1	0.2—0.3
325	Hunisa	a25—104	—	—	—	—	—	14	—	0	—	5	—	—	—	14.0:0.0			
326	Hunisa	a26—51	—	—	—	—	—	15	—	0	—	7	—	—	—	15.0:0.0			
328	Hunisa	a26—91	—	—	—	—	—	4	—	4	—	13	—	—	—	1.0:1.0			

Table 5

Percentage of hermaphroditic males (of the total number of male seedlings) in each progeny obtained by crossing *V. vinifera* female Z30—45 with 8 different F_1 males from 030—44 selfed

Population number	Female parent	Male parent	Total number of mature male seedlings	Hermaphroditic males % 1967	1968
e104	Z30—45	a26—27	77	85.7	93.5
e105	Z30—45	a26—30	74	73.0	84.0
e106	Z30—45	a26—35	8	100.0	75.0
e107	Z30—45	a26—48	31	77.4	93.6
e108	Z30—45	a26—63	14	92.9	92.9
e109	Z30—45	a26—66	30	100.0	100.0
e110	Z30—45	a26—83	12	100.0	100.0
e111	Z30—45	a26—90	5	60.0	100.0

population excluded, of 24 F_1 males tested, 9 were homozygous male to 2 heterozygous males.

Sex conversion in male seedlings in the progeny of each of the crosses of female *V. vinifera* 'Z30—45' with 7 pure males (a26—27, a26—30, a26—35, a26—48, a26—66, a26—83, a26—90) and one hermaphroditic male (a26—63) from 030—44 selfed progeny was studied in detail in 1967 and 1968. Table 5 shows that the percentages of hermaphroditic males (of the total number of male seedlings) in 1967 and 1968, respectively, were 85.7 and 93.5 in population e104; 73.0 and 84.0 in population e105; 100.0 and 75.0 in population e106; 77.4 and 93.6 in population e107; and 60.0 and 100.0 in population e111. In population e108, 92.9 % of the males and in populations e109 and e110, 100.0% of the males were hermaphroditic males in both years. It is interesting to note that unlike 030—44 selfed progeny, more than 60 % of the male seedlings in the progeny of each of these crosses were hermaphroditic males in both years. The percentage of fruitful clusters, % seeded berry set and average normal seeds per berry in these hermaphroditic males (Table 6*) were, in general, significantly higher than those in the vines of first and second vegetative propagation of clone 030—44 (Negi and Olmo 1970) and in hermaphroditic males of 030—44 selfed progeny. In some years, certain hermaphroditic males of these crosses, with more than 9 % seeded berry set, appeared as fruitful as hermaphroditic vines of cultivated varieties. Such seedlings noted in Table 6 are: e25—120, e26—16, e26—44, e26—52, e26—56, e27—3, e27—51, e28—54, e28—57, e28—60, e29—10, e29—21, e29—32, e29—33, e29—41, e29—46, e29—54, e29—56 and e29—64. In population e104, 3 seedlings; in population e105, 8 seedlings; in population e107, 2 seedlings and in population e108, 1 seedling were pure males in both years of observation. Thus, the increase in the number of hermaphroditic males and increase in their fruitfulness on outcrossing (female × F_1 males) and occurrence of pure males for 2 consecutive years in the progeny of some of these outcrosses lend further evidence that the degree of sex conversion from functionally male to functionally

*) Data on percentage of fruitful clusters, % seeded berry set and average normal seeds per berry were obtained in 1967 and 1968 in all male seedling vines in the progeny of each of the crosses of female Z30—45 with 8 different F_1 males from 030—44 selfed. Table 6 contains such data for only a few representative seedlings.

Table 6

Fruitfulness and average number of normal seeds per berry of some male seedlings (e series) in each cross of *V. vinifera* female Z30—45 with 8 different F_1 males from 030—44 selfed

Seedlings of e series	Fruitful clusters, %		Seeded berry set, %		Normal seeds/berry, avg.	
	1967	1968	1967	1968	1967	1968
Z30—45 × a26—27 (Pop. e104)						
e25—120*	95.0	88.2	11.71	5.75	1.05	1.10
e25—132	23.8	93.9	0.49	3.67	1.10	1.00
e26—8	0.0	0.0	0.00	0.00	—	—
e26—16*	94.4	70.0	13.67	1.31	1.25	1.75
e26—44*	95.0	86.1	9.71	3.50	1.55	2.05
e26—52*	95.4	93.5	9.75	5.02	1.80	2.50
e26—56*	93.7	77.8	11.31	3.76	1.80	1.70
e27—3*	94,7	90.5	9.43	5.99	1.55	1.55
Z30—45 × a26—30 (Pop. e105)						
e27—4	21.4	50.0	0.15	2.35	1.00	1.90
e27—8	37.5	28.6	0.26	0.66	1.00	1.25
e27—24	13.6	82.3	0.04	3.35	1.00	1.60
e27—38	66.7	80.0	0.85	5.89	1.00	0.85
e27—51**	81.8	93.9	11.50	12.31	1.80	1.80
e28—7	0.0	0.0	0.00	0.00	—	—
e28—14	0.0	46.1	0.00	0.63	—	1.00
Z30—45 × a26—35 (Pop. e106)						
e28—15	95.0	90.3	7.08	3.81	1.35	1.55
e28—25	20.0	50.0	0.21	1.20	0.90	1.00
e28—33	6.7	66.7	1.37	2.73	1.10	2.00
Z30—45 × a26—48 (Pop. e107)						
e28—34	66.7	90.9	1.35	3.03	1.75	1.00
e28—47	0.0	0.0	0.00	0.00	—	—
e28—54*	95.0	90.9	14.39	2.86	2.40	2.75
e28—57*	50.0	96.3	4.01	11.02	1.35	2.50
e28—60*	96.1	96.1	11.20	4.63	2.20	2.75
e29—2	25.0	23.1	0.28	0.37	1.75	1.00
Z30—45 × a26—63 (Pop. e108)						
e29—5	50.0	73.3	1.14	4.07	1.25	2.00
e29—10*	92.6	92.7	9.32	6.11	1.40	2.85
e29—10*	91.7	97.1	11.26	7.94	2.20	2.05
e29—26	75.0	62.5	2.41	1.47	1.20	1.25
Z30—45 × a26—66 (Pop. e109)						
e29—27	70.8	52.4	2.29	1.16	1.85	1.50
e29—32*	96.8	98.7	10.82	4.84	1.70	2.00
e29—33*	92.6	96.7	9.88	3.48	2.05	2.50
e29—41*	75.0	77.8	3.08	17.43	1.70	2.50
e29—46*	89.5	89.4	10.17	5.62	2.40	2.75
e29—54*	90.0	88.6	0.73	11.60	1.05	2.65
e29—56*	81.2	98.6	12.95	5.33	1.90	2.50
e29—59	92.3	96.1	4.83	7.09	1.85	2.00
Z30—45 × a26—83 (Pop. e110)						
e29—60	71.4	67.6	0.98	2.04	1.00	1.15
e29—64*	94.7	85.7	9.72	6.86	1.45	2.25
e30—3	27.8	58.8	0.19	1.06	1.00	1.00
e30—8	85.7	84.1	7.60	3.60	1.30	1.10
Z30—45 × a26—90 (Pop. e111)						
e30—9	20.0	60.0	0.08	3.08	1.00	1.20
e30—11	0.0	50.0	0.00	0.88	—	1.80
e30—21	6.7	22.2	0.08	0.09	0.92	0.92

*) Hermaphroditic males with more than 9 % seeded berry set in one of the two years of observation.

**) Hermaphroditic male with more than 11 % seeded berry set in both the years of observation.

hermaphroditic flowers is influenced by genetic modifiers. The wide variation in percentages of fruitful clusters and seeded berry set from season to season on the same male seedling and from seedling to seedling in the same season

Table 7

Flower types and pollen viability of some homozygous and heterozygous males in the 030—44 selfed progeny

Male seedling	Genetic constitution	Flower types								Stainability test (1968)			Germination test (1968)		
		1964		1966		1967		1968		Total pollen grains observed	Pollen grains stained	Stained normal pollen grains, %	Total pollen grains counted	Pollen grains germinated	Pollen germination %
		Majority	Minority	Majority	Minority	Majority	Minority	Majority	Minority						
a26—27	Homozygous	1,2	3	1,2	3	1,2	3	1,2	3	600	533	88.8	600	226	37.7
a26—30	Homozygous	1,2	3	1,2	3	1,2	3	1,2	3	600	541	90.2	600	192	32.0
a26—35	Heterozygous	1,2	3	1,2	3	1,2	3	1,2	3	600	551	91.8	600	200	33.3
a26—63	Heterozygous	1,2,3	4	1,2,3	4	1,2,3	4	1,2,3	4	600	548	91.3	600	224	37.3
a26—66	Homozygous	1,2	3	1,2	3	1,2	3	1,2	3	600	555	92.5	600	210	35.0
a26—83	Homozygous	1,2	3	1,2	3	1,2	3	1,2	3	600	531	88.5	600	220	36.7
a26—90	Heterozygous	1,2	3	1,2	3	1,2	3	1,2	3	600	562	93.7	600	211	35.2

emaphasizes the important role of still unknown environmental conditions on sex conversion.

Flower types and pollen viability of some homozydous and heterozygous males in the 030—44 selfed progeny:

Data on flower types of 4 homozygous males numbered a26—27, a26—30, a26—66, a26—83 and 3 heterozygous males numbered a26—35, a26—63 and a26—90 were obtained in 1964, 1966, 1967 and 1968. Pollen viability of these seedlings was also tested in 1968. The data are presented in Table 7. The heterozygous male a26—63 had the majority of flowers of types 1 to 3 and a few functionally hermaphroditic flowers of type 4 in all 4 years. Other heterozygous males and all the homozygous males had flowers of types 1 und 2 predominantly and a small number of type 3 flowers in all 4 years. Thus, homozygous males can not be differentiated from heterozygous males on the basis of their flower types. Percent normal pollen grains, as detected by staining and the % pollen germination were not significantly different in these 2 groups of males. Morphological characteristics of the homozygous and heterozygous males were also similar and no distinguishing features could be found, even though the seedlings were grown side by side.

Sex ratios in the progeny obtained by crossing some *V. vinifera* females with some hermaphroditic male vines of the clone 030—44:

Several hermaphroditic male vines of the clone 030—44 were crossed with female *V. vinifera* 'Hunisa' and with female siblings of 030—44. Some progeny did not blossom. However, a few seedlings blossomed in 4 such crosses in 1968 which were classified for flower sex types. The fruitfulness of male seedlings could not be determined because the vines had to be removed. The data presented in Table 8 show that the ratios of males to females fitted a 1:1 ratio with no significant deviations. No completely hermaphroditic vines were found in the progeny of these crosses. These findings support the conclusoins drawn above that (1) sex conversion in the clone 030—44 is not due to germinal mutation of the major gene for sex; (2) in *Vitis vinifera* L. *(sylvestris),* sex is inherited as a simple Mendelian factor.

Chromosomal studies in male and female vines of *V. vinifera* L. *(sylvestris):*

The availability of vines, homozygous and heterozygous for male transmission, as well as the female types, provided an exceptional opportunity to search for possible diffferences in morphology of sexdetermining chromosomes.

Table 8

Sex ratios in the progeny obtained by crossing some *V. vinifera* females with some hermaphroditic male vines of the clone 030—44 (1968)

Population number	Female parent	Male parent	Males	Females	Undetermined	Ratio of males to females	χ^2	χ^2 analysis d.f.	P
330	Hunisa	M5:13	24	20	34	1.2:1.0	0.36	1	0.5—0.6
331	Hunisa	M5:25	13	13	24	1.0:1.0			
341	033—60	M5:29	5	4	11	1.2:1.0	1.12	1	0.2—0.3
351	034—46	M5:33	7	6	10	1.2:1.0	0.76	1	0.3—0.4

Different stages of microsporogenesis were studied in male vines of the clone 030—44; female vines of the clones 033—60 and 034—46; homozygous and heterozygous males and females in the 030—44 selfed progeny. No differences in chromosomal behavior in the meiotic divisions of these vines could be established, nor was a heteromorphic pair of chromosomes observed. Thus, the sex determining chromosomes are not differentiated morphologically or structurally in *V. vinifera* L. *(sylvestris)* at the usual magnifications of the light microscope.

Discussion

The present results prove that the sex conversion from functionally male to functionally hermaphroditic flowers in the male *V. vinifera (sylvestris)* clone 030—44 is not due to germinal mutation of the major gene for sex. These findings rule out the postulation of NEGRUL (1936) and LEVADOUX (1946) that the occurence of hermaphroditic males in grapevines is due to mutation(s) of the male determining gene(s). OBERLE's (1938) assumption that the occurence of mutations in some of the many genetic factors controlling sex may be responsible for the occurence of hermaphroditic males also does not seem to be correct.

Findings on the frequency of sex conversion in the F_1 males of 030—44 selfed progeny, and in the males of each of the crosses of female 'Z30—45' with 8 different F_1 males of 030—44 suggest that the sex conversion is due to the influence of genetic modifiers as well as environmental conditions, both local and seasonal. However, it was not possible to estimate the number of modifying genes involved with the data at hand. The assumption of the influence of genetic modifiers on sex conversion in male vines is further supported by the following observations made by the authors. Three other male seedling sibs of the clone 030—44 showed natural sex conversion while one (034—54) did not in 3 consecutive years. Sex conversion has been observed in the male vines of certain other species such as *V. longii* '5427'*, '5429' and *V. riparia* '5421', '49175' and the species hybrid "Ganzin 1". It was not observed in male vines of certain species; namely, *V. cinerea* '5009', *V. girdiana* '3816' and *V. rotundifolia* 'Male'. Within the species *V. rupestris,* the clone 'Constantia' showed sex conversion while the clone 'du Lot' ('St. George') never showed this phenomenon during 5 consecutive years. Other workers (BREIDER and SCHEU 1938, BETHMANN 1939, LEVADOUX 1946) observed similar variations of sex expression in male clones and species of *Vitis.* BARRETT (1966) reported that sex conversion in the male *V. riparia* 'Deam' was not due to a mutation. He opined that it was due to environmental factors and/or gene complexes located on the autosomes. Sex conversion was reported to be affected by genetic modifiers and environmental factors in *Carica papaya* (HOFMEYR 1939 a, STOREY 1953) and in *Asparagus officinalis* (RICK and HANNA 1943). The latter authors speculated that at least two modifying genes influence this character in *Asparagus.* SNEEP (1953) assumed that sex conversion in *Asparagus officinalis* depends on some dominant factors.

Segregation of the 030—44 selfed progeny into 3 males and 1 female and obtaining a ratio of 1 homozygous male to 2 heterozygous males when 24 F_1 males of 030—44 were tested by crossing with *V. vinifera* females prove conclusively the following facts in *V. vinifera* L. *(sylvestris):* (1) the male sex is heterogametic and is inherited as a simple Mendelian factor, maleness being completely dominant; (2) homozygous males are as viable as heterozygous males;

*) The numbers refer to the accessions of individual vines from native populations.

(3) the pure males and hermaphroditic males are genetically alike as far as the major gene for sex determination is concerned. Obtaining an approximate ratio of 1 male to 1 female in each progeny of the crosses of some *V. vinifera* females with hermaphroditic male vines of 030—44 support the conclusion that sex in *V. vinifera* L. *(sylvestris)* in inherited as a simple Mendelian factor. Bethmann (1939) had obtained an approximate ratio of 3 males to 1 female in the progeny of self-pollinated hermaphroditic male vines of 'Couderc 1616' *(Solonis* × *V. riparia)* and 'Teleki C' *(V. berladieri* × *V. riparia).* His results also indicate that the male sex in these species hybrids is heterogametic and is inherited as a simple Mendelian factor, maleness being dominant. Barrett (1966), however, reported obtaining a ratio of 1 male to 1 female on selfing a hermaphroditic male *V. riparia* 'Deam'. This is in disagreement with the ratio obtained by Bethmann (1939) and that presented here. It is possible that he obtained this ratio because of insufficient progeny.

The data presented here in *Vitis vinifera* L. *(sylvestris)* are the first evidence in *Vitis* that homozygous males are as viable as heterozygous males. These two groups of males could not be differentiated on the basis of their flower types, pollen viability, and other morphological characteristics. Results parallel to the present were reportet in *Thalictrum* (Kuhn 1939) and *Asparagus officinalis* (Rick and Hanna 1943). In *Mercurialis annua* the homozygous males differed from heterozygous males by producing more sterile pollen (Gabe 1939, Kuhn 1939). In *Carica papaya,* homozygous males are inviable (Hofmeyr 1939 b, Storey 1953). As stated by Rick and Hanna (1943), the equal viability of homozygous and heterozygous males suggest a genetically active Y chromosome or male allelomorph bearing chromosome in these plant species in contrast to the frequently inert Y chromosome in animals. Thus the dioecious condition is apparently much more superficial in these plants than it is generally in animals. According to Westergaard (1958), plant species with viable homozygous males are the most primitive type and they may be not more (but probably also not less) than 2 mutational steps removed from their bisexual ancestor.

Chromosomal studies in homozygous and heterozygous males, as well as female vines of *V. vinifera* L. *(sylvestris),* revealed that there were no essential differences in chromosomal behaviour in the meiotic divisions of these vines, nor was a heteromorphic pair of sex chromosomes observed. None of the previous workers (Hirayanagi 1929, Kobel 1929 a, b; Nebel 1929, Negrul 1930, Branas 1932, Husfeld 1932, Ghimpu 1932, Olmo 1937, Shetty 1959 and several others) have noticed heteromorphic sex chromosomes in *Vitis.*

Thus, in *Vitis,* sex is determined by genes located on undifferentiated autosomes. As mentioned in the introduction, there are several schemes for sex determination in *Vitis,* (Valleau 1916, Müller-Thurgau and Kobel 1924, Negrul 1936, Breider and Scheu 1938, Oberle 1938, Bethmann 1939, Levadoux 1946). However, the symbols used by Valleau (1916) and Levadoux (1946) are not in accord with current genetic usage. Two pairs of linked factors, the hypotheses proposed by Müller-Thurgau and Kobel (1924), Oberle (1938) and Bethmann (1939) lack experimental evidence. However close the factors may be, occasional recombination of them should produce neuter and hermaphroditic plants in the progeny of self-pollinated males. Such plants were not found in the present 242 progeny derived from selfing the male *V. vinifera* clone 030-44. Assumption of seve-

ral linked factors for sex by NEGRUL (1936) is also hard to prove. BREIDER and SCHEU (1938) had assumed the presence of X and Y sex chromosomes, which have not been observed cytologically in any *Vitis* species.

As has been pointed out by OBERLE (1938), the original or most ancient form in *Vitis* was probably hermaphrodite. In related and more primitive genera, such as *Cissus,* only hermaphroditic species are known. The presence of all sexual parts in the flowers of both male and female plants indicates that the origin of the male flower arises by the partial suppression of the gynoecium, and the origin of the female flower arises by the partial suppression of the androecium. Since suppressor genes are thus concerned, current terminology suggests using the symbol Su.

The present results in *Vitis vinifera* L. *(sylvestris)* can be explained on the basis of a single allelic pair, Su^F and Su^m. The gene Su^F suppresses the development of functional female organs and thus determines maleness. Its allele Su^m in homozygous condition suppresses the development of male organs and thus determines femaleness. The gene Su^F is completely dominant over its allele Su^m. The genetic formulae for heterogametic and homogametic males are Su^F Su^m and Su^F Su^F respectively. The female is homogametic with the genetic formula Su^m Su^m. 030—44 is a heterogametic male with the genetic constitution Su^F Su^m, which on selfing, produced 1 Su^F Su^F : 2 Su^F Su^m : 1 Su^m Su^m, i. e., 3 males to 1 female. One-third of the males are homogametic, which on crossing with normal females should produce all male progeny. The other two-thirds are heterogametic, which on crossing with normal females should produce male and female offspring in about equal numbers. In the sample of 24 F_1 males crossed to females, 9 (about $^1/_3$ of the total) were homozygous, which agrees with the expectation. It can be deduced that one-third of the total male seedlings from 030—44 selfed are homogametic and the other two-thirds are heterogametic males.

Since the native vines of *V. vinifera (sylvestris* form) are dioecious and most of the cultivated varieties of *V. vinifera (sativa* form) are hermaphroditic and self-fruitful (RATHAY 1888-1889, BAILEY 1934, NEGRUL 1936, LEVADOUX 1946), the question of the origin of the latter forms (hermaphrodites) arises. MÜLLER-THURGAU and KOBEL (1924) speculated that the cultivated *V. vinifera* hermaphrodites have originated in the crosses of females and males due to crossing-over between the sex genes in the males. According to some authors (NEGRUL 1936, OBERLE 1938, BREIDER and SCHEU 1938, LEVADOUX 1946), hermaphrodites have originated from males by mutation. The possibility of the origin of hermaphrodites from females has also been recognized (BREIDER and SCHEU 1938, OBERLE 1938). However, none of these authors has produced *V. vinifera* hermaphrodites experimentally. In the present study, no completely hermaphroditic vines were obtained in the progeny of self-pollinated hermaphroditic male clone 030-44, in the progeny of the crosses of vinifera females Hunisa and Z30—45 with different F_1 males from 030-44 selfed, and in the progeny of the crosses of *V. vinifera* females with some hermaphroditic male vines of the clone 030—44. However, one seedling numbered e27—51 had more than 11% seeded berry set in both the years of observation. Such hermaphroditic male seedlings, particularly e27—51, may tentatively be classed as p a r t i a l h e r m a p h r o d i t e s. Their definite sexuality, however, must be determined by further genetic studies and by observing their

behaviour for additional years. It is quite likely that by raising further progeny from these so-called partial hermaphrodites, we may be able to select for complete and stable hermaphrodites in advanced generations. Thus the segregation of modifying genes may be one method by which hermaphrodites can arise without mutation of the major suppressor genes. It could also explain the occurence of unusual sex ratios not conforming to a simple allelic hypothesis.

Summary

Sex conversion in the male *V. vinifera (sylvestris)* clone 030—44 could not be attributed to germinal mutation of the major gene for sex. The differences in sex expression among male seedling vines can best be explained on the basis of an undetermined number of minor modifying genes able to shift a very sensitive threshold. The frequency of sex conversion within a given seedling vine was greatly influenced by environmental, both local and seasonal, conditions.

The following facts were established in *Vitis vinifera* L. *(sylvestris):* (1) the male sex is heterogametic and is inherited as a simple Mendelian factor, maleness being completely dominant; (2) homozygous males are as viable as heterozygous males, they are phenotypically alike and can only be identified by breeding tests; (3) pure and hermaphroditic males are genetically alike as far as the major gene for sex is concerned; (4) sex chromosomes are not differentiated, either morphologically or physiologically.

A pair of alleles, Su^F and Su^m, were used to explain the results obtained on selfing the hermaphroditic male clone 030—44. The gene Su^F determines maleness and its allele Su^m in homozygous condition determines femaleness. The gene Su^F is completely dominant over its allele Su^m. The genotypes of the homozygous and heterozygous males are Su^F Su^F and Su^F Su^m respectively, and that of the female is Su^m Su^m.

The evolution of cultivated *V. vinifera* hermaphrodites in the light of present results is discussed.

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