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THE ORIGIN OF VARIATION IN "WILD" *RAPHANUS SATIVUS* (*CRUCIFERAE*) IN CALIFORNIA

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Analyses of populations of *Raphanus* growing in the central part of California, from the Sierra Nevada foothills to the Pacific coast, show that pure *R. raphanistrum* can be found only in the Central Valley, while over the remainder of the area populations of the so-called "wild" (weedy) *R. sativus* occur. More detailed morphological studies of a number of populations in this area have revealed that the populations of "wild" *R. sativus* originated by hybridization of the cultivated forms of this species (the radish) with another introduced species, already a weed, *R. raphanistrum.* The composition of each hybrid population with respect to the proportion of characters of the one or the other species depends upon the habitat it occupies and its geographic location. Populations in inland areas display a high proportion of *R. raphanistrum* characters, while in those near the coast *R. sativus* characters predominate.

Artificial hybrids between *R. raphanistrum* and a cultivated form of *R. sativus* exhibited about 50% pollen fertility and were heterozygous for a reciprocal translocation. Examination of "wild" populations of *R. sativus* revealed that plants heterozygous for a reciprocal translocation are present in varying proportions. Experimental evidence is produced to show that this translocation is identical with that separating *raphanistrum* from cultivated forms of *sativus.* Thus a cytological proof of the introgression is added to the morphological evidence. Introgression of *raphanistrum* characters appears to have been a major factor in converting the erstwhile crop plant, *R. sativus,* into a highly successful weed in California.

Introduction

It has been pointed out by Howell (1949) that in many areas of California the native flora has become almost vestigial. The place of the native species has been taken by the descendants of plants brought intentionally or accidentally from the Old World by man. A number of these introduced species have become serious weeds (in whatever sense the term is used). The success of an introduced plant even above

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its abundance in its native habitat may be attributed to any or all of three factors (BAKER, 1962, 1965).

Firstly, there may be a greater abundance of suitable habitats for it than in its native region.

Secondly, the plant may be freed from pests, diseases or competitors which seriously hamper it in its native home.

Thirdly, genetic changes may have taken place which give it greater vigor or greater ecological tolerance in its new weedy form.

Morphological and cytological studies of wild *Raphanus* populations in central California carried out in the course of this investigation have provided evidence for the hypothesis that the wild populations in central California consist of swarms brought about by hybridization of escaped specimens of the cultivated *Raphanus sativus* L. with the equally introduced but already weedy *R. raphanistrum* L.

The two species of *Raphanus* were introduced from Europe during the 19th century, as may be estimated from the first gatherings of herbarium specimens (cf. ROBBINS, 1940). *Raphanus sativus,* the radish, is an important crop plant and appears to have originated in cultivation, and is not listed as an indisputable wild plant in any flora so far written. It has been suggested that *R. sativus* was domesticated in China (cf. DE CANDOLLE, 1883; TROUARD-RIOLLE, 1914; STURTEVANT, 1919). In Europe it has been known as a crop plant since the time of the Roman Empire (HEGI, 1935). According to TROUARD-RIOLLE (1914) and others, although there are many varieties of radish, their common characteristics include the following:

1. A swollen root (actually the upper part of the tap root and the hypocotyl), which, however, varies in color and shape.

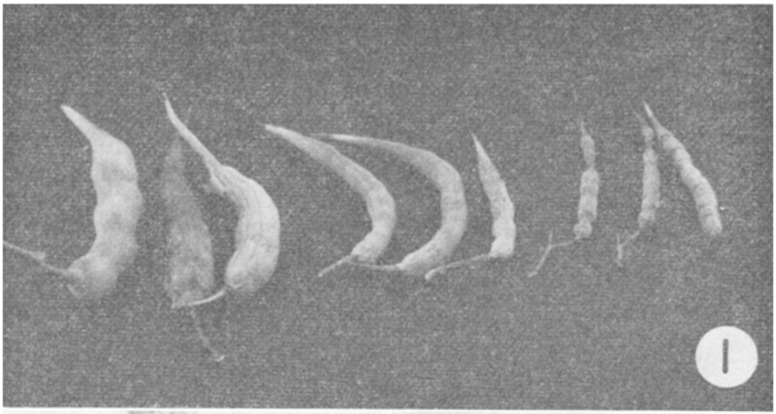
Figure 1. Three fruits of *Raphanus sativus* ("Cincinnati Market" cultivar) (left), three of *R. raphanistrum* (right) and three of the F1 hybrid between them (center).

Figure 2. Metaphase I in pollen mother cell of artificial F_1 hybrid between *R. sativus* (from Berkeley) and *R. raphanistrum* (× 1700).

Figure 3. Metaphase I in pollen mother cell of artificial F_1 hybrid between *R. sativus* (cultivated) and *R. raphanistrum* (× 1700).

Figure 4. Metaphase I in pollen mother cell of plant from natural population 5 at Rockaway Beach, San Mateo County, California (× 1700).

Figure 5. Metaphase I in pollen mother cell of plant from natural population 7 at College of Holy Names, Oakland, Alameda County, California (× 1700).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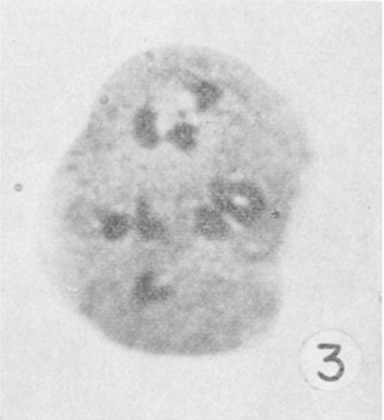
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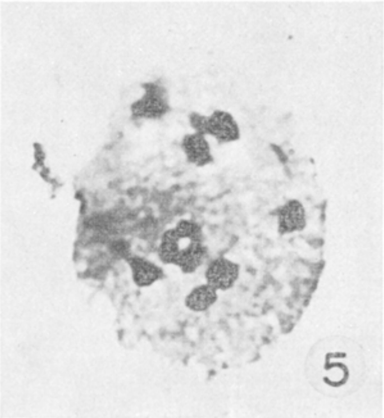
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2. The lomentum type of fruit (Fig. 1). This measures from 4.0 to 6.5 cm. in length and 0.9 to 1.1 cm. in diameter, and is capped by a long beak (which may be 2–3 cms. in length). Ripe fruits become spongy and corky in structure and crush easily in the hand.
3. Flowers with a variety of colors and color patterns. The background petal color varies from white to pink or violet but does not include yellow. Superimposed may be a number of different vein-colors, again utilizing only anthocyanin pigments.

In Europe, *R. sativus* has formed semi-wild populations after escaping from cultivation (TROUARD-RIOLLE, 1914). The plants in these populations resemble their cultivated ancestors, except for their roots which show a tendency to be more slender and branched.

In California, it is reported by JEPSON (1909–1936), ABRAMS (1944), and MUNZ (1959) that *R. sativus* has been naturalized, coming as a "wild" plant from Europe. The descriptions of the "wild" *R. sativus* by each of these authors show discrepancies, particularly concerning the root, fruit shape and structure, and the petal color. On the other hand, ROBBINS (1917), ROBBINS & RAMALEY (1933), and HOWELL (1949) consider *R. sativus* to have been introduced into California by the early settlers as a cultivated plant and that subsequently it escaped from cultivation in the areas where it was planted. In other parts of the United States, the authors of floras have treated "wild" *R. sativus* as an escape from cultivation (e.g., SMALL, 1933; DAVIS, 1952; JONES, 1945; GLEASON, 1952; and J. P. ANDERSON, 1959).

Raphanus raphanistrum appears to have been introduced into North America by seed mixed with the seeds of useful crops. This species is widely distributed in Europe as well as in North Africa and Asia Minor, but is notably absent from China and India (DE CANDOLLE, 1883; TROUARD-RIOLLE, 1914; HEGI, 1935). In many features *R. raphanistrum* resembles *R. sativus* but the following characteristics identify it as a distinct taxon:

1. It forms a penetrating tap root, with frequent branching.
2. The fruits (Fig. 1) measure 4–8 cm in length and 0.4 to 0.6 cm in diameter, with a conical beak of only about 1.0 cm length.
3. The fruit, when ripe, shrinks in diameter between the seeds and finally breaks in fragments, each containing a single seed. The fruit coat is longitudinally grooved and hard (not crushable, except between the seeds, by hand pressure).

4. The laminae of the petals are yellow or white, while their veins may be pigmented with yellow, green, brown or blue or may lack any differentiating pigment.

The somatic chromosome number of both species is $2n = 18$ (KARPECHENKO, 1924, 1928). The same base number of 9 occurs throughout the genus *Raphanus* (cf. DARLINGTON & WYLIE, 1955, etc.) and is also found in the neighboring genus *Brassica*.

Artificial crosses between the two *Raphanus* species have been made successfully by a number of investigators, and it has also been shown that hybridization can take place naturally. In Germany, FOCKE (see HEGI, 1935), in the cross *R. sativus* × *R. raphanistrum*, found 50% of abortive pollen grains in the plants of the F_1 generation. A similar result was obtained by TROUARD-RIOLLE (1914) in a cross which she made in France.

By contrast, FROST (1923) made a cross between "wild" *R. sativus* and *R. raphanistrum* (both from material found growing in Southern California) in which the F_1 generation was fully fertile. KOBABE (1959) showed that the two species cross naturally in Germany, and that F_1 plants there are also fully fertile, suggesting that the chromosomes of the parents are homologous.

Thus, there is need for an investigation of the nature of the populations of *Raphanus* growing without cultivation in central California, their variability and the causes of their variability, especially in view of the possibility that interspecific introgression has played a role.

DISTRIBUTION OF THE TWO SPECIES AND COMPOSITION OF THE WILD *Raphanus* POPULATIONS

A rough survey of Californian wild *Raphanus* populations was undertaken at the beginning of this study in order to trace the distribution of the two species, as well as to get some idea of the composition of populations in different parts of the State. Color of the petals and the diameter of the ripe fruits were chosen from among the several characters distinguishing the two species. These two characters are the more conspicuous and appear to be resistant to direct environmental influence. Besides these two main characters, however, a number of other features were taken into consideration, so that their

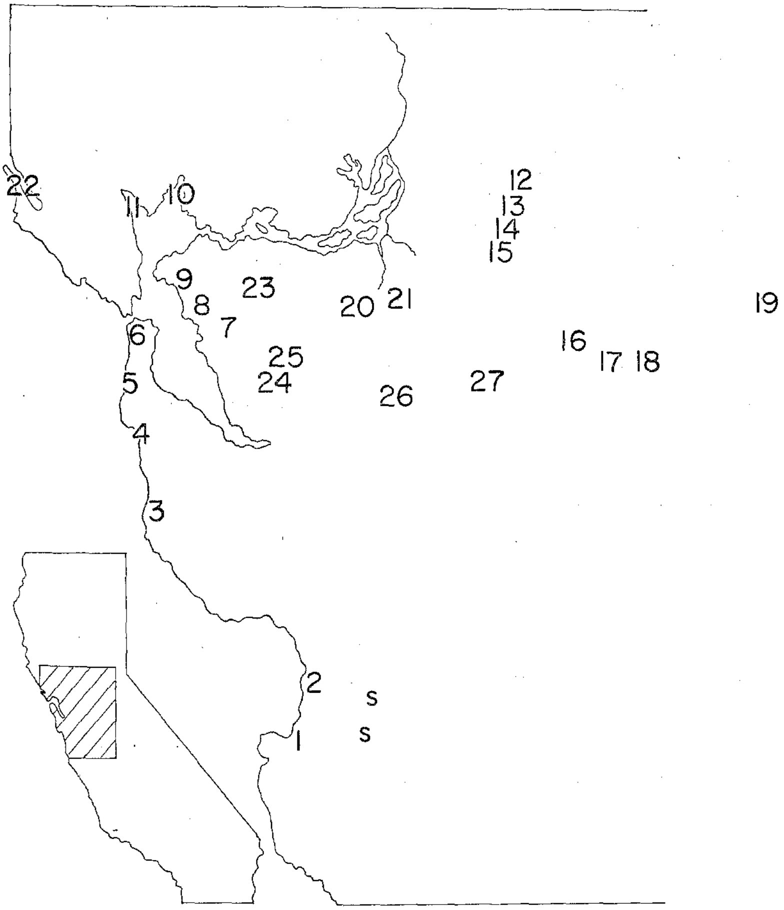

Figure 6. Map of central California (inset shows position of the mapped area in the state as a whole) with locations of the natural populations sampled (the numbers correspond with localities in Table 1) and the two cultivated populations of *R. sativus* (marked with S) which were studied.

value for subsequent intensive population studies might be estimated.

Twenty seven populations were sampled and analysed in this preliminary survey. They were located along the central California coast of the Pacific Ocean and in a number of stations inland as far as the floor of the Central Valley. (See Fig. 6). Within each region, populations growing in a variety of habitats were sampled: near the seashore (in the case of coastal populations), at the edges of cultivated fields in which water was provided during the dry season of the year, in waste places and along roadsides. The collection and scoring of the flowers and fruits could not coincide in time for most populations. Consequently, all flower colors were scored in the spring, and fruits were examined during the summer of the same year. When all the population samples were considered the correlation coefficient relating the proportion of plants with carotenoid pigments in the petals in each population to the proportion of plants in the same population having fruits less than 0.6 cm in diameter was found to be + 0.91.

From the results obtained (Table 1) we may draw the following conclusions for the populations studied.

In populations growing in the immediate vicinity of the coast of the Pacific Ocean, characters of *R. sativus* predominate (i.e. anthocyanous petal color and large fruit diameters). Between populations in the same general area, however, considerable variation is noticeable.

Population 3 (see Fig. 6) is the closest to the cultivated forms of *R. sativus* in all characters studied. Impressive in this population (which grows at the edge of a cultivated field at Pescadero, San Mateo County and enjoys part of the care taken for the artichoke crop) is the high proportion of plants (about 15%) with swollen roots. It is not possible to say whether this population has recently escaped from cultivation or by some means has preserved some characters like those of the presumptively parental cultivated crop. However, the former explanation seems the more likely.

Another population in the same general area (Population 5) is growing in a waste place at Rockaway Beach. An exceptionally high proportion of *raphanistrum* characters was scored here. The more xeric nature of this habitat (related, apparently, to the sandy soil) was indicated by the early drying of the plants each summer, while others nearby on more retentive soil remained green until July or August. Only a very small proportion of swollen "roots" (about 0.5%) was observed among plants of this population. Another exceptional population occurs on Point Reyes Peninsula (no. 22), where the high proportion of yellow flowers can be related to the pastoral nature of the agricultural operations in this area. Radishes have never been grown as a crop plant here and the weeds are recent imports from farther inland even from the Central Valley itself (BAKER, unpubl.).

In the Berkeley Hills, the two more fully studied populations (7

TABLE 1

LOCATIONS OF POPULATIONS IN CALIFORNIA WHICH WERE SAMPLED AND THEIR COMPOSITIONS IN TERMS OF PETAL PIGMENTATION

Population	Habitat	County & location	Percent			
			Anthocy.	Carot.	None	Both
1	Waste place	Monterey, Monterey	78	2	18	2
2	Rear of beach	Monterey, Moss Landing	78	2	19	1
3	Edge of arable field	San Mateo, Pescadero	80	0	20	0
4a	Rear of beach	San Mateo, Half Moon Bay	62	5	32	1
4b	Roadside	San Mateas, Half Moon Bay	85	0.4	14	0.8
5	Waste place	San Mates, Rockaway Beach	33	15	50	2
6	Waste place	San Francisco, Point Lobos	78	3	14	0
7	Waste place	Alameda, Holy Names College	40	22	35	3
8	Waste place	Alameda, Spruce St., Berkeley	47	13	36	4
9	Waste place	Contra Costa, Arlington Blvd. El Cerrito	32	16	50	2
10	Roadside	Solano, 2 mi. W. of Vallejo	62	16	20	2
11	Bridge foundations	Sonoma, Petaluma Creek, Rt. 37	14	72	11	3
12	Roadside	San Joaquin, 1 mi. W. of Clement, Rt. 88	10	55	32	3
13	Edge of field	San Joaquin, 12.5 mi. N.E. of Stockton, Rt. 88	0	93	7	0
14	Sorghum field	San Joaquin, 12 mi. N.E. of Stockton Rt. 88	0	85	15	0
15	Edge of bean field	San Joaquin, 10 mi. N.E. of Stockton, Rt. 88	8	52	38	2

(Continued)

Population	Habitat	County & location	Percent Anthocy.	Carot.	None	Both
16	Roadside	San Joaquin, 2 mi. E. of Escalon, Rt. 120	3	64	31	2
17	Roadside	Stanislaus, 1 mi. E. of Oakdale, Rt. 120	36	37	24	3
18	Roadside	Stanislaus, 4 mi. E. of Oakdale, Rt. 120	18	32	49	1
19	Roadside	Tuolumne, Jamestown	89	6	5	0
20	Roadside	Contra Costa, 6.25 mi. W. of Middle River, Rt. 4	71	4	21	4
21	Roadside	San Joaquin, 3.15 mi. W. of Uncle Tom's, Rt. 4	78	3	8	11
22	Roadside	Marin, Point Reyes Peninsula	12	48	37	4
23	Roadside bank	Alameda, Euclid Ave., Berkeley	52	10	32	6
24	Orchard	Contra Costa, Between Orinda and Moraga	30	9	58	4
25	Roadside	Contra Costa, St. Mary's College, Moraga	39	15	40	7
26	Roadside	San Joaquin, Corral Hollow	24	25	48	3
27	Roadside	Stanislaus, San Joaquin River crossing, Rt. 132	13	54	27	6

and 9) show an increase of *raphanistrum* characters over the coastal ones (excepting no's. 5 and 22 already mentioned). This is particularly clearly expressed in Population 7 (where swollen "roots" are absent). Plants in Population 9 stay green about one month longer than those in Population 7 and a number of them bear roots which are swollen like those of the cultivated crop plants.

A number of populations growing in the Central Valley were sampled and showed a predominance of *raphanistrum* characters. Thus, they contain a high proportion of plants with yellow petals and fruits of less than 0.6 cm diameter (both of which are *raphanistrum* characters). However, pure populations without any suggestion of *sativus* are not easily found, and only numbers 13 and 14 appear to be nearly pure *R. raphanistrum.*

It seems that environmental conditions in the Central Valley suit the requirements of *R. raphanistrum,* but the introduction by man of *R. sativus* for crop purposes may have changed the constitution of the populations through hybridization, particularly around towns, villages and settlements where radishes were cultivated. An intrusion of *sativus* characters is particularly obvious along the delta of the San Joaquin-Sacramento rivers (populations 20 and 21). Here coastal climatic influences penetrate into the Central Valley and are responsible for the presence there of a number of otherwise coastal taxa (STEBBINS and BAKER, unpubl.) and radish crops were also grown here.

A population sample taken in the Sierra Nevada foothills (No. 19) and other observations suggested that an increase of *sativus* characters is found as one climbs out of the Central Valley. No populations were sampled at higher elevations in the Sierra Nevada but HALL & HALL (1912) found only what they identified as *R. raphanistrum* on the floor of the lower Yosemite Valley.

Artificial Crosses

MATERIAL AND METHODS

Plants of *Raphanus raphanistrum* and *R. sativus* (both weedy and cultivated forms) were used to make a series of artificial crosses. The larger number of cultivars of *R. sativus* do not differ from each other in most morphological characters. Thus, they agree in general habit of the plant, in leaf shape, in fruit shape and structure, and in pubescence of the various aerial parts. Moreover, there are no known cytological differences between the cultivars. Therefore, the use of a single cultivar to represent the cultivated forms in the hybridization programme was not inappropriate. The variety chosen was "Cincinnati Market", seeds of which were supplied by the Ferry Morse

Company of San Francisco in the spring of 1961. In addition, plants of the weedy form of *R. sativus* were grown from seeds collected in the summer of 1961 from populations 4 and 6 occurring along the Pacific Coast.

Seeds of *Raphanus raphanistrum* were collected in the summer of 1961 from populations growing in the Central Valley of California. Population 13 (Fig. 6) was considered to represent the species satisfactorily. The judgment was based upon a comparison of plants of this population with taxonomic descriptions of the species, with specimens available in the herbarium of the University of California, Berkeley, and with a large number of herbarium specimens from other states of the U.S.A. and from Eastern Canada (kindly loaned to us by the Plant Research Institute of the Canada Department of Agriculture). In addition, seeds of *R. raphanistrum* were collected in the same year from Population 16, growing in the Central Valley.

All plants used for crosses were kept in an insect-free greenhouse on the Berkeley campus of the University of California. In order to avoid self-pollination, emasculation of experimental flowers was carried out before any artificial cross was made. The anthers were removed the day before the flowers opened and pollen of the desirable kind was unloaded on the stigmas.

Meiotic chromosome studies of parental plants and hybrids were made on material that had been fixed in a mixture of three parts absolute alcohol to one part of glacial acetic acid for 24 hours and stored in 70% alcohol. The material was stained by the acetocarmine method. Staining was improved considerably by adding to the acetocarmine a few drops of 45% acetic acid saturated with iron acetate (DARLINGTON & LA COUR, 1950). Slides were made permanent by using the dry-ice method of CONYER & FAIRCHILD (1953).

Pollen fertility in the same plants was studied by using fresh pollen from flowers which had opened on the same day as the examination was made. Anthers of different flowers of the same plant were smeared together in acetocarmine and a minimum of 200 pollen grains were counted from each sample. Seed fertility was estimated by comparing the number of ovules, estimated by the count of ten flowers, with the average number of seeds per pod of ten artificially cross-pollinated flowers of the same plant.

ARTIFICIAL CROSSES MADE IN BERKELEY

A comprehensive series of intra-and inter-specific crossings was carried out (Table 2).

Results

"CULTIVATED" *Raphanus sativus* AND *R. raphanistrum* (Crosses 1 and 2 in Table 2)

These two sets of interspecific crosses used *Raphanus raphanistrum* parents from two different Central Valley populations (14 and 16) with *R. sativus* ("Cincinnati Market"). Whereas the first cross was

TABLE 2

CROSSINGS CARRIED OUT AND THE ORIGINS OF THE PLANTS USED IN THEM

Cross Number	Ovule parent	Pollen parent	Remarks
1	*Raphanus raphanistrum* popn. 16 (R.r.)	*R. sativus* "Cincinnati Market" (R.s.c.)	Carried out reciprocally
2	*R. raphanistrum* popn. 14 (R.r.)	*R. sativus* "Cincinnati Market" (R.s.c.)	
3	F_1 (R.s.c. × R.r.) from cross no. 1	F_1 (R.s.c. × R.r.) from cross no. 1	
4	F_1 (R.s.c. × R.r.) from cross no. 1	*R. sativus* "Cincinnati Market" (R.s.c.)	
5	*R. sativus* "Cincinnati Market" (R.s.c.)	*R. sativus* wild, popn. 4 (R.s.w.)	
6	*R. raphanistrum* popn. 14 (R.r.)	*R. sativus* wild, popn. 4 (R.s.w.)	The same plant of R.s.w. involved as in cross no. 15
7	*R. raphanistrum* popn. 16 (R.r.)	*R. raphanistrum* popn. 14 (R.r.)	

made reciprocally, the purpose of the second cross was mainly to find out whether similar results would be obtained by using as the *R. raphanistrum* parent a plant derived from a population growing thirty miles away from the first one and it was made in one direction only. All crosses were successful and the number of seeds formed in each fruit was as great as in the case of intraspecific crosses, indicating that there is no isolating mechanism, such as would prevent fertilization or seed development, operating between the two species.

The morphological characters studied in the F$_1$ hybrids were those which were considered likely to be useful in the subsequent intensive examination of wild populations.

Root: In none of the plants in these hybrid generations did the swollen "root" of the cultivated parent appear. All plants developed a slender, well branched root, more like that of the *raphanistrum* parent.

Fruit: The diameter of the fruit (measured at its broadest part) varied from 5.5 to 6.5 mm (Fig. 1). The surface of the fruit was smooth and the fruit wall was hard (i.e., difficult to crush between the fingers). At maturity the seeds were arranged in a single row, but no con-

TABLE 3

PETAL COLOR OF PARENTS AND THEIR F$_1$ GENERATIONS

Cross number	Ovule parent	Pollen parent	F$_1$ Generations				Total Plants
			Antho-cyanous	White	Yellow	Bronze	
1	R.s.c. Rhodamine pink 527/2 *)	R.r. Sulphur yellow 1/2 *)		3	—	3	12
1 recipr.	R.r.	R.s.c.	5	3	—	2	11
2	R.s.c. Rhodamine pink 527/2 *)	R.r. Sulphur yellow 1/2 *)	5 **)	15			20

*) Classification of the parents' petal colors, according to the color chart of the Royal Horticultural Society.

**) Anthocyanin was restricted to the tips of the petals, while the rest of the lamina was white.

strictions were formed between them so that the moniliform appearance of the ripe fruit of *R. raphanistrum* was not achieved.

Among the 43 plants of this generation, two set occasional seeds in the valves of the fruit in addition to the usual production of seeds in the beak. This character was also observed in the *raphanistrum* parent.

Flowers: No two plants showed the same shape to the petals, pointing up the variation for this character which exists in each of the two species. The color of the petals was also variable and this was expressed more in the progeny of the first cross than in the second (where the plants were almost uniformly provided with white petals). (See Table 3).

Trichomes: In both crosses the *sativus* parents lacked trichomes on the stem, pedicels, sepals and fruit coat, while the *raphanistrum* parents had hairs on those parts. Almost all of the F_1 plants developed hairs on their stems, pedicels and sepals which, in some cases, were even more dense than on the *raphanistrum* parents. In a restricted number of plants a few hairs were developed on the fruit walls.

Flowering time: In all plantings a record was kept of the duration of the period of vegetative growth from germination to flowering. For a comparison of the length of this period in the hybrids with that in their parents a sample of parental seeds was always sown at the same time as the hybrid seeds. It appears that there is some variation between plants of the same species in the duration of this period but despite this, there is a distinguishable difference between the two species. Thus, *R. raphanistrum* comes into bloom more than one month earlier than "cultivated" *R. sativus.* When F_1 seed was sown (either in summer or winter) it produced plants which bloomed earlier than those of the *sativus* parent but later than those of *R. raphanistrum* (Table 4).

Fertility: In all three interspecific F_1 groups the pollen fertility of individual plants was examined and found to range from 46 to 62% ($\bar{x} = 57\%$). Seed fertility was also estimated for the same plants and ranged from 65 to 90% ($\bar{x} = 81\%$).

Cytology: Cytological examinations of a sample of four plants from each F_1 group was made, particular attention being given to meiotic behavior in the pollen mother cells. Observations were made particularly on cells in late diakinesis and both anaphases. In all samples a configuration of seven bivalents and a quadrivalent (forming either a ring or a chain) could be found (Figs. 2 and 3).

TABLE 4

CHRONOLOGY OF GROWTH OF F_1 HYBRID GENERATIONS AND THEIR PARENTS

	Number of plants	Date of germination	Time from germination to flowering (days)	Mean number of days
R. sativus (cultivated)	10	June 25–27	62–76	70
R. raphanistrum	10	June 25–27	30–36	33
R. raphanistrum × *R. sativus* (cult.)	23	June 25–27	41–64	51
R. sativus (cultivated)	10	Dec. 2–4	110–140	128
R. raphanistrum	10	Dec. 2–4	60–80	64
R. raphanistrum × *R. sativus* (cult.)	20	Dec. 2–4	80–125	103

From each preparation 40 cells were scored and it was estimated that the quadrivalent association was present in 85% of the cells. It was concluded, therefore, that the plants of the F_1 hybrid generation were heterozygous for a reciprocal translocation. The high frequency of occurrence of the quadrivalent indicates also that the re-arrangement is of considerable size. In a few cases it was observed that, at the first anaphase, unequal chromosome distribution was taking place, with the result that in the first telophase nuclei were formed containing eight and ten chromosomes, respectively.

F_2 GENERATION

(Cross 3 – Table 2)

The artificial crossings between F_1 hybrid plants which were carried out to produce Cross no. 3 in Table 2 provided a large number of seeds. A sample of 18 seeds was sown and all germinated after six to eight days to produce an F_2 generation. Space was not available for more plants. The main purpose of raising this generation was to find out if any obvious species-isolating barriers would find expression in this generation. In addition, some morphological and cytological observation were made possible.

Morphology:

Root: Variation appeared among the plants of this generation with respect to the development of the "roots". These varied from slender, well branched roots to swollen structures closely resembling those of the cultivated radish.

Fruits: Each plant developed fruits which were different from any other of the same generation with respect to their size, shape, diameter, crushability, number of seeds and their arrangement in the fruit. The diameter of the fruits ranged without grouping from 5.2 to 7.5 mm. This appears to indicate polygenic determination of this character.

Three groups in respect to the crushability of the fruit wall were recognized for the sake of classification. Two plants had fruits in which the wall was not crushable in the hand, seven were intermediate (the wall could be crushed with effort), and nine were soft (easily crushed). In five plants, the seeds in the fruit were displaced alternately and in thirteen plants the seeds were in a single row. None of the plants bore fruits with constrictions between the seeds. One plant had fruits which matured one seed in the valves besides the usual set of seeds in the beak.

Flowers: Both F_1 plants involved in making this cross bore yellow petals, but the F_2 plants showed a wide range of variation in petal color. One plant produced petals with a similar pink color to that of the original cultivated *sativus* parent, but all others had different degrees and shades of a combined anthocyanous and carotenoid pigmentation in the petals.

Flowering time: The lapse of time between germination and the opening of the first flowers ranged from 70 to 195 days in the different plants. However, three distinct groups could be identified rather clearly:

1. Early flowering 70 to 90 days ($\bar{x} = 86$), 5 plants
2. Medium 100 to 122 days ($\bar{x} = 112$), 10 plants
3. Late 140 to 195 days ($\bar{x} = 164$), 3 plants

Fertility: Pollen fertility was examined. In ten plants it ranged from 38 to 58% ($\bar{x} = 50\%$), while in eight plants it ranged from 85 to 100% ($\bar{x} = 92.4\%$). Two clear groups are apparent. Seed fertility also followed this pattern with the same 10 plants having 50 to 80% fertility ($\bar{x} = 70\%$) while the other eight plants were fully fertile.

Cytology: Three plants from each of the two fertility groups were examined cytologically, by studying the meiotic behavior of pollen mother cells. It was observed that each plant with reduced fertility tended to form seven bivalents and a quadrivalent (with the same frequency as in the F_1 parents). In the plants with high fertility two formed nine bivalents while the third possessed an extra chromosome (i.e., was a trisomic). The three more or less homologous chromosomes in the latter plant formed a trivalent 80% of the times. In the remaining cases the extra chromosome remained single or, very rarely, there was an association of five chromosomes. It was concluded that this trisomic was a tertiary one resulting from the unequal segregation of a quadrivalent, such as was seen in the F_1 hybrid generation.

The 10 : 8 ratio with respect to the fertility found in the F_2 generation is not significantly different from the expected 1 : 1 ratio from a cross between two plants which are heterozygous for one reciprocal translocation (i.e., $NT \times NT \rightarrow 1NN : 2NT : 1TT$).

The plants which developed swollen "roots" (like those of the commercially cultivated varieties) were the same plants which showed late flowering. These three plants were also fully fertile and one of them that was examined cytologically produced nine bivalents at meiosis. Contamination of the seeds sown could not explain this apparently complete linkage because all three plants were different from the cultivated variety in a number of morphological characters such as petal color, pubescence, fruit structure and shape, etc.

Crosses 4, 5, and 7 (Table 2)

Various other crosses were made utilizing the same *R. raphanistrum* parents but crossed with plants of *R. sativus* which originated from weed populations. In almost all characters these plants of *R. sativus* resembled the cultivated radish, except for their lack of a swollen "root".

The results obtained are given in Tables 5 and 6. In every respect they are comparable with the results from Crosses 1, 2 and 3, allowing for the difference in root structure between cultivated and wild *R. sativus*.

Studies of wild populations

A number of wild populations that had already been sampled in the first part of this investigation were analyzed more thoroughly after

TABLE 5

MORPHOLOGICAL CHARACTERS OF THE PARENTS AND THEIR PROGENIES FROM CROSSES NUMBER 4, 5, 6 AND 7 IN TABLE 2

		Root			Fruit wall			Petal color			
Cross Number		Swollen	Intermediate	Slender	Soft	Intermediate	Hard	Anthocyanous	Yellow	Bronze *)	White
#5	*R. sativus* (cult.) ×										
	R. sativus (wild)										
	Parents	×			×			×			
			×			×		×			
	Progeny	5	5		4	6		10			
#7	*R raphanistrum* ×										
	R. raphanistrum										
	Parents			×			×		×		
				×			×		×		
	Progeny			21			21		21		
#4	F₁ × *R. sativus* (cult.)										
	Parents			×			×		×		
		×			×			×			
	Progeny	4	5		9			6		2	1
#6	*R. raphanistrum* ×										
	R. sativus (wild)										
	Parents			×			×		×		
			×			×		×			
	Progeny			14			4	6	6	2	

*) Bronze color is due to the presence of both anthocyanin and carotinoid pigment.

the artificial crosses between the two species of *Raphanus* had been carried out. The study of the artificial hybrids provided the morphological and cytological bases for these more refined analyses.

MORPHOLOGICAL STUDIES

The morphological studies of the wild populations were based on the pictorialized scatter diagram methods introduced by E. ANDERSON

TABLE 6

FERTILITY, CYTOLOGY AND FLOWERING TIME OF THE PARENTS AND THEIR PROGENIES FROM THE CROSSES 4, 5, 6 AND 7 IN TABLE 2

(II = bivalent; IV = quadrivalent)

Cross Number	Pollen fertility	Chromosome configuration at meiosis	Flowering time		
			Early	Middle	Late
#5 *R. sativus* (cult.) *R. sativus* (wild)					
Parents	= 98%	9 II		1	
	= 98%	9 II			1
Progeny	All plants = 98%	All plants = 9 II		5	5
#7 *R. raphanistrum* × *R. raphanistrum*					
Parents	= 98%	9 II	1		
	= 98%	9 II	1		
Progeny	= 98%	9 II	21		
#4 F_1 × *R. sativus* (cult.)					
Parents	= 98%	9 II			1
	= 55%	7 II & 1 IV		1	
Progeny	5 plants = 48 to 60%	5 plants = 7 II & 1 IV		5	
	4 plants = 98%	4 plants = 9 II			4
#6 *R. raphanistrum* × *R. sativus* (wild)					
Parents	= 98%	9 II		1	
	= 98%	9 II	1		
Progeny	All plants = 55%	All plants = 7 II & 1 IV	5	9	

(1949, 1953, 1954). The parents and the F_1 plants were examined (Fig. 7) and the same methods were then applied to wild populations (Figs. 8–11).

To represent the cultivated radish in these diagrams a sample of a population of the "Cincinnati Market" variety being grown in the Salinas area (Monterey County) for seed production by the Ferry Morse Company was used (Fig. 6). For *R. raphanistrum*, population sample no. 13 (12.5 miles northeast of Stockton on State Route 88) was used.

From each sub-region (i.e., Pacific coast, Berkeley and Oakland

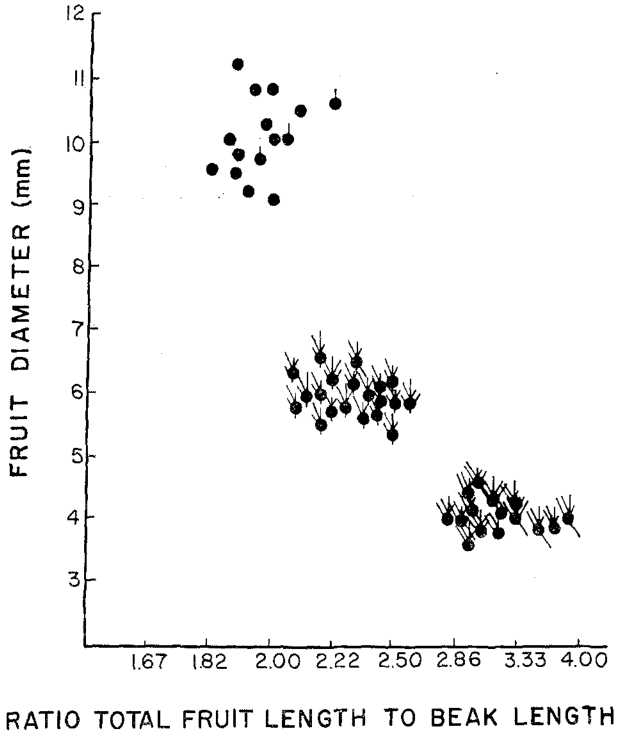

Figure 7. Scatter diagram (see text, page 263, for explanation of symbols) of samples of *R. sativus* (cultivated) (upper left), *R. raphanistrum* (population 13) (lower right) and F_1 hybrids between them (center).

Hills; Central Valley) within the general area of investigation, several populations were sampled in the detailed morphological studies.

Characters used: The selection of characters was based upon the frequency of their occurrence in the two species. Characters with strikingly different means and lack of overlap in the parental species were used. The diameter of the fruit and the ratio of the sterile part of the beak to the total fruit length were chosen to give the main axes of the scatter diagrams because they showed a wide range and yet could be measured accurately. Six other characters that were scored are indicated by rays from each circle (representing one plant) in the scatter diagrams (Figs. 7–11). Long rays represent one extreme, short rays an intermediate condition and no rays the other extreme. These characters were

1. Hairs on the stem	● Dense	● Sparse	● None
2. Hairs on the pedicel	● Dense	● Sparse	● None
3. Hairs on the fruit	● Dense	● Sparse	● None
4. Hardness of the fruit wall	● Hard	● Medium	● Soft
5. Seed-arrangement in the fruit	● Moniliform	● One row	● Two or more rows
6. Seed position in the fruit	● Valves and beak		● Beak only

With respect to the first three characters, involving the occurrence of hairs, three classes were recognized (viz., dense, sparse, none).

The hardness of the fruit wall is a character without clear-cut classes Furthermore, one can only estimate hardness in the absence of refined mechanical methods of analysis. Nevertheless, three classes were arbitrarily distinguished (viz., Fruit wall soft: Crushes easily by hand pressure. Intermediate: Crushes by hand pressure but with effort. Hard: Very difficult to crush by hand pressure, or even impossible).

"Seed-arrangement" refers to the manner in which the seeds are set in the fruit. Three classes are easily distinguished (viz., two rows or irregular arrangement of the seeds; seeds arranged in one row, but without severe constriction of the fruit wall between each seed; moniliform arrangement).

"Seed-position" refers to the part of the fruit in which the seeds are set. In this character only two classes were recognized (viz., seeds formed in valves as well as those set in the beak of the fruit, Fig. 1).

METHODS OF HANDLING

All sampling and scoring of characters was carried out in the field. The best time for this is before the plants start to dry and the fruits are almost ripe (although some open flowers are still present). Later in the season the fruits may fall off (except from plants resembling the cultivated parent, which keep their ripe fruit even after the stems are dead). The middle of May or a little later, depending on the year, is the best time for the sampling of Central Valley populations, while for the populations on the Coast Range hills the beginning of June is appropriate and for coastal areas the beginning of July is favorable.

From each population, 40 to 50 plants were scored for all eight characters. For a random collection, whilst walking across the population, one plant was picked up every five to eight steps, depending on the shape and the size of the population. Populations of more or less square shape were divided by three to four transects and plants were collected walking along those transects.

The diameter of the fruit was measured by calipers to a tenth of a millimeter. This measurement was taken always on the broadest ripe fruit of the plant, and at its widest part.

For the estimation of the ratio of beak length to total fruit length, the largest fruit on the plant was always measured, because it was considered that it more nearly represented the full expression of the potentiality of the particular plant.

All characters involving hairs were scored by careful inspection of the plants. Because hairs do not usually appear on all fruits and pedicels of the same plant, the classification was based on the density of hairs where these did develop.

"Seed-position" was easy to score by simple inspection of the fruits. In this connection, it should be mentioned that not all fruits on a plant develop seeds in the valves even when the potentiality is there. As long as a number of them were to be seen however, the plant was put into this class. The lack of consistency in this seed-development is probably due to failure of all ovules to be fertilized.

INTERPRETATION OF THE SCATTER DIAGRAMS

The scatter diagrams for each population (Figs. 7–11) verify the presence of the variation originally detected, and show more clearly that hybridization between the two *Raphanus* species has contributed to it. Whether or not hybridization has occurred in any population and to what degree it has influenced each species depends not only upon the climatic factors but also upon the length of time during which the two species have been in proximity to each other.

Along the Pacific Coast, Population 4 (Fig. 8), growing near the seashore at Half Moon Bay, shows some introgression of *raphanistrum* characters into a population of *sativus*. Populations 8 and 7 (Figs. 9 and 10) grow in the Berkeley and Oakland hills, respectively, and differ from each other in that Population 7 has a greater resemblance

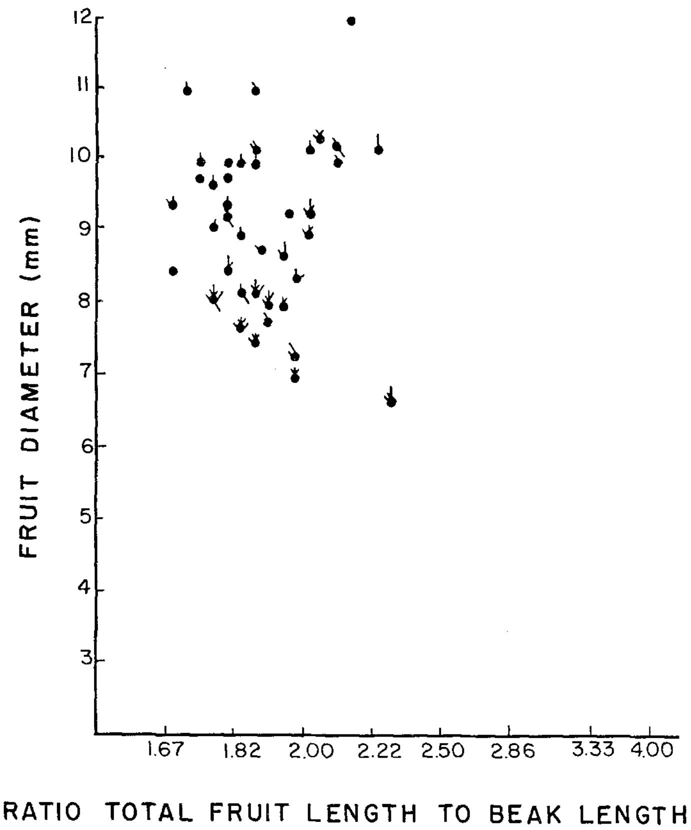

Figure 8. Scatter diagram of sample from population 4, Half Moon Bay, San Mateo County, California.

to *R. raphanistrum*. Population 8 grows on Euclid Avenue, Berkeley, while Population 7 occurs about five miles east of San Francisco Bay, at the entrance to the College of Holy Names, Oakland. The soil in which the latter plants are rooted dries about one month earlier in the summer than that of Population 8 (about 6 miles away, at the same elevation) indicating a more xeric habitat for Population 7.

Population 16 (Fig. 11) (two miles east of Escalon) is the only extra one in the Central Valley of California studied intensively. In the scatter diagram it appears to be at an opposite extreme to Population 4 (Fig. 8), in the sense that it is a *raphanistrum* population modestly introgressed with *sativus* characters. The population grows between

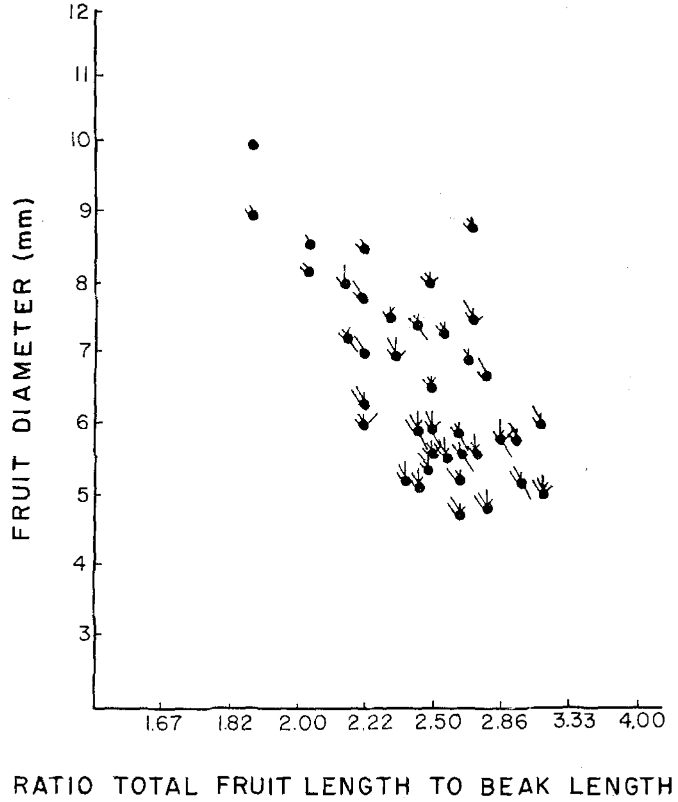

Figure 9. Scatter diagram of sample from population 8, Berkeley, Alameda County, California.

State Highway 120 and an irrigation ditch. The climate of this area of the Central Valley is semi-arid with mild moist winters and dry summers and with mean annual rainfall of 13.6 inches (3400 mm) per year, distributed from September to May. At the beginning of summer most herbaceous plants which are not watered artificially dry up or go dormant. Population 16, however, because of the moisture available at the margins of the irrigation ditch, stays green longer than other populations in the same area.

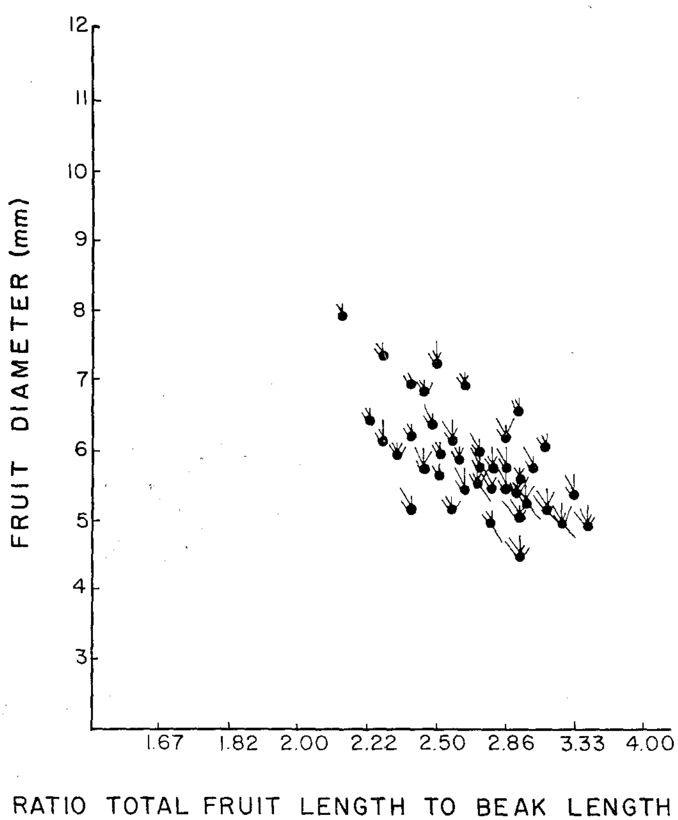

Figure 10. Scatter diagram of sample from population 7, Oakland, Alameda County, California.

CYTOLOGICAL STUDIES

In the artificial crosses, it was found that the two species of *Raphanus* differ in one reciprocal translocation (and their hybrids, being heterozygous for this translation, form a quadrivalent association in meiosis). Any cross of the F_1 hybrids with each other, or any backcross to the parents, will result in progenies half of which will be chromosomally homozygous with a configuration of nine bivalents in meiosis and half heterozygous for a translocation (with a tendency to form quadrivalents). On this assumption it was expected that any wild population that has been exposed to hybridization in recent times would include plants that exhibit meiotic irregularities of this

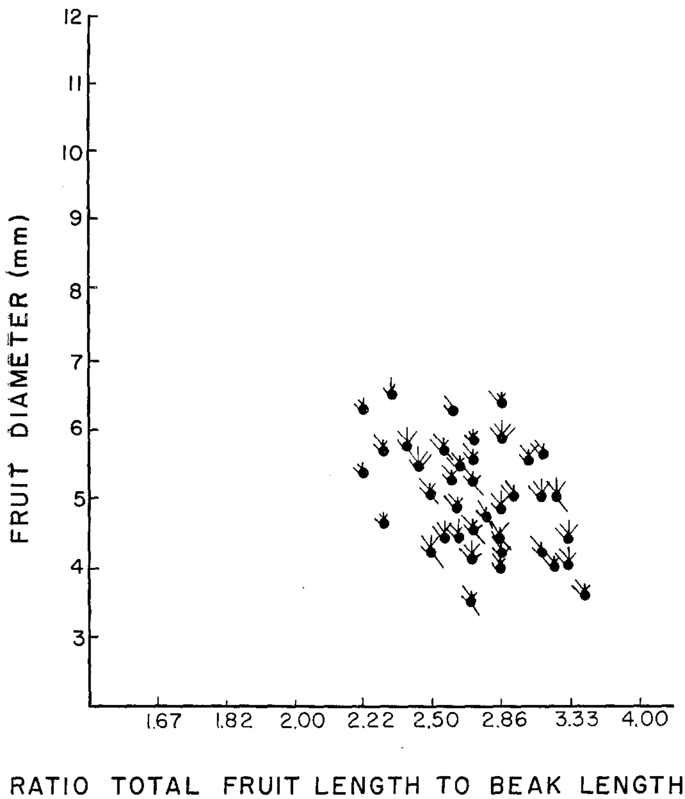

Figure 11. Scatter diagram of sample from population 16, two miles east of Escalon, San Joaquin County, California.

type. Taking into account that the two species were introduced separately into California in the 19th century, hybridization between them since then detected by morphological characters should be confirmable by this cytological means. In this connection, two populations were examined intensively (populations 7 and 8), while some other populations were studied by examination of pollen fertility only.

During the spring of 1963, and while the plants were in full bloom, flowers were collected from plants that were labeled previously with colored ribbons and numbered tags so that it was easy to find them again whenever this was necessary. The pollen fertility of each plant was ascertained and recorded. Buds were collected from each plant and were fixed directly in the field for cytological examination.

Results from these studies showed that all wild populations sampled along the Pacific coast and in the Coast Ranges contain plants with reduced fertility (always around 40 to 60% pollen fertility, except in a few cases where a further reduction to 30% was recorded). In Population 4 (Half Moon Bay), 10% of the plants examined showed reduced fertility (to the same level); in Population 5 (Rockaway Beach), the figure was 20%; and in Population 7 (College of the Holy Names, Oakland), it was 25%. A number of other populations not listed in the tables, but from the San Francisco Bay area, were examined and always plants with reduced fertility were found (although in varying proportions).

Cytological examination of plants from Populations 7 and 8 showed that the reduction of fertility is due to the fact that these plants are heterozygous for a reciprocal translocation (Figs. 4 and 5). The frequency of quadrivalent formation in meiosis was more or less the same as in the artificial hybrids between the two species. Plants with normal fertility always showed a configuration of nine bivalents.

To test whether the translocations found in wild populations were homologous with those found in the artificial hybrids, the following test was carried out. Pollen grains from a *Raphanus sativus* plant of wild origin, heterozygous for a translocation, were used to pollinate two F_1 hybrid plants. Seeds obtained from these crosses were sown and 25 plants were raised to maturity.

Examination of these 25 plants showed that 13 of them had pollen fertilities ranging from 49–60% while 12 were fully fertile. Once again, two clearly distinct classes, with full and half fertility, are indicated. Cytological examination of pollen-mother cells in a number of the plants with normal and reduced fertilities, respectively, showed that the former always produced nine bivalents at meiosis, while the latter always showed a configuration of seven bivalents and an association of four chromosomes.

The equal proportions of the two cytological classes of plant shows that the translocation found in the wild plant tested is the same as that discovered in the artificial F_1 hybrids between the two *Raphanus* species. Heterozygosity for it presumably came about by natural hybridization of the two species in Calfifornia.

Discussion

"Cultivated" *Raphanus sativus* has been known for thousands of years as a cultivated plant, while its wild ancestor is unknown. During this time, it has been subjected to selection by man for characters that have perfected it as a cultivated crop plant. The most important of these is the structure of the root, but characters of the rest of the plant have also been affected. A delay in the formation of the flowering shoot is important so that materials may be made available for the growth of the edible root. In addition, fruits which stay on the plant long after maturity and open easily for the extraction of the seeds were favored in artificial selection. Each of these characters, on the other hand, is equally disadvantageous for a successful weed, as "wild" *R. sativus* appears to be now in California. Thus, a swollen root is sensitive to diseases and to mechanical injuries, while its limited formation of laterals and consequently poor absorbing capacity will restrict the species to rich and moist soils.

The long period from germination to flowering and to fruit maturity requires a climate with mild temperatures in the spring, as well as moisture and humidity throughout the summer, for the plants to be able to carry through their reproductive cycle. The varieties grown for seed by the Ferry Morse Seed Company in the Salinas area of Monterey County are sown in the spring and harvested in August; they are irrigated during the summer. The fruits, being soft, are an easy prey for birds, which eat the seeds. FROST (1923) reports that linnets at Riverside (in southern California) destroy large quantities of fruits this way. The same destruction was observed in the experimental plot in Berkeley, where the two species of *Raphanus* were growing together. Birds ate preferentially almost all the seeds of "cultivated" *R. sativus,* while fruits of *R. raphanistrum,* being hard-walled and falling early from the plants, were not attacked.

The results of the artificial crossings carried out in this investigation showed that fruit structure and flowering time are polygenically controlled characters, while swollen root is oligogenically determined (probably by two major genes with a number of modifiers). It was also shown that the genes for root and fruit-structure in "cultivated" *R. sativus* are recessive to the corresponding alleles in *R. raphanistrum* with the F_1 hybrids almost intermediate between the parents in the duration of the period from germination to flowering. These findings exclude the possibility that escaped plants from cultivation acquired weedy properties by accumulation of the many mutations needed because the time lapse since *R. sativus* was introduced in California is less than two centuries and seemingly inadequate for such an accumulation. We should also exclude the possibility that hidden variability existing in the cultivated material was released, because of the recessive nature of the characters of the cultivated type and the long period during which the species has been subjected to artificial selection for them. Consequently, the most plausible manner of explaining the origin of "wild" *R. sativus* and its weedy properties is the hybridization of "cultivated" *R. sativus* with a wild related species capable of providing these characters.

HEISER (1947) in his studies on the genus *Helianthus*, was able to show that *H. annuus* (which was introduced in northern California as a crudely cultivated plant) has hybridized with *H. bolanderi* (which was previously a distinct species restricted principally to serpentine areas in northern California). The introgression of *H. annuus* genes into *H. bolanderi* produced a vigorous weedy variant which is now spreading in the area as well as possibly helping the local adaptation of *H. annuus*. E. ANDERSON (1949) reported a case of spontaneous hybridization between cultivated lettuce (*Lactuca sativa*) and the wild lettuce (*Lactuca serriola*) near Pullman, Washington, demonstrated by MARION OWNBEY. In this case, by using leaf color as a marker, OWNBEY was able to show extensive introgression from the garden lettuce into the weedy wild lettuce. Thus, there are precedents for the kind of hybridization shown by *Raphanus*.

The only closely related species to the cultivated *R. sativus* available in California is *R. raphanistrum*. However, as an already successful weed, it is supplied with the appropriate characters to offset the features which make *R. sativus* such a valuable crop plant but potentially such a poor weed. *R. raphanistrum* is characterized mainly by its slender and well-branched tap root, the shortness of the period from its germination to its flowering, the structure and the shape of its fruit, and by the fragile connection of its fruits with the supporting pedicel. These properties make *R. raphanistrum* a successful weed of dry areas where a relatively short vegetative period is demanded (as at higher altitudes and latitudes). It is also adapted to dry and hot

Mediterranean climatic conditions such as those of the Central Valley of California (where favorable conditions are gone by the end of May).

The artificial crosses have shown that there are no barriers to fertilization between *R. sativus* and *R. raphanistrum* and that their F_1 and F_2 hybrid generations are fertile and produce large numbers of seeds. It was also observed in the experimental plot at Berkeley, where both species were grown together, that bees and other insect visitors go from one species to the other. It is to be expected, therefore, that whenever the two species come in contact hybridization will occur. Introduction of *R. sativus* by European man as a cultivated crop into areas of California where *R. raphanistrum* had already been introduced as a weed of cultivated land was the means of bringing the two species together. The occurrence of plants with swollen roots in weedy populations of *R. sativus* suggests that hybridization of *R. raphanistrum* with the cultivated radish has taken place recently. Otherwise such a recessive and unfavorable character would hardly still be found. In the first part of this study, a coastal population was described (Table 1, pop. 3) in which about 15% of plants possessed roots of the cultivated type. In the Central Valley of California, populations were also found with a high proportion of "cultivated" *R. sativus* characters, especially around cities and settlements, suggesting that the genesis of the weed population is going on polytopically.

The artificial crosses between the two species showed that they differ in a reciprocal translocation, and it appears that genes determining root structure and flowering time are very closely linked and located on the chromosomes involved in the translocation. Thus, the translocation has been important in evolution, keeping in the same linkage group genes of high biological significance.

Wild populations characterized as being of hybrid origin by morphological studies were found to contain different proportions of plants heterozygous for this reciprocal translocation. This not only shows the validity of the methods used in the morphological studies, but provides a further indication that hybridization has taken place in recent time. Heterozygosity for the reciprocal translocation reduces pollen-grain fertility to about 50%; consequently, the translocation would be expected to be eliminated from populations in time and a fixation of the one or the other arrangement would take place.

Artificial crossings between *R. sativus* and *R. raphanistrum* have been carried out in the past by a number of investigators and it is interesting to consider their results in the cytological context. FOCKE (see HEGI, 1935) in Germany and TROUARD-RIOLLE (1914) in France found that plants of the F_1 hybrid generation produced about 50% abortive pollen grains. On the other hand, FROST (1923) in southern California and KOBABE (1959) in Germany did not find any reduction of fertility in their F_1 hybrids. It may be concluded that in the two first cases the reduction of fertility is due to the presence of a translocation; presumably the *raphanistrum* plants used by FOCKE and TROUARD-RIOLLE have the same chromosome arrangement as ours in central California. The high fertility found in the hybrids produced by FROST and by KOBABE can be attributed to their having crossed *R. raphanistrum* with "wild" *R. sativus* strongly introgressed with *raphanistrum* characters and the translocation may have become homozygous in their populations.

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