HYBRIDS OF ♀ *RAPHANUS SATIVUS* L. × ♂ *BRASSICA OLERACEA* L.

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(With Two Plates and Three Text-figures.)

Cytological investigations of *Cruciferae*, begun by the author in 1922, proved that *Raphanus sativus* L. and *Brassica oleracea* L. have the same number of chromosomes. As several hybrids of these genera had been described formerly, but not yet cytologically investigated, similar crosses were made by the author and unexpectedly resulted in giving 123 hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. Thus not only the discovery of interesting facts concerning the behaviour of parental chromosomes in the process of formation of sexual cells was made possible, but also the establishment of the morphological peculiarities of the hybrids.

The crosses were made in June of 1922. Altogether 202 flowers of radish (*Raphanus sativus* L. *prol. niger* Pers.) were emasculated and pollinated with pollen from different cabbages: *Brassica oleracea* var. *capitata* L., var. *gongyloides* L., var. *sabauda* L. and var. *gemmifera* D.C. The flowers were isolated in muslin bags, and almost all of the pollinated stigmas set fruit and developed pods with 2 to 4 small shrivelled or normal seeds. These seeds were planted 31 March, 1923, in a box in a greenhouse. The seedlings came up at intervals, taking on an average eight days[1], but most of the seeds did not germinate at all. On April 10 the seedlings were transplanted into pots and kept in a cooler part of the hothouse, and later on under cold frames. Many plants differed from the radish from the very beginning, having less conspicuously nerved cotyledons, leaves less dissected, and less pubescent or glabrous, and in crosses with red cabbage, an anthocyan colouring of stems and leaves. Although differing in vigour, all hybrids towards the end of May had the appearance of strong cabbage seedlings with 6–7 leaves, and were transplanted into a field-plot at a distance of 1¼ metres from each other. Here they began to develop and their differences became more apparent, some hybrids remaining till the autumn small and stunted, others on the contrary reaching an enormous size. Towards the end of July several hybrids were in bloom and about the middle of October out of 123

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[1] Whereas seeds of radish and cabbage come up on the 4th day.

hybrids 46 flowered, and 25 plants had flower-buds[1]. Some plants developed pods which did not ripen and gave no seeds.

The hybrids were crossed with their parental forms and with each other, and material was fixed for cytological investigation. In the middle of October of 1923 the plants were minutely studied morphologically and dug up. After the leaves were cut away the roots were stored for planting next spring (1924).

MORPHOLOGY OF THE HYBRIDS.

Respecting the first hybrid of *Raphanus sativus* L. × *Brassica oleracea* L., made by Sagaret in the middle of the last century, we know that it flowered abundantly but gave only two normal pods, one of which resembled a cabbage pod and the other a radish pod. Seeds of these pods developed weak plants which Sagaret did not study. The second hybrid described by Gravatt in 1914 was of striking vigour. Its branched stem grew out through the ventilator of the roof of the greenhouse and a short way down it on both sides. It had big glabrous leaves resembling those of a cabbage, it blossomed abundantly, bore flowers, but was completely sterile and gave no pods. 15 per cent. of the flowers had eight stamens; the ovaries appeared to be 2-celled like those of the cabbage parent. One slide of the hybrid pistil showed sections of an ovary with three cells. As to the hybrids *Raphanus sativus* × *Brassica oleracea* made lately by Baur we know only that they were very vigorous but completely sterile. This is about all we know concerning the morphology of radish-cabbage hybrids.

For a proper analysis of a hybrid plant a sufficient number of the hybrids and a statement of the characters which most differentiate the two parents are required. We had an unusually large number of hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. and there were no difficulties in establishing the characters necessary for an analysis, as both genera differ sharply in regard to roots, leaves, flowers and fruits, and at the end of the period of vegetation in their general habit also. We begin our description of parental forms and of their hybrids with their habit of growth.

Habit of growth. The maternal forms of radish, grown under the same conditions as the hybrids, were in bloom towards autumn, their flower-stalks being 1·7–1·9 metres long, the lower leaves being 30–40 cm. in length, the length of the flowering axis 25–45 cm.

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[1] Cabbage flowers in general in its second year of growth whereas radish often bears seeds in its first year, especially if sown early.

The cabbages did not flower and had an ordinary appearance: hearting cabbages had a short stem, the leaves, with exception of a few lower ones, forming the heart; kohlrabi had a thick stem in form of a bulb; Brussels sprouts had a longer stem with big leaf buds in form of small heads; Savoy cabbage had a short stem with crinkled leaves forming a loose bunch. The length of the lower leaves of cabbages was 40–60 cm.; the height of the longest Brussels sprouts did not exceed 70–90 cm.

The radish-cabbage hybrids towards autumn appeared to be strictly polymorphic in their habit of growth and, irrespective of difference in the variety of cabbages participating in the crossing, were generally of three types. The 1st type was a small plant with a very short stem about 30 cm. in length, densely covered, especially at the base, with small leaves; the stem was often branched and bore short flowering shoots 15–17 cm. in length with clustered flowers. The 2nd type of plant consisted of big leafy rosettes of 1½ metres in diameter, or bushes with short thick branches and big leaves. They flowered sometimes, developing small clusters of flowers.

The 3rd type was represented by plants of a vigorous growth with a strong stem with many branches of 2–2½ metres in height. They flowered abundantly and their flowering shoots reached a length of 1½ metres and more. These plants were like wild Cruciferae such as *Sisymbrium* or *Barbarea*, but of a gigantic size. The difference between the hybrids is clearly seen in our photograph (Pl. XXI, fig. 1).

Hybrids of type I are obviously stunted in growth never attaining the size of a flowering radish or cabbage. The stems of cabbages grown beside the hybrids were 1·2–1·5 metres in height. Hybrids of the second

TABLE I.

No.	♀	♂	No. of crossings	No. of hybrids obtained	No. of blossoming hybrids I year	No. of hybrids with outgrowth on roots	No. of hybrids			Remarks
							I	II	III	
1	*Raphanus sativus* L. *prol. niger* Pers.	*Brassica oleracea* L. var. *capitata* L.	127	55	35	37	14	29	12	Two intermediate hybrids between the II and III type are included in the II type
2	,,	*Brassica oleracea* L. var. *gongyloides* L. (Kohlrabi)	32	33	23	21	11	7	15	—
3	,,	*Br. oleracea* L. var. *sabauda* L. (crinkled leaf cabbage)	27	4	4	1	—	2	2	—
4	,,	*Br. oleracea* L. var. *gemmifera* D.C. (Brussels sprouts)	16	31	9	16	15	14	2	Two hybrids of an intermediate type between the I and the II are included in the I type
		Total	202	123	71	75	40	52	31	

and third type exceed their parent in vigour and were alike in this respect; possibly their difference is due to a slower development of stems in plants of type II.

Transitional forms between these three types occur occasionally as exceptions. This polymorphism of hybrids has been observed for crosses with all varieties of cabbages. Table I shows the respective figures.

There are more rosettes or low "bushes" (type II) in crossings with hearting cabbage; stunted plants occur more often in crossings with Brussels sprouts; developed plants of great vigour are numerous in crosses with kohlrabi. Sometimes in a group of crosses there are only two types. Table II shows, e.g., that the F_1 of the 17^{22} crossing was represented by two groups of plants, viz. nine small ones with small leaves and six very full ones with big leaves, but in crosses 23^{22} and 4^{22} there are all three types of hybrids. The difference between representatives of the three types in one cross was very great. Thus in the cross No. 23, we had plants with a height of 5 cm. only, with small leaves no longer than 26 cm., but we had also in the same cross rosettes with leaves 68 cm. long and plants 250–260 cm. high. This divergence in F_1 was observed in all crosses notwithstanding the small number of hybrids. Their number is too small to establish any definite relations between the three types.

Stem. The stem of a flowering cabbage is always glabrous, with a waxy efflorescence, and has a normal pith. The stem of a radish is pubescent or hairy, and hollow; it has often a reddish hue near the nodes and at the base of branches. The hybrids had stems with a normal pith; they were glabrous with a slight waxy efflorescence; almost all plants had a reddish colouring at the nodes, but only 15 plants of the 1st type had pubescent stems. Hybrids of red hearting cabbage were coloured as their parental forms all along their stems. The broadening of stems in kohlrabi is dominant in inter-racial crosses. It is caused by a development of vascular fibres surrounded by parenchyma and connected with the normal peripheral system of fibres only in its upper and lower part of the enlargement (Lund and Kjärskou). Half of the hybrids of radish × kohlrabi developed such bulbs on the stem, but it is interesting that an anatomical investigation of several such "enlargements" in hybrids did not discover any central systems of vascular fibres, and only in one core were there several bunches of fibres, whereas ordinarily they consisted simply of parenchyma, and the vascular fibres were located only along the periphery of the stem. Perhaps it was on

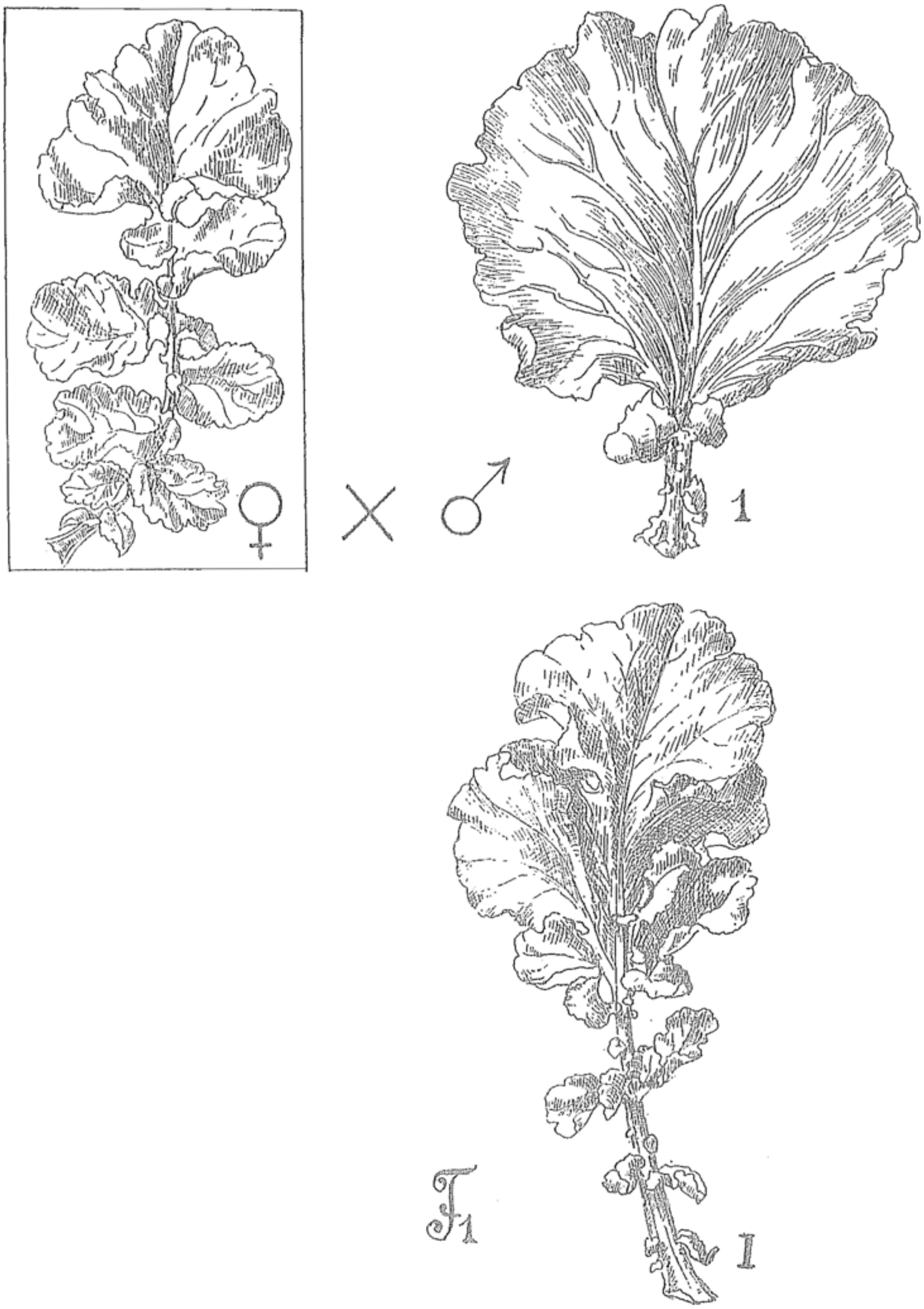
TABLE II.

No. of crossings	Name of crossed plants ♀ and ♂	Types of F_1	No. of hybrids of each type	Height of hybrids in cm.	Size of lower leaves in cm.	No. of hybrids with leaves of radish				No. of hybrids with leaves of an intermediate character				No. of hybrids with leaves like cabbages				No. of hybrids flowering during the 1st year	No. of hybrids with outgrowth on roots
						with a waxy effl.		without a waxy effl.		with a waxy effl.		without a waxy effl.		with a waxy effl.		without a waxy effl.			
						Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous		
17^{22}	*R. sativus* L. *prol. niger* Pers. ×	I	9	35–90	8/20–10/28	.	.	5	4	.	.	.	.	.	.	.	.	7	6
	B. oleracea L. var. *capitata* L.	II	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Radish × hearting cabbage	III	6	220–253	15/40–18/52	.	1	.	.	1	2	1	.	1	.	.	.	6	2
23^{22}	*R. sativus* L. *prol. niger* Pers. ×	I	3	5–23	8/26– 9/29	.	.	3	.	.	.	.	.	.	.	.	.	1	.
	B. oleracea L. var. *gongyloides* L.	II	3	*9–88	17/47–20/68	.	.	.	.	1	.	1	.	1	.	.	.	1	2
	Radish × Kohlrabi	III	4	230–260	9/22–13/38	.	.	.	.	1	.	.	.	.	1	.	2	4	3
4^{22}	*R. sativus* L. *prol. niger* Pers. ×	I	6	12–38	10/24–12/33	.	.	4	1	.	1	.	.	.	.	.	.	2	5
	B. oleracea L. var. *gongyloides* L.	II	2	*10	23/70	.	.	.	.	.	1	.	1	.	.	.	.	.	1
	Radish × Kohlrabi	III	6	107–250	7/18–11/39	.	.	.	.	2	.	.	1	.	2	.	1	6	4
16^{22}	*R. sativus* L. *prol. niger* Pers. ×	I	5	8–54	6/16–11/32	.	.	5	.	.	.	.	.	.	.	.	.	1	1
	B. oleracea L. var. *gemmifera* D.C.	II	2	25–90	18/56	.	.	.	.	.	1	.	.	.	1	.	.	1	1
	Radish × Brussels sprouts	III	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

* Rosettes of leaves, no stems.

TABLE III.

Types of F_1	Medium height in cm.	Medium size of leaves in cm. br./leng.	No. of hybrids of each type	No. of hybrids with leaves of the *radish* type				No. of hybrids with leaves of an *intermediate* type				No. of hybrids with leaves of a *cabbage* type				No. of hybrids flowering during their 1st year	No. of hybrids with out-growths on roots
				with a waxy efflorescence		without a waxy efflorescence		with a waxy efflorescence		without a waxy efflorescence		with a waxy efflorescence		without a waxy efflorescence			
				Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous	Pubescent	Glabrous		
I	28·7	9·9/28·2	40	1	.	23	13	3	.	.	.	.	.	.	.	16	22
II	48·7	16·0/57·1	52	3	2	1	2	18	14	5	4	2	1	.	.	24	36
III	180·4	14·0/38·6	31	.	1	.	.	8	6	1	3	1	6	.	5	31	18
		Total	123	4	3	24	15	29	20	6	7	3	7	.	5	71	76

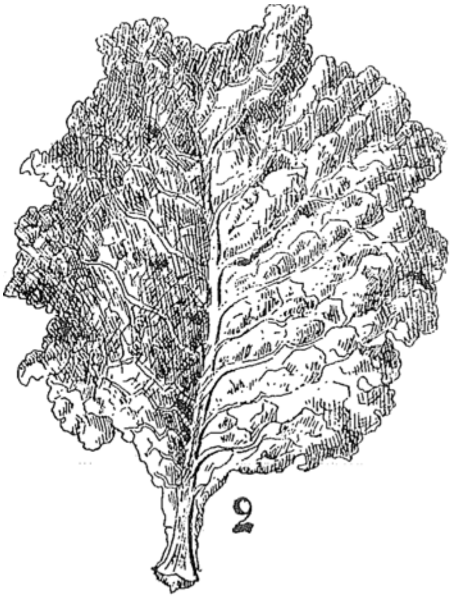


Text-fig. 1. LOWER LEAVES:

♀ *Raphanus sativus* L. *prol. niger Persival.*

♂
1 *Brassica oleracea* L. v. *capitata* L.
2 *Brassica oleracea* L. v. *sabarida* L.
3 *Brassica oleracea* L. v. *gemmifera* D.C.
4 *Brassica oleracea* L. v. *gongyloides* L.

F_1
I ♀ *Raph. sat. niger* × ♂ *Brass. ol. capitata* L.
II ♀ *Raph. sat. niger* × ♂ *Brass. ol. sabarida* L.
III ♀ *Raph. sat. niger* × ♂ *Brass. ol. gemmifera* D.C.
IV ♀ *Raph. sat. niger* × ♂ *Brass. ol. gongyloides* L.



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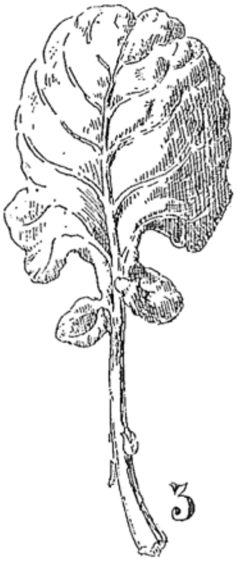

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4

II

III

IV

account of an insufficient development of the conducting system that hollows existed in bulbs of the hybrids of radish × kohlrabi, with numerous additional roots. Other peculiarities in the development of stems of cabbages do not appear in hybrids of radish × cabbages. No hearts were formed, and instead of the small hearts in Brussels sprouts, their hybrids developed only many side branches—dormant buds thus changed into developing ones[1] (see Pl. XXI, fig. 2).

Roots. The radish has a thick central root and a cabbage has thin and numerous roots. The hybrids had one central more or less enlarged root, but also many side roots which were freely distributed in the soil, or, perhaps because of a rather prolonged cultivation in garden pots, were tightly twisted around the central root (Text-fig. 2 *D*). In taste all roots of the hybrids were like radish but less pungent. It is interesting that most hybrids (see Tables I, II and III) had peculiar outgrowths of different shapes and size on their roots. These outgrowths covered sometimes all side roots and gave leaf-bearing shoots, which sometimes appeared on the surface of the soil (see Pl. XXI, fig. 3). Neither radish nor cabbage ever develop such shoots. These outgrowths are of an exogenous origin and consist of a completely solid parenchyma, rich in starch grains and traversed by vascular fibre bunches; the leaflets acquire sometimes, even underground, a differentiation of tissues. These outgrowths were observed on flowering and on non-flowering plants alike, on vigorous and stunted hybrids, and are met in all crosses (see Table II). An analogous development of outgrowths and leaf shoots has been observed before on hybrids of *Brassica Napus* L. × *Brassica Rapa* L. (Lund and Kjaerskou, Wilson, Kajanus). This parallelism, if there is no infection by bacteria[2], is very interesting in connection with similar hereditary variability in genetically related species (homologous series of variations of Vavilov and Baur).

Leaves. The difference between leaves of radish and cabbage is very great. Radish leaves are coarse and hairy, dissected with long unevenly dentated sections. Cabbage leaves are glabrous with a waxy bluish efflorescence; they are more fleshy, dissected in a lyre-form so that their terminal section is much greater than the lateral sections. Hybrids resembled radish or cabbage (seldom) or were of an intermediate type. Intermediate types are shown along with parental forms in Text-fig. 1.

Pubescence or a waxy efflorescence was observed separately and

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[1] In interracial crossings of cabbages dormant buds and heads are dominant.

[2] Dr Peklo (Prag) found in outgrowths of *Br. Napus* × *Br. Rapa* bacteria which were determined by Kajanus as *Bact. tumefaciens* (1917).

sometimes together, or not at all. Pubescence was seldom observed on leaves of a cabbage type, or a waxy efflorescence on leaves of a radish type. Table II shows that hybrids of a single cross present all possible combinations of parental characters. There is no indication of the degree or strength of the characters in the table: therefore we point out that on cabbage leaves the pubescence was slighter than on radish leaves just as a waxy efflorescence was in them stronger than on radish leaves. The table shows that small plants had almost always leaves of a radish type, hybrids of the 2nd type had intermediate leaves and hybrids of the 3rd type had often real cabbage leaves. This is obvious in Table III where the 123 hybrids are ranged according to their types and leaves. The stunted type is thus linked up with maternal radish leaves, and a vigorous type with cabbage leaves of the parental type. As in interracial crosses, the reddish colouring in crosses of radish × red head cabbage dominated, and 3 out of 4 hybrids of radish × Savoy cabbage had also crinkled leaves. Hybrids of the 1st type were of a light green colour, and hybrids of the 2nd and 3rd type dark green, but as in general the colour of leaves changes easily its exact definition is difficult.

Flowers. The flower-clusters of radish and cabbage have on the average about 20–40 flowers. Clusters of hybrids of type I had 15–35 flowers and hybrids of type III developed in separate clusters as many as 170–190 flowers and had still other flower-buds. But simultaneously no more than 7–10 flowers were in bloom, the rest fell and they could be counted only by their stems. Probably this abundant flowering was due to the fact that only a few pods were set. The calyx in radish is pubescent, of the cabbage smooth like those of the hybrids. Petals of radish are about 1·5–1·8 cm. long generally, and white with dark nerves or purple. Petals of cabbages are 2–2·3 cm. long, yellow. Their flowers are shown on Text-fig. 2 *A* and *B*. Petals of hybrids were of 1–2 cm. in length of an intermediate shape (Text-fig. 2 *C*) and of various colours: white, purple or of a pale-cream colour (one plant)[1]. Hybrids and parents were analysed by N. E. Prokopenko in order to determine the presence of flavone and anthocyanin. The presence of either pigment was determined according to a change in colour of petals in vapours of ammonia: anthocyanin changes into blue, flavone into yellow, a mixture of both changes into green. Petals of radish appear to possess both flavone and anthocyanin and the last one is present in nervures espe-

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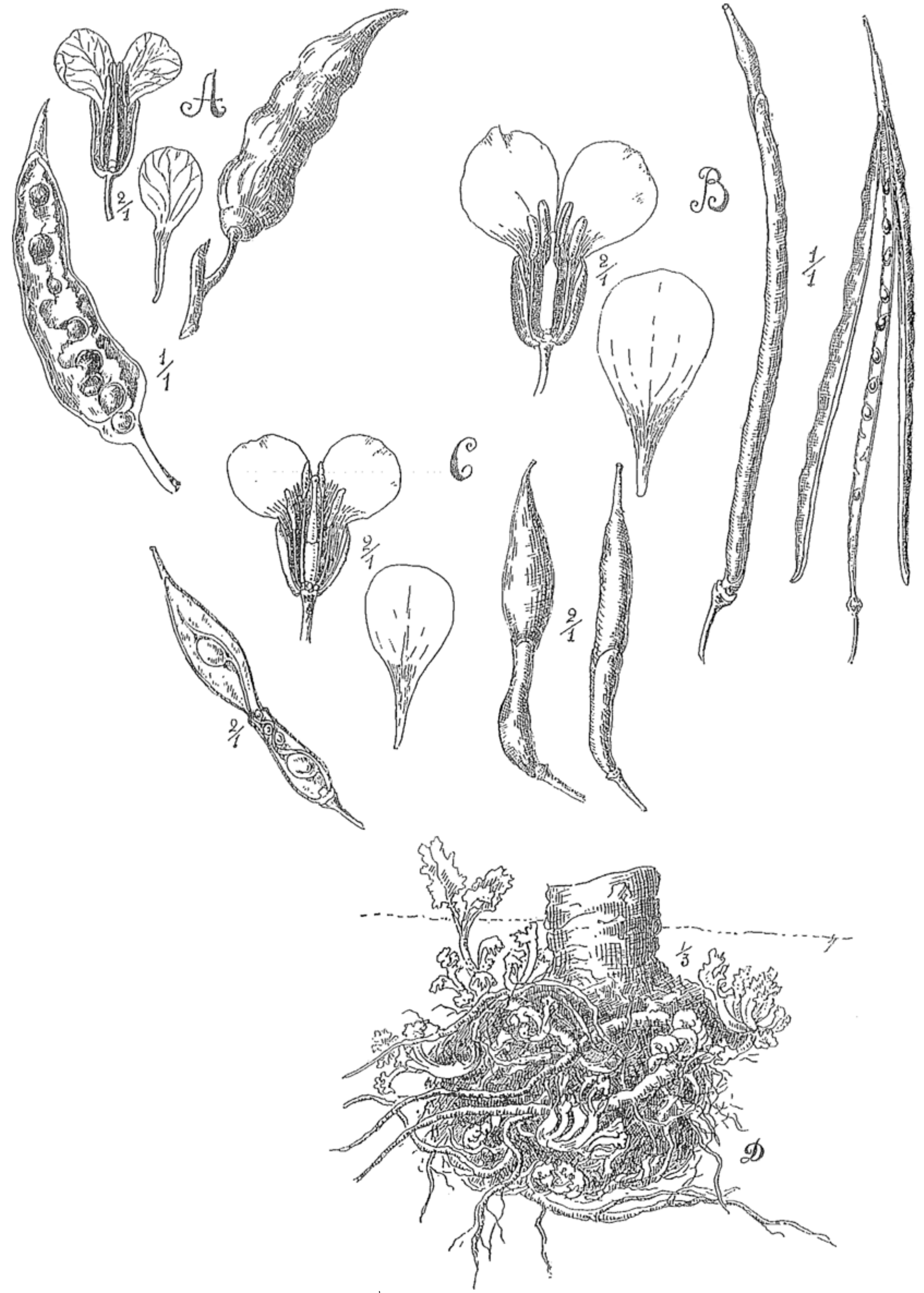
[1] In crosses of *Raphanus sativus* L. × *Raphanus raphanistrum* L. white colour dominates or prevails (Focke).



Text-fig. 2. Fruits and Flowers: *A. Raphanus sativus* L. *B. Brassica oleracea* L. C. Of the hybr. ♀ *Raph. sat.* × ♂ *Brass. ol.* *D.* Root of the hybr. ♀ *Raph. sat.* × ♂ *Brass. ol.*

cially; the cabbage possess only flavone in petals and nervures alike. Hybrids are different and sometimes peculiar, e.g. one hybrid of radish × hearting cabbage had in all flowers flavone only in nervures and at the base of the claw of petals and anthocyanin only in a little spot at the outer end of petals. Parents of this hybrid did not show any similar distribution. Gravatt, as we said, found eight stamens in some flowers of his hybrid. Such changes did not occur in our cases[1]: like all Cruciferae they had six stamens, their pollen was not powdery but stuck together, and it was difficult to use it when crossing.

The ovaries of radish consist of two segments: a lower small one containing a single ovule, and a long upper one gradually passing into the pistil—with 8–12 ovules. Cabbages have one single ovary with 30–35 ovules, and pass directly into the pistil. The hybrids have 2-celled ovaries but, in contrast to the radish, both segments were equally developed and had 3–6 ovules: the upper segment gradually narrowing into the pistil (see Text-fig. 2 *C*). As in the parental forms there is a longitudinal partition; the kind of ovaries determines the shape of the pods.

Pods. Radish has a spindle-like, inflated pod which does not split and consists of two parts, of a lower, smaller one, often sterile or containing one seed only and an upper one enlarged by a spongy mesocarp contracting towards the end, more or less narrow between the seeds (see Text-fig. 2 *A*). Cabbages have a linear pod splitting into two valves and elongated into a sterile or one-seeded apex (see Text-fig. 2 *B*). Our hybrids had no ripe seeds. Most of the ovaries fell off soon after pollination and only some, especially in hybrids of radish × hearting cabbage "kubyshka," developed for some time. Text-fig. 2 *C* shows young hybrid pods magnified twice: they are very peculiar as their lower part is distinctly 2-celled like a cabbage pod, but the upper part is more like a radish pod. An anatomical study of these hybrid pods shows that the longitudinal partition is pushed aside towards one or other wall of the pod, and goes over into the upper part; the sponge-like mesocarp develops in the upper part of the pod, but occurs also in the lower part. The resemblance to radish or cabbage pods depends upon the development of seeds in the lower or upper part of the pod; but the structure of pods is alike in all hybrids, and they would be considered by a systematist as a definite independent form. The difference of the pods in Sageret's case was probably due to their outward appearance only.

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[1] Only one flower in a hybrid was abnormal, having six petals in calyx, six petals, seven stamens and a deformed pistil.

The morphology of the pods of the hybrids seems clear: botanists like Pomel, Hayek, Schulz and others who have studied Cruciferae believe that the small segment of the pod in *Raphanus* is due to a reduction of a two-valved pod (*Valvarglied*) and the pod of radish is in reality only a developed manyseeded apex (*Stylarglied*). In *Brassica oleracea*, conversely, the two-valved part is developed and the apex remains small. Hybrids naturally receive both characters and we have really a pod where both segments are equally developed. Thus hybridisation confirms the conceptions of morphologists—a method rarely used till nowadays.

Conclusion of the morphological description of hybrids.

Thus a description of hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. shows that F_1 is quite uniform as to the form of pods and flowers but differs in form, pubescence, waxy efflorescence of leaves and in colour of flowers, while in habit of growth and vigour it is divided into two or three types. *Raphanus sativus* L. and *Brassica oleracea* L. are cross-fertilizers and therefore heterozygous; F_1 in their interracial crossings is always polymorphic, thus cabbages which were crossed with radish in 1922 were also intercrossed with each other and their F_1 was different in shape and leaves and length of stem. But in separate crosses the difference among racial hybrids of cabbages or radishes is insignificant and quite unlike that found in the generic crosses of *Raphanus* × *Brassica*. Their striking divergence in some characters and the habit of growth is probably due not only to their parents having been heterozygous but also to the peculiar nature of hybrids. We have here in one organism two quite different haploid collections of chromosomes, whose gens are mostly different and do not pair up with each other; they give no allelomorphs and act independently and separately. Thus no dominance or recessiveness can be observed and the heterozygosis of the parental forms is obvious, but at the same time the normal development of characters and of the whole organism is injured. The morphology of hybrids is determined in this case not only by gens but also by their more or less harmonious action. A further study of the hybrids is required for a detailed determination of their morphogenesis. The question about the divergence in F_1 is very complicated and requires great caution in its solution[1].

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[1] For various theories see Atkinson, "Sorting and blending of unit characters," *Zeit. f. ind. A.* 1916. Bd. xvi.

CYTOLOGY OF HYBRIDS[1].

The nuclear division in somatic cells of hybrids.

Most of the hybrids of the stunted type, if they did not flower, differed from radish only by their weaker development, by their more branched roots and by outgrowths on them. It was natural to inquire: are not the cabbage chromosomes eliminated in them under an influence of an unfamiliar protoplasm? A similar phenomena has been observed by Baltzer in fertilization of eggs of a sea-urchin of the genus *Strongylocentrotus* by sperm of the genus *Sphaerechinus*; chromosomes of the foreign spermatozoid were thrown out of the spindle and did not get into the daughter cells. Nor did they participate in the further development of the egg, which reached the Pluteus state, and had a purely maternal type of skeleton.

I have studied the nuclear division in cells of the root-tip and of stem-shoots of two radish × cabbage hybrids of the stunted type, most alike to radish, and of one hybrid of the vigorous type, intermediate in its morphological characters. It appeared that the division was normal in all three hybrids. According to my investigations in 1922, *Raphanus sativus* L. and varieties of *Brassica oleracea* L. have a diploid number of chromosomes equal to 18 and their haploid number is thus 9. The hybrids in somatic cells have 18 chromosomes, the sum of the haploid numbers of their parents. Thus there is no diminution in numbers of chromosomes or abnormalities in divisions of nuclei in somatic cells of the hybrids.

The reduction of the chromosomes in maternal cells of pollen.

The reduction of the chromosomes in anthers of *Raphanus sativus* L., *Brassica oleracea* L. and their hybrids can be observed in small buds about 2 mm. in length fixed when the weather is warm and favourable for growth. The time is of no importance as I found heterotypic division in materials fixed alike in the morning, during the day, and in the evening. In preparations of such buds one sees ordinarily synapsis and tetrads of pollen, but sometimes also other stages of heterotypic mitoses are seen which are evidently proceeding with greater rapidity. In the

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[1] The material for cytological investigations was fixed by a mixture according to S. G. Navaschin: 15 parts of 1 per cent. of chromic acid + 1 part of glacial acetic acid + 3 parts of 16 per cent. of formalin + 17 parts of dist. water. Microtome sections were 5–10μ thick; for staining iron haematoxylin was used.

two outer, shorter anthers the division begins later than in the four inner, longer anthers (e.g. when in the shorter anthers the first division takes place, in the longer one the pollen tetrads are already formed). Separate nests of anthers have also sometimes different stages of division, although in closer limits; they are later in upper parts of the nest. The reduction of the chromosomes proceeds alike in *Raphanus sativus* L. and *Brassica oleracea* L.[1] This division is shown in Figs. 1–12 of Pl. XXII, made from preparations with Abbé's drawing apparatus. It is difficult to say anything definite about the behaviour of chromatin threads in the synapsis (Fig. 1) because of the minuteness of the nuclei in *Raphanus* and *Brassica*. At first thin and long, these threads get contracted, thicker, and at last fall into separate segments or chromosomes, which lie in pairs (Fig. 2). There appears in the synapsis besides the first one, also another nucleolus, which is less intensively coloured by haematoxylin and is almost always near the first one as if "coming out" of it.

The next stage after synapsis is diakinesis when in *Raphanus sativus* L. (Fig. 4) and in *Brassica oleracea* (Fig. 3) alike a full conjugation of the pairs of chromosomes takes place, resulting in a formation of nine "bivalent chromosomes." The nucleoli sometimes are still there, or disappear. The nuclear membrane gets dissolved and the chromosomes are distributed in one plane. The metaphase of the first division then begins. If the spindle in this stage is seen from above (from the pole) the chromosomes are quite round and can be easily counted (Figs. 5 and 6), but if seen sidewise one can observe only the equatorial plane of the chromosomes (Fig. 7). Here a half of the bivalent chromosomes divides from each other, and later in anaphases of the first division (Fig. 8) a quite regular parting of them takes place towards the poles, nine to each pole. The parted chromosomes build daughter nuclei in the stage of the telophase (Fig. 9): a nucleus cover appears again and nucleoli are formed. But there is no resting stage, as the telophases of the first division pass into prophases of the second division: the nucleoli disappear and the chromosomes become twisted like 8 (Fig. 10). The nuclear membranes are again dissolved and in the metaphase of the second division two spindles are built in different planes (Fig. 11); the chromosomes split, and their halves proceed towards the poles, building four nuclei placed in a tetrahedron. Again cell membranes testiailes appear and four separate cells emerge (Fig. 12)—the tetrads of pollen developing later on into pollen grains. Thus the heterotypic mitoses in *Raphanus*

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[1] I studied *Brassica oleracea* L. var. *sabauda* L.

sativus L. and *Brassica oleracea* L. proceed quite normally according to the scheme usual for *Dicotyledonae*[1].

The reduction of the chromosomes has been observed by me in anthers of six hybrids: in four hybrids of radish × kohlrabi and in two of radish × hearting cabbage; in all plants the figures of division were alike. In synapsis (Pl. XXII, fig. 13) the hybrids do not differ from the maternal forms; but later on the spireme of chromatin threads divides into chromosomes (Fig. 14) which do not conjugate during diakinesis but remain lying separate and disorderly along the periphery of the nucleus (Figs. 15 and 16). Whereas in the parental forms we have in diakinesis nine bivalent chromosomes, in hybrids because of an absence of conjugation, there are nine radish and nine cabbage univalents or 18 chromosomes (Figs. 3, 4, 15 and 16). Sometimes one observes 17 or 16 chromosomes but there are no typical "gemini," and some chromosomes are simply hidden behind others or are covered by the nucleolus. As the nuclei are very small and the chromosomes are heaped together, this is very probable.

This absence of conjugation of parental chromosomes is also evident in the next stage, the metaphase of the first division. The parent plants have in this stage 9 big bivalent chromosomes but the hybrids have 18 small univalent chromosomes, corresponding in size to chromosomes of the anaphase of the parents (Fig. 17, compare with Figs. 5 and 6 with Fig. 8). The form of chromosomes in the hybrids is less definite than in *Raphanus* and *Brassica*. The chromosomes do not form in metaphases normal nuclear plates, although they are heaped in cells more or less; some of them are sometimes seemingly thrown towards the very borders of the cell (Figs. 17, 18). In the side view of the metaphase the hybrids have no spindles of division, and only sometimes one observes separate fibres (Fig. 19, compare with Fig. 7). There are no typical anaphases—the chromosomes proceed towards the poles irregularly (Fig. 20). The telephases have often in the beginning the form of 8 (Fig. 21) and then the daughter nuclei are formed (Fig. 22). An unequal number of chromosomes is used: from 6 till 12. Separate chromosomes and those which remain behind during the separation to the poles—often do not get into the daughter cells and remain included in the plasma as separate inclusions of chromatin. In some cases chromo-

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[1] Rarely one observes in *Brassica* such anomalies as e.g. a separation of a pair of chromosomes in the anaphase from the others which travel to the poles. These separated chromosomes remain in the protoplasm and do not participate in the formation of the nuclei.

somes did not separate at all during the first division and formed one big nucleus (Fig. 23).

Already in the telophases the second division begins—a longitudinal splitting of chromosomes (Fig. 24). In the metaphase of the second division a normal development of spindles is often observed as also regular metaphases with a different number of chromosomes (Fig. 24). In anaphases chromosomes are distributed unevenly, some of them are often thrown out altogether from the sphere of division (Figs. 25, 26), the spindles are long and not always rightly adjusted; sometimes we observed a fusion, as it were, of the two spindles (Fig. 27). Telophases have often a form of a tetrahedron, but with outward inclusions of chromatin (Fig. 28). A precise determination of the number of chromosomes in anaphases and telophases of the 2nd division is impossible because of their accumulation. The protoplasm in pollen mother cells of hybrids is quite normal.

The hybrids have therefore besides normal pollen tetrads (Fig. 29) groups of two, three and even seven cells (Figs. 30, 31). The cells are not alike in form and size, they have nuclei of different dimensions, and some of them contain chromatin only in separate grains.

Young pollen developed even out of small cells grows, but later its contents degenerate, the protoplasm and nuclei disappear and only wall membranes remain. But some pollen grains are preserved and they develop. The first division in pollen takes place (Fig. 32), generative cells are separated, and in some cases even a division of the generative nucleus (Fig. 38) has been observed as well as perfectly mature pollen with two male nuclei (Fig. 34). The protoplasm in such pollen is quite normal.

Thus we observe definite anomalies in the process of heterotypic division of pollen-mother-cells—in hybrids of radish × cabbage, proper to all hybrids of distant forms. A complete absence of conjugation of parental chromosomes in diakinesis was first observed by Federley in interspecific crossings of the moth *Pygaera*. The author could not find in them the stage of synapsis, and the univalents behaved differently from those in hybrids of *Raphanus* × *Brassica*: they split in the first and second division and their halves were evenly distributed so that each spermatozoid had a diploid or nearly diploid number of chromosomes[1]. Chromosomes did not conjugate also in the prophase of a

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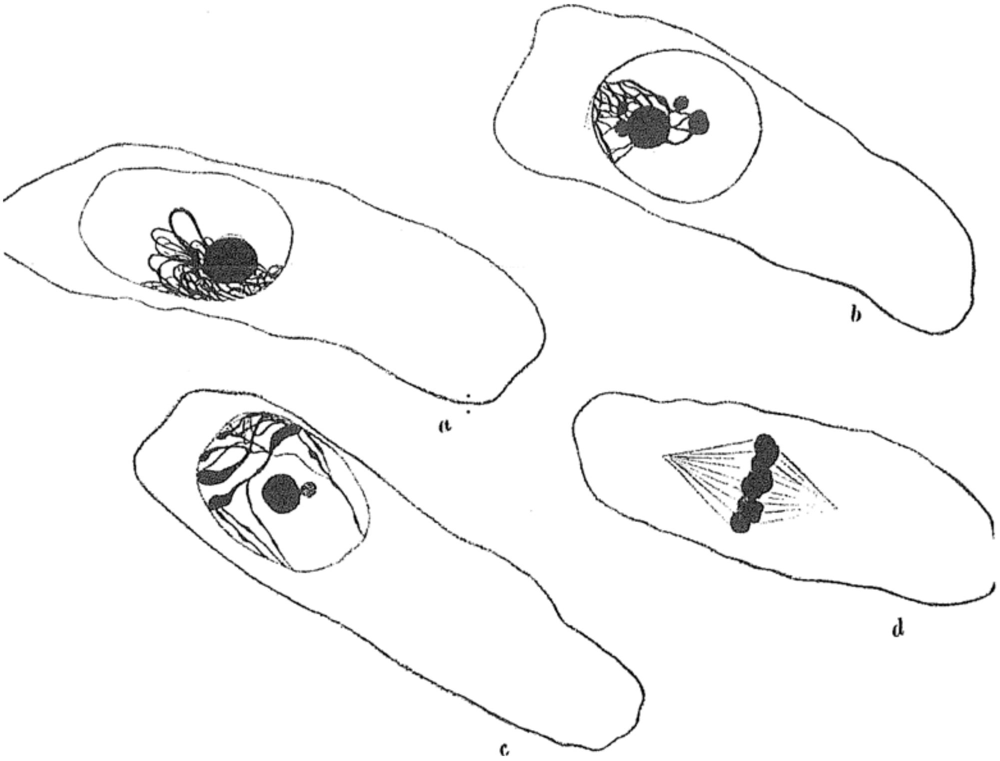
[1] Only separate chromosomes conjugated sometimes in diakinesis. An absence of conjugation observed also Doncaster and Harris in hybrids of butterflies *Bistoninae*. *Journ. of Genetics*, 1914.

heterotypic division according to Poll's studies of hybrids of pea-fowls with guinea-hens.

In plants this phenomena was first observed by Haase-Bessel in interspecific hybrids of *Digitalis purpurea* × *Digitalis lutea*. Hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. are in this respect a second example. Anomalies in the development of both hybrids are almost alike.



Text-fig. 3 (*a–d*). Reduction division in embryosac mother cell. *a.* Synapsis in *B. ol.* L. *b.* Synapsis in hybr. of *R. sat.* L. × *B. ol.* L. var. *capitata* L. *c.* Deployment of the synapsid knot in hybr. of *R. sat.* L. × *B. ol.* L. var. *capitata* L. *d.* Metaphase of the first division in hybr. of *R. sat.* L. × *B. ol.* L. var. *gongyloides* L.

The reduction of the chromosomes in the ovules.

The reduction in the ovule of hybrids of *Raphanus sativus* × *Brassica oleracea* begins in buds of 3–4 mm. in length like that of the parental forms, i.e. when tetads are developed in anthers and young pollen is built[1]. Synapsis in the ovule of hybrids has been observed by me several times (Text-fig. 3 *a* and *b*), but the deployment of the chromatin knot

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[1] *Brassica oleracea* f.e. is decidedly protogynous.

proceeds anomalously (Text-fig. 3 *c*). Out of a great number of ovaries I found only once a very clear preparation with a metaphase of the first division, where it was proceeding quite normally—there was a normal spindle and one could see in the equatorial plate nine bivalent chromosomes (Text-fig. 3 *d*). In the same bud pollen was quite anomalous. No other stages of heterotypic division could be found, and I never observed the development of an embryo sac. Possibly the development of the megaspore mother cell ceases in hybrids in the stage of the prophase or after the parting of chromosomes—in the anaphase of the first division. A different behaviour of chromosomes in anthers and ovules was pointed out by Rosenberg for *Hieracium Pilosella*, by Täckholm for the *Rosa canina* group, and by Overeem for hybrids of *Oenothera gigas* × *Oenothera semigigas*. In previously mentioned studies Federley and Poll investigated only spermatogenesis, and Hasse-Bessel observed in *Digitalis* during diakinesis an absence of conjugations of chromosomes in ovaries and anthers alike.

Cases of different conjugations of chromosomes in the development of male and female gametes, the influence of external factors (see Tischler), and new data of Federley (1917) showing that racial hybrids of some butterflies do not differ in behaviour of chromosomes from interspecific and generic hybrids, may possibly indicate that the conjugation of chromosomes does not depend on gens but on some other causes.

Sterility of hybrids and reciprocal crossings of ♀ Brassica oleracea L. × ♂ Raphanus sativus L.

Sageret's statement that radish × cabbage hybrids set seeds was not confirmed by Gravatt and Baur. My numerous intercrossings of hybrids among themselves and with the parental forms gave no results. The ovaries sometimes developed did not contain any embryos. Experiments with germination of pollen in various solutions all failed (Gravatt).

Sterility of gametes in hybrids is probably due to an occasional distribution of parental chromosomes during the reduction of the chromosomes, why most gametes never get a haploid collection of chromosomes, necessary for their development.

Cases when active parental gametes may be formed may be too rare to be observed in experiments. Possibly during a prolonged vegetative propagation of hybrids they may acquire some fertility as observed by Wettstein in *Sempervivum* hybrids. No reciprocal crossings of *Brassica oleracea* L. × *Raphanus sativus* L. did succeed, as shown by attempts of Sageret, Herbert, Gravatt, Baur and my attempts of 1923. I crossed also *Brassica oleracea* L. with *Raphanus raphinistrum* L. and *Raphanus odessoinus* Spreng. but without results.

Anatomical investigations of ovaries of cabbages pollinated by radish pollen and fixed on the 3rd or 5th day after do not disclose any development of embryos in ovules; only destroyed embryo-sacs are seen in preparations. Pollen on stigmas and in pistils gives only short tubes, or does not germinate at all. Evidently this failure is due to physiological peculiarities of the stigmas of cabbages, hindering a normal germination of radish pollen.

SUMMARY.

In summarising the above data one has to state that a detailed study of generic hybrids of ♀ *Raphanus sativus* L. × ♂ *Brassica oleracea* L. establishes some new facts of a general genetical interest. These facts can be emumerated as follows:

1. Hybrids of F_1 of *Raphanus sativus* × *Brassica oleracea* differ much among themselves in vigour, habit of growth, in leaves and in colour of flowers. In separate crossings occur exceptionally vigorous and also stunted plants, the latter being mostly of a maternal character.

2. This polymorphism of hybrids does not affect the structure of flowers and fruits. Those characters are intermediate for all hybrids.

3. The roots of hybrids have outgrowths and leaf shoots just as hybrids of *Brassica Napus* L. × *Brassica Rapa* L.

4. The nuclear division in somatic cells (somatic mitoses) proceeds quite normally.

5. The heterotypic mitosis in pollen-mother-cells is quite anomalous: the parental chromosomes do not conjugate in diakinesis, and their distribution during the first and second division is anomalous; besides the pollen-tetrads, groups consisting of two, three and even seven cells are formed.

6. Of the grains of pollen only a few are developed, and pollen mostly degenerates.

7. In cells of archesporium the early stages of a meiosis were observed: synapsis and the dissolution of the knot of chromatin threads; once a normal metaphase of the first division was observed with a normal spindle and bivalent chromosomes. Other stages of division were not observed. No formation of embryo-sacs in ovules of hybrids could be detected.

8. Hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. remain sterile when intercrossed or crossed with parental forms.

9. A reciprocal crossing between ♀ *Brassica oleracea* L. × *Raphanus sativus* L. ♂, does not succeed.

I have to acknowledge and to express my gratitude for kind help and interest in my work to Prof. S. J. Jegalov, to A. G. Nikolaeva and U. N. Sveshnikova. I am also much indebted to Prof. S. G. Navaschin for his valuable suggestions and to Prof. N. J. Vavilov for his help in publishing this article.

EXPLANATION OF PLATES.

PLATE XXI. Fig. 1. General view of the whole collection of hybrids: *Raphanus sativus* L. × *Brassica oleracea* L. In the foreground is a hybrid of the 1st type; then is a series of hybrids of the 2nd type; the left row and one plant at right are hybrids of the 3rd type (tallest). Photographed in October, 1923.

Fig. 2. Hybrids: *Raphanus sativus* L. × *Brassica oleracea* L. var. *sabauda* L. (at right), *Raphanus sativus* L. × *Brassica oleracea* L. var. *gemmifera* D.C. (in the centre), and *Raphanus sativus* L. × *Brassica oleracea* L. var. *capitata* L. (at left). Photographed in October, 1923.

Fig. 3. Outgrowths and leaf shoots on roots of hybrids *Raphanus sativus* L. × *Brassica oleracea* L.

PLATE XXII. The drawings have been made with Abbé's drawing apparatus.

Figs. 1–31 on Pl. XXII and Text-fig. 3 were done with Zeiss, 1/12 homog. immer. + compens. ocular 12.

Figs. 32–34, Pl. XXII at 1/12 homog. immer. + compens. ocular 8.

On reproduction the figures were × $\frac{2}{3}$.

Figs. 1–12. Reduction division in pollen-mother-cells in *Brassica oleracea* L. and in *Raphanus sativus* L.

Fig. 1. Synapsis in *B. ol.* L.
Fig. 2. The first stages of diakinesis in *B. ol.* L.
Fig. 3. Diakinesis in *B. ol.* L.
Fig. 4. Diakinesis in *R. sat.* L.
Fig. 5. Metaphase of the first division in *B. ol.* L.
Fig. 6. Metaphase of the first division in *R. sat.* L.
Fig. 7. Metaphase of the first division in *B. ol.* L. (viewed from the side)
Fig. 8. Anaphase of the first division in *B. ol.* L.
Fig 9. Telophase of the first division in *B. ol.* L.
Fig. 10. Prophase of the first division in *B. ol.* L.
Fig. 11. Metaphase of the second division in *R. sat.* L.
Fig. 12. Pollen-tetrads in *B. ol.* L.

Figs. 13–31. Reduction division in pollen-mother-cells of hybrids of *R. sat.* L. × *B. ol.* L.

Fig. 13. Synapsis in hybrid of *R. sat.* L. × *B. ol.* L. var. *capitata* L.
Fig. 14. The beginning of diakinesis in hybrid of *R. sat.* L. × *B. ol.* L. var. *capitata* L.
Figs. 15, 16. Diakinesis in hybr. of *R. sat.* L. × *B. ol.* L. var. *capitata* L.
Figs. 17, 18. Metaphases of the first division in hybr. of *R. sat.* L. × *B. ol.* L. var. *gongyloides* L.

Fig. 19. Metaphase of the first division in hybr. of *R. sat.* L. × *B. ol.* L. var. *capitata* L.

Fig. 20. Anaphase of the first division in hybr. of *R. sat.* L. × *B. ol.* L. var. *gongyloides* L.

Fig. 21. The beginning of telophase of the first division in hybr. of *R. sat.* L. × *B. ol.* L. var. *capitata* L.

Figs. 22, 23. Telophase of the first division in hybr. of *R. sat.* L. × *B. ol.* L. var. *capitata* L.

Fig. 24. Metaphase of the homoeotypical division in hybr. of *R. sat.* L. × *B. ol.* L. var. *capitata* L.

Figs. 25, 26, 27. Anaphases of the homoeotypical division in hybr. of *R. sat.* L. × *B. ol.* L. var. *capitata* L.

Fig. 28. Telophase of the homoeotypical division in hybr. of *R. sat.* L. × *B. ol.* L. var. *capitata* L.

Figs. 29, 30, 31. Stage of the "pollen-tetrads" in hybr. of *R. sat.* L. × *B. ol.* L. var. *gongyloides* L.

Figs. 32–34. Pollen grains in hybr. of *R. sat.* L. × *B. ol.* L.

Fig. 32. The first division in pollen grain in hybr. of *R. sat.* L. × *B. ol.* L. var. *gongyloides* L.

Fig. 33. The second division in the pollen grain in hybr. of *R. sat.* L. × *B. ol.* L. var. *gongyloides* L.

Fig. 34. Normal pollen grain with two male nuclei in hybr. of *R. sat.* L. × *B. ol.* L. var. *gongyloides* L.

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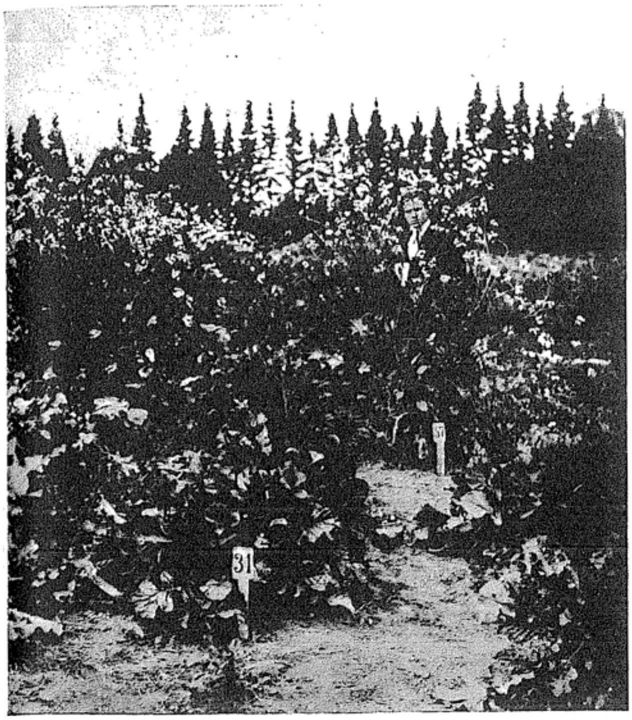

Fig. 1.

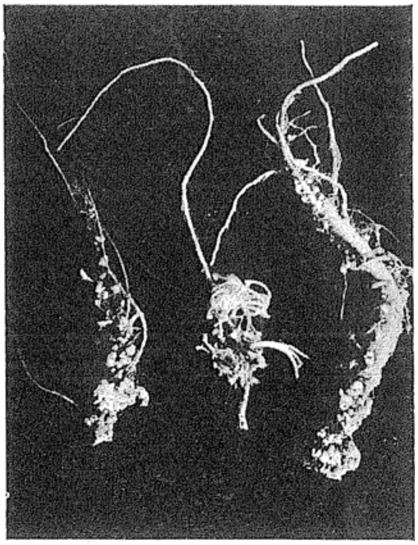

Fig. 3.

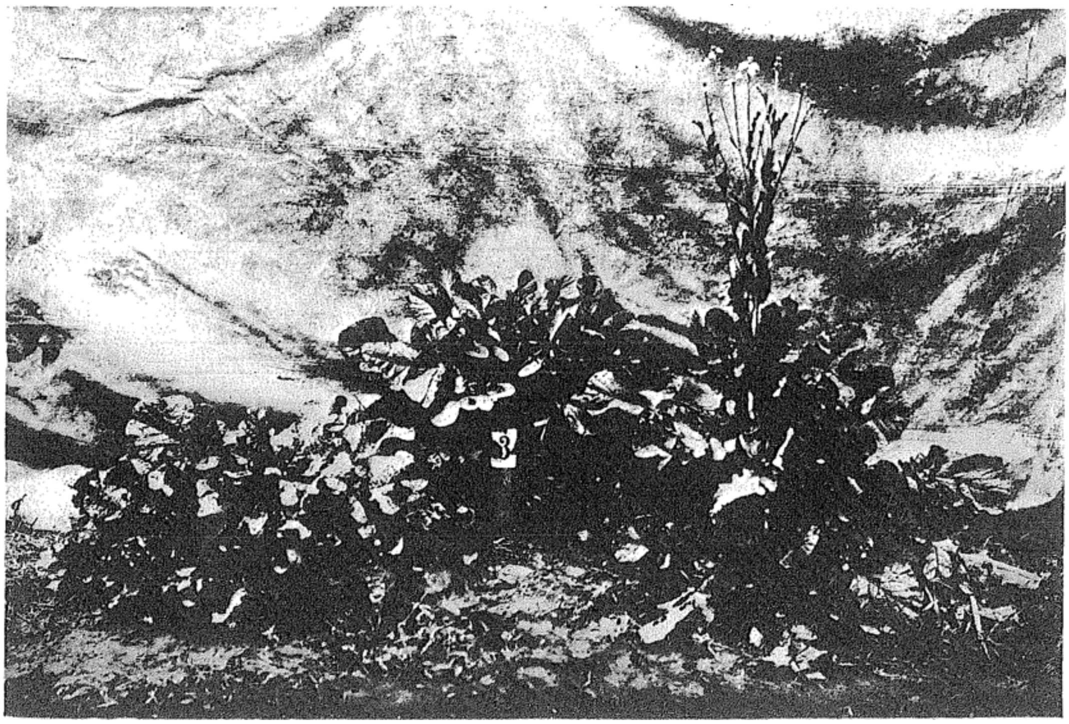

Fig. 2.

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PLATE XXI