INVESTIGATION ON F_1 AND F_2 HYBRIDS BETWEEN *BRASSICA CARINATA* AND *RAPHANUS SATIVUS*

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Introduction

The cross between *Brassica carinata* (Abyssinian mustard) and *Raphanus sativus* (Radish) has been recorded by Karpechenko (1929), Morinaga (1933) and Nagaharu (1935). Morinaga mentions that a *Carinata-Raphanus* hybrid (F_1) produces no constant bivalents in micro-sporogenesis. He has, however, drawn a place at first metaphase showing 22 univalents and two bivalents. Nagaharu confirms the complete absence of pairing.

In the present investigation two hybrids belonging to F_1 and F_2 generations were raised in 1935 and 1936 respectively at Cambridge (England) and were cytologically examined. The meiotic chromosomes were studied exclusively on smear preparations fixed in La cour 2B followed by the Iodine-Gentian-Violet method of staining.

2. Meiosis in Parental Species.

(a) *B. carinata* (*2n=34*). This species was cytologically examined by a few who have recorded its chromosome number to be 34 and meiotic process regular. No multivalents were observed at prophase of meiosis. In the present investigation twelve nuclei were analysed at diakinesis, of which five showed two tetravalents (Fig. 1), six only one tretravalent in each and one no multivalent. At anaphase numerical segregation of chromosomes is regular. (Fig 2).

(b) *R. sativus* (*2n=18*). At diakinesis in this species nine bivalents are regularly observed. Maeda and Sasaki (1934), however, report the presence of multivalents in two horticultural varieties.

3. F_1 Hybrid

The hybrid grew quite vigorously with glabrous stem and red tinge at the nodes. The leaves were lobed and smooth. The petals were white and the stigma very much swollen. The proportion of dehiscent to non-dehiscent part in the fruit was approximately 2:1.

Meiosis.

It was examined chiefly from diakinesis onward. Four pollen mother cells were studied at mid-diakinesis, but exact analysis of all the chromosome configurations in any one of them was difficult. Roughly the number of bivalents varied between 6 and 9. After undergoing pro-metaphase the nucleus passes into metaphase when again a variable number of bivalents is seen. Owing to the stretched condition of bivalents along the spindle axis in side view and their larger size in polar view they are easily discriminated from the univalents (Figs. 3 and 4). Twenty-six plates were studied at this stage and the results are shown below:—

Bivalents	0	1	2	3	4	5	6	7	8	9	Total
P.M.Cs.	—	—	2	—	5	5	4	4	2	4	26

In a side view plate one heteromorphic bivalent was also observed (Fig. 5). Its absence from other plates may suggest that the chromosomes concerned might have remained unpaired. Secondary associations between bivalents and univalents are quite frequently observed. In all the three polar views with nine bivalents two groups of two secondarily associated univalents were constantly observed (Fig. 3).

At anaphase the bivalents disjoin quite regularly while the univalents show the following behaviour:—

(1) Either the univalents reach the equator along with the bivalents in which case two types of behaviour are observed: (a) The univalents travel to the opposite poles possibly at random, but sometimes they may split and the halves may travel to the opposite poles or the splitting may occur on their way to the poles in which case the chromatids possibly travel to the same pole. This occurs more or less simultaneously with the disjointing of the bivalents. (b) Several univalents are seen lagging on the equator which sometimes show their division. Such a condition may sometimes lead to the formation of a restitution nucleus (Fig. 6).

(2) Or the univalents do not reach the equator and may join the nearer constituents of the bivalents at the opposite poles. Occasionally they remain scattered between the poles forming a sort of bridge which may possibly result in a restitution nucleus (Fig. 9).

Figs. 1—9. Meiosis in *Brassica carinata* (2n=34), ×3,200. 1. Diakinesis, two tetravalents and thirteen bivalents and a nucleolus. 2. I anaphase showing regular segregation of chromosomes. Meiosis in F1. *B. carinata.* × *R. sativus.* × 3,290. 3. I metaphase polar view showing nine bivalents and eight univalents. Notice secondary associations among bivalents and univalents. 4. I metaphase side view showing nine bivalents and eight univalents. 5. I early anaphase with six bivalents (one of which is heteromorphic) and fourteen univalents. 6. I late anaphase, notice the splitting of lagging univalents. 7. II metaphase polar views where some univalents have divided at I division. 8. II division spindles, notice the lagging of univalents. (Meiosis in F1. *B. carinata*×*R. sativus.* ×3,200,) 9. restitution nucleus with 29 chromosomes.

Transitional conditions may also be expected. At second division, the univalents of a restitution nucleus (Fig. 9) are seen arranged on the equatorial plate and undergoing regular division (Figs. 10 and 11). Occasionally lagging and losing of one or two univalents are also observed. Such a pollen mother cell results in a dyad, with 2n or nearly 2n number of chromosomes. If a restitution nucleus is not formed the two groups of chromosomes without undergoing interkinesis enter into the second division when again lagging is sometimes observed. (Fig. 8). This results in a tetrad. The hybrid produced dyads and tetrads roughly in equal numbers.

After a certain stage the anthers began to dry off and they were not observed to dehisce. 1—2% good pollen was observed by teasing out the anthers in aceto-carmine.

4. F_2 Hybrid

The F_1 hybrid was extremely sterile. It was growing along with several other Brassica hybrids, but produced no fruits even by open pollination. Since the anthers did not dehisce they were teased out in drops of tap-water on a slide and pollination was then effected by simply dipping the stigma into this water, which was always examined under the microscope to ensure that sufficient pollen was present. But the flowers were not bagged. By such a method two feebly developed pods were obtained (but this does not altogether exclude the possibility of an out-cross) which yielded only one small round seed (somewhat red) which gave the present F_2 hybrid.

It produced hairy and lobed leaves and a small greenish white bulb. In other characters it entirely resembled the F_1. In this hybrid the anthers, as a rule, ruptured quite normally.

The hybrid showed 28 chromosomes in the root-tip cells fixed in La cour 2B followed by the Iodine-Gentian-Violet method of staining.

Meiosis.

At very early diakinesis (or late diplotene) one nucleus and at late diakinesis also one nucleus were studied which showed the following configurations.

Early diakinesis: 1 tetravalent, 6 bivalents and 12 univalents. (Fig. 13).

Late diakinesis: 1 trivalent, 8 bivalents and 9 univalents.

At I metaphase seven polar views were examined when again variable pairing was observed as shown below. At this stage some bivalents can be distinctly marked but all of them cannot be definitely ascertained, hence the number of bivalents was always

confirmed by subtracting the observed number from the somatic number 28. Trivalent which was observed only twice could be distinctly made out. Occasionally at this stage nucleolar fragments were also detected. Secondary associations were quite often observed.

P.M.Cs.	Trivalents.	Bivalents.	Univalents.
1	—	10	8
2	I	10	5
*3	—	10	8
4	—	6	16
5	—	8	12
6	—	10	8
7	I	5	15

* Drawn in Fig. 14.

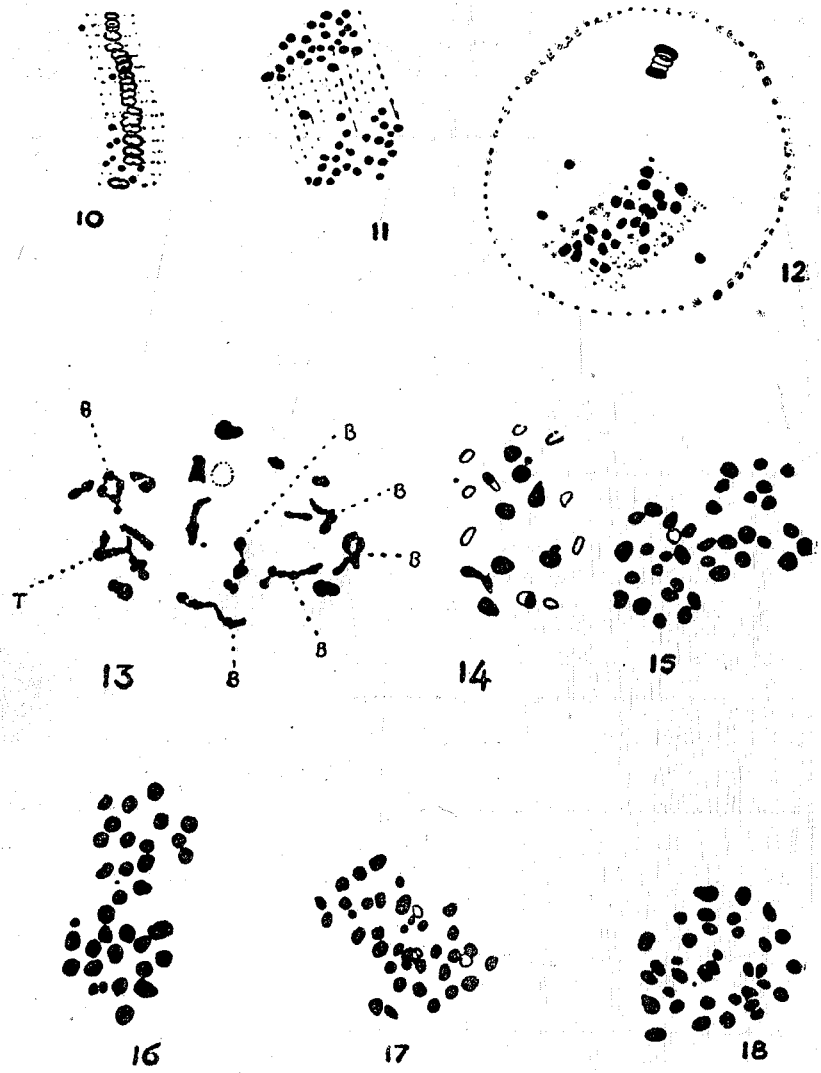
Very often plates showing more than 28 univalents are observed (Figs. 15-18). They possibly represent different types towards the formation of restitution nucleus when variable numbers of univalents have divided. Chromosomes were counted from nine such plates which gave the following distribution:

No. of univalents ..	34	35	36	37	38	39	40
Frequency of P.M.Cs. ..	1	2	3	1	—	—	2

At I anaphase the univalents behave in a similar manner as observed in F1 (see page 138). At interkinesis, when the univalents were seen scattered within the cavity and had not lost their boundary, chromosome number was determined from three such pollen mother cells in both the daughter nuclei as shown below. The division of univalents at this stage was quite often observed.

P.M.Cs.	I Pole	II Pole	Total
1	18	10	28
2	12	16	28
3	14	18	32

At II division some irregularities were quite often noticed, such as the lagging of several chromatids and the fusion of spindles.



Figs. 10—18. Side view of a restitution nucleus 11. late anaphase. 12. II division. (Meiosis in F_2 *B. carinata.* ×*R. sativus.* ×3,200.) 13. Early diakinesis showing one tetravalent (T) six bivalents (B) and 12 univalents. (14) I metaphase with ten bivalents and 8 univalents and two nucleolar fragments. Various types towards the formation of restitution nucleus in F_2 (2n=28) when variable numbers of univalents have divided. ×3,200. 15. With 36 chromosomes. 16. With 35 chromosomes and possibly a necleolar fragment. 17. With 40 chromosomes. 18. With 35 chromosomes.

The hybrid produced dyads, triads and tetrads in the following percentages, examined from one preparation only:

Dyads	Triads	Tetrads
18	6	76

The pollen was examined in acetocarmine and two types were distinguished: 1. Large pollen which took the red stain. 2. Small ones which did not stain and were yellow. Some variation among these two types was also noticed. Pollen counts were made on three different days as shown below.

	Date.	Large pollen		Short pollen	
		Actual No.	percentage.	Actual No.	Percentage.
1.	25-5-1936.	26	12%	189	88%
2.	28-5-1936.	17	7%	222	93%
3.	29-5-1936.	26	14%	159	86%

By open pollination only one seed was obtained from this hybrid.

Summary

The paper deals with the cytology of *Brassica carinata* (2n=34), *Raphanus sativus* (2n=18) and their F_1 and F_2 hybrids.

At diakinesis in *B. carinata* occasionally one or two tetravalents were observed. No such multivalents were formed in *R. sativus*.

In F_1 at meiosis variable pairing, up to nine bivalents was seen. Heteromorphic bivalent was also observed once among twenty-six plates examined.

The F_2 hybrid showed 28 chromosomes (2n). At meiosis a variable number of bivalents up to 10, with occasional multivalents (trivalent and tetravalent) was observed.

The hybrids in both the generations were extremely sterile.

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