Interspecific Hybridization in *Brassica*

VI. The cytology of F_1 hybrids of *B. juncea* and *B. nigra*¹⁾

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Brassica oleracea L. and *Brassica nigra* Koch. seem to represent two extreme types in *Brassica*. As *B. oleracea* may be assumed to be the type of the genus, *B. nigra* was excluded therefrom by several authors, and was placed in *Sinapis*.

The interspecific hybridization of *B. nigra* and other species named in *Brassica* has been tried by a few workers. Moreover all the attempts made by SINSKAIA to cross *B. nigra* with *B. juncea*, *B. campestris* or *B. napus* had no results. SINSKAIA therefore thought that *B. nigra* occupied an entirely isolated position in the genus (6). According to SASAOKA, however, NAGAI succeeded in obtaining a hybrid seed from the crossing of *B. juncea* with *B. nigra* (5). In regard to my own experiments *B. nigra* produces hybrids with certain other species of *Brassica*, though with some difficulty.

In this paper, I will first report the karyological observations made on the F_1 hybrid *B. juncea* Coss. $\times$ *B. nigra* Koch., then I will discuss briefly the genomic interrelation of various cultivated species in *Brassica*.

Materials and Methods

The parental species: One of the parental species *B. juncea* Coss. (Katuona) is cyto-genetically identical with *B. cernua* Hemsl., and both species were referred more than once in this series of works (3, 4). The seeds of the other parent *B. nigra* Koch. were obtained in 1933 through the Nishigahara Agricultural Experimental Station, and it was understood that they were from an old sample that had been introduced from Russia. The gametic number of chromosomes of this species was determined as 8.

The F_1 plant: In the spring of 1933, 201 flowers of *B. juncea* were castrated and to them the pollen-grains of *B. nigra* were applied. Ten

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seeds were obtained in all, out of which two germinated. One plant which grew up very vigorously was later proved to be a true hybrid by its intermediate appearances. The materials for the karyological studies were taken exclusively from this plant. Some outstanding characters of the parents and the F_1 plant are shown in the following table.

Table 1. Some outstanding characters of *B. juncea*, *B. nigra* and their F_1 hybrid.

	<i>B. juncea</i>	<i>B. nigra</i>	F_1
Leaves	Thick, dark green, without hairs; long obovate in outline, unlobed.	Thin, soft green, with dense stiff hairs; short obovate in outline, margin lobed toward the base.	Thick, dark green, with sparse stiff hairs; long obovate in outline, margin lobed toward the base.
Flower	Sepals spreading; petal's claw stout and erect.	Sepals spreading; petal's claw very slender and spreading.	Sepals spreading; petal's claw stout and erect.
Inflorescence	Long; pedicels oblique to the axis of inflorescence.	Short; pedicels erect or almost parallel with the axis of inflorescence.	Short; pedicels in intermediate position.
Silique	Cross section circular.	Cross section diamond shaped.	Cross section circular.

The F_1 plant produced some seeds in natural condition, but no seeds were obtainable when the inflorescences were bagged.

The anthers, at the correct stage of development, were fixed with BENDA's or BOUIN's fixative. As a trial, the aqueous solution of picric acid in the latter fixative was in some cases replaced with its absolute alcohol solution, and with commendable results. Sections 10-15 thick were made, according to the paraffin method, and stained with iron-alum-haematoxylin.

Results of Karyological Observations

Heterotypic division: The history of the chromosome complement in the F_1 hybrid was followed chiefly from the diakinesis. The diaphasic nucleus contains 8 bivalent and 10 univalent chromosomes, and both kinds of chromosomes can easily be distinguished from each other by their size and shape. Small satellite-like chromatic bodies appear at this stage connected with bivalents. Fairly often two bivalent chromosomes are kept in loose contact like a tetravalent (Fig. 1). In the heterotypic metaphase, I counted always 8 bivalent and 10 univalent chromosomes. The bivalents, now on the equatorial plate, arrange

themselves in two manner viz. 7 bivalents in a ring and 1 bivalent in the center, or 6 in a ring and 2 near the centre. These configurations

Fig. 1. A nucleus in diakinesis, showing 8 bivalents (2 of which are loosely connected) and 10 univalents. $\times 4,000$.

coincide perfectly with those of the 8 floating magnets in MAYER's experiments (2). Apparently the configuration of bivalents on the equatorial plate are not at all affected by the univalents scattering by chance near the surface of the spindle substance. In Figs. 2-5, the polar views of 4 metaphasic plates are presented to show the relative positions of the 8 bivalent and 10 univalent chromosomes. The univalents shown as projected among the bivalents are actually situated far from the equator.

In the heterotypic anaphase, the bivalents separate first, and then the univalents, which have been situated by chance near the equator, start to split. Fig. 6 shows an early anaphasic spindle where all the bivalents have been disjoined while the univalents still remain intact.

Figs. 2-5. Polar views of heterotypic metaphase showing the configurations of 8 bivalents and 10 univalents. $\times 4,000$. Fig. 2. 6_{II} in a ring and 2_{II} in central position. 2_I nearly on the plate, 3_I above, and 5_I below the plate. Fig. 3. 6_{II} in a ring and 2_{II} in the center. 10_I nearly on the plate. Fig. 4. 7_{II} in a ring and 1_{II} in the center. 8_I nearly on the plate, 1_I above and 1_I below it. Fig. 5. 7_{II} in a ring and 1_{II} in the center. 9_I nearly on the plate and 1_I above it.

The anaphasic spindles at slightly later stages are shown in Figs. 7 and 8. The univalents that take up their position far away from the equator do not show any signs of splitting. The interkinesis does not proceed far, and the daughter nuclei pass into the homotypic prophase without losing the sharp outline of chromosomes.

Homotypic division: The homotypic spindle contains two kinds of chromosomes, dyads and monads. The dyad chromosomes appear in their normal sizes, but the monad chromosomes show a marked reduction in size thus making a striking contrast (Fig. 9). It was fairly difficult to stain all of those small chromosomes sharply. The frequency distribution of the chromosome numbers in the homotypic metaphase was as follows.

Table 2. Frequency distribution of chromosome numbers in homotypic metaphase.

Number of chromosomes on the homotypic spindle	11	12	13	14	15	16	17	18	19	Average
Frequency	1	3	7	13	13	11	1	0	1	14.5

Figs. 6 and 7. Two polar views of heterotypic anaphase depicted respectively at 4 different foci a, b, c and d, from upper to lower. $\times 4,000$. Fig. 6 shows 2 groups of 8 disjoined bivalents and 10 intact univalents. Fig. 7 shows 2 groups of 8 disjoined bivalents, and 7 split and 3 intact univalents.

Fig. 10 is an exceptional, giant homotypic plate formed occasionally by the union of two spindles. In the homotypic anaphase, most of the monads lag behind the main groups of split chromosomes. The cytokinesis proceeds normally, and 4 microspores result, though they very rarely seem to develop into matured pollen-grains.

Figs. 8-10. A side view of heterotypic anaphase and 2 polar views of homotypic metaphase. $\times 4,000$. Fig. 8. Heterotypic anaphase showing 2 groups of 8 disjoined bivalents, and 8 split and 2 intact univalents. Fig. 9. Homotypic metaphase showing 15 chromosomes, 10 dyads and 5 monads. Fig. 10. A giant homotypic plate formed by the fusion of two spindles.

General Remarks

In short, the chromosome behaviours in the F_1 hybrid of *B. juncea* ($n=18$) and *B. nigra* ($n=8$) also belong to the *Pilosella* type of the *Drosera* scheme. The hybrid presents consistently, in the heterotypic metaphase, 8 bivalent and 10 univalent chromosomes. On the other hand, the F_1 hybrid, *B. juncea* $\times$ *Raphanus sativus* is known to produce no bivalent chromosomes in its microsporogenesis (1). It is, no doubt, impossible for the *juncea* chromosomes to undergo synapsis inter se in the F_1 microsporocyte, if a dissimilar genome only is contributed by the other parent. Thus, for the 8 constant bivalents found in the microsporocyte of *B. juncea* $\times$ *B. nigra*, allosynapsis, i.e. the conjugation of the whole *nigra*-chromosomes with 8 *juncea* ones, seems to be the only explanation. I have already analysed the chromosome complement of *B. juncea* into two different elemental chromosome sets **a** and **b** which are respectively composed of 10 and 8 chromosomes. The **a** set was, on that occasion, identified with the set contained in *B. chinensis* and with various other species that have 10 chromosomes. Then the *juncea* chromosomes which make synapsis with the *nigra* chromosomes must be those composing the **b** set. The **b** set chromosomes are, thus, identified with the *nigra* chromosomes.

Based on the results of my cyto-genetical investigations, the cultivated species of *Brassica* are classified into 6 groups as shown in Table 3.

Table 3. Genomic constitution of cultivated *Brassica*.

Group	Chromosome number (n)	Genomic constitution	Species included	Remarks
I	10	aa	<i>B. campestris</i> L. <i>B. Rapa</i> L. <i>B. chinensis</i> L. <i>B. pekinensis</i> Rupr. <i>B. japonica</i> Sieb.	Elemental species I
II	8	bb	<i>B. nigra</i> Koch.	Elemental species II
III	9	cc	<i>B. oleracea</i> L. <i>B. alboglabra</i> Bailey	Elemental species III
IV	18	aabb	<i>B. juncea</i> Coss. <i>B. cernua</i> Hemsl.	Amphidiploid species from I and II
V	19	aacc	<i>B. napus</i> L. <i>B. Napella</i> Chaix.	Amphidiploid species from I and III
VI	17	bbcc	<i>B. carinata</i> Braun.	Amphidiploid species from II and III

A full discussion concerning this classification will be made elsewhere on some future occasion.

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