**Cytogenetic Studies in Artificially Raised Interspecific Hybrids of *Papaver*.
VIII. F_1 plants of *P. bracteata* $\times$ *P. lateritium*.¹⁾**

By

Kono Yasui

Tokyo Imperial University
(With 22 Text Figures)

Received November 20, 1940

Introduction

In previous investigations, I have shown that neither of the plants *P. bracteata* and *P. lateritium* have any chromosome capable of association with those of *P. somniferum* in the PMCs of either F_1 plant *somniferum* $\times$ *bracteata* or *somniferum* $\times$ *lateritium* (YASUI 1927, 1937).

Between *P. bracteata* and *P. lateritium* I have succeeded in making a cross when *bracteata* was used as the female plant (Fig. 1), while the reciprocal crossing, *lateritium* $\times$ *bracteata*, has not yet been successful.

In the present inquiry I have endeavoured to find on one hand the genetic relationship between the parent plants themselves and on the other to determine whether these parents have such chromosomes as are capable of association between themselves in the meiosis of the PMCs of the F_1 plants, and also to study the behaviour of such chromosomes in their meiosis, especially of univalents if present.

Material and Method

The behaviour of the chromosome in the PMCs was studied with either aceto-carmin smear material or that fixed with NAVASHIN's fluid. Paraffin sections were stained after NEWTON's gentian violet method and also with HEIDENHAIN's iron-alum haematoxylin. Photomicrographs were taken with Zeiss 1/12 H.I. $\times$ 100 and Leitz peripl. oc. $\times$ 10. All drawings were made by the use of a camera lucida with Zeiss 1/12 H.I. $\times$ 100 obj. and Komp. Ok. $\times$ 12.

1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 264.

Fig. 1. a, *Papaver lateritium*, (♂); b, *P. bracteata*, (♀); c, *F*₁ plant.

Observation

External characters in the *F*₁ plants. Some external characters in the *F*₁ plants are intermediate between those of the parent plants, but many characters of *P. lateritium* dominated as shown in Table 1.

The chromosomes. The somatic chromosome number in the female parent, *P. bracteata*, is 14 (Fig. 2) (cf. YASUI 1937). They form 7 gemini in the meiosis. The male parent, *P. lateritium*, also has 14 somatic chromosomes which form also 7 gemini in the meiosis of the PMCs (Fig. 3) (cf. YASUI 1927, SUGIURA 1940). The behaviour of the chromosomes in the meiosis of PMCs in both parent plants is quite regular and produces healthy pollen grains. The female parent is highly self-sterile, while the male is self-fertile. The somatic chromosome number of the *F*₁ plants is also 14 which is the sum of the gametic chromosome numbers of the parent plants.

Table 1. External characters in the parents and F₁ plants

Characters	Female parent <i>P. bracteata</i>	Male parent <i>P. lateritium</i>	F ₁ plant
Height of plant	About one meter	About 1/2 meter	About one meter
General appearance	Sturdy	Somewhat slender	Slender
Aerial shoot	Simple, uniflorous	Branched	Branched
Young peduncle	Erect	Nodding	Nodding
Bract	Prominent	Absent	Absent
Colour of petal	Spectrum red	Bittersweet orange	Flame scarlet to genadine
Size of flower	Large	Small	Intermediate
Shape of ovary	Spherical	Obovate	Obovate
Trichomes on floral axis	Stiff, decurrent	Soft, erect	Soft, long, erect; some hairs stiff like those of the female parent
Flower season	Once in spring	Flowering all year round	Flowering all year round
Fertility	Highly self-sterile	Self-fertile	Almost sterile

The chromosome behaviour in meiosis of PMC of F₁ plants.

In the early prophase several chromosomes are gathered around a large nucleolus, while later they recede one by one from the nucleolus except 4 large which remain attached until a later prophase just before the nucleolus disappears from the nucleus. Two of the latter have each a small satellite; one of the non-satellited chromosomes is attached at the region of the constriction to the nucleolus and is associated with the distal end of its larger arm with a small chromosome to form a bivalent, and the remaining 4th one also is associated with a small chromosome, which is smaller than the above mentioned, to form another bivalent (Fig. 9). There was observed a bivalent two components of which resembled each other and were associated side by side (Fig. 9, a). So that there were 3 bivalents, 2 unequal and one rather homologous one and 8 univalents in the PMC (Figs. 6a-c, 10, 12, 13), but sometimes there were found 5 bivalents and 4 univalents. Consequently we can see that the parent plants have more than 3 pairs of the chromosomes with more or less homologous parts between them. Some of these bivalent components were found associated with each other in the early stage but soon after they separated leaving no sign of the previous association and behaved as if they were univalent chromosomes (Figs. 4 and 5).

The 3 bivalents mentioned above generally came together into the central region of the equator of the atractosome, and a few univalents, generally less than 4, came into the periphery of the equator (Figs. 12 and 13), while other chromosomes did not enter into the formation of the equatorial plate at all, but were distributed

Figs. 2-8. Photomicrographs of PMCs ($\times 1000$), except 5, a camera lucida drawing ($\times 1200$). All figures except 6a-6c are from aceto-carmin smear material; 6a-6c, NAVASHIN's fixation. 2, polar view of the last metaphase of a PMC of *P. bracteata*; 3, the same of *P. lateritium*; 4, 5, PMCs of F_1 plants, later prophase; 6a-c, three optical sections from a paraffin section, a, bivalents at the equator; 7a-c, a mechanically pressed PMC at different optical levels; 8a-c, a PMC at different optical levels, univalents in the upper polar region show traction fibers well.

irregularly in the atractosome and were connected with one pole of the latter with traction fibers and proceeded into the pole earlier than the components of the bivalents (Figs. 6-8, 10-13). The connection between the univalents and the poles with the traction fibers was observed very clearly especially in the aceto-carmin smear preparations (Figs. 7, 8 and 11). A PMC was pressed mechanically during the investigation so that the chromosomes in a

pole (the upper pole in Figs. 7a-c) were pushed out from the cell, but the connection with the traction fibers clearly remained. At that time a bivalent (a bivalent in outline on the left hand side in Fig. 10) was pushed downward, so that the traction fiber from that pole became elongated and curved, as is to be seen also in the figures 7b, c and 10. The bivalent components had separated generally and

Figs. 9-18. Camera drawings of PMCs of F_1 plants. 9, 12-16, NAVASHIN's fixation; 10, 11, 17 and 18 aceto-carmin material. In 10-13 bivalents are shown in outline. 9, later prophase, 2 satellited univalents, 2 bivalents attached to nucleolus, *a*, a bivalent having 2 homologous components associating side by side; 10, same with 7, *a-c*; 3 bivalents at the equator, one of which is displaced by the mechanical press, having elongated and curved upper traction fibers; 4 univalents in upper polar region are shown in their normal position before being pressed mechanically; 11, same with 8a-c, 2 unequal bivalents at the equator, traction fibers and their connections between univalent chromosomes and a pole; 12, same with 6b, *a*, 3 bivalents and 4 univalents at the equator, 2 univalents near the upper pole, and the remaining 2 proceeding toward the lower pole; 13, 3 bivalents and 2 univalents at the equator, 3 univalents connected with upper pole. 2 at the other polar region, one proceeding to the same pole; 14, almost all the chromosomes reaching one pole, a few proceeding to other pole; 15, 5 univalents at lower pole, 6 at upper, one unequal bivalent at equator; 16, interkinesis, daughter chromosome groups having the chromosomes in the same number, but unequal in sizes; in one group a univalent is divided into two (*u*); 17, fibers from one pole distributed on the surface region of the atractosome are shown; 18, a depressed PMC, the atractosome becoming crescent. $\times 1200$.

proceeded into the poles later than some univalents, but often in the form of bivalent.

The mode in which the univalents proceeded toward the poles was at random, consequently the chromosome numbers of the daughter nuclei of the 1st meiotic division were different generally, in an extreme case one daughter nucleus having only 3 chromosomes which may be the separated components of the bivalent which formed the equatorial plate, and in another 11. In some nuclei they showed the same number of chromosomes, though even in such cases the sizes of the chromosomes in the nuclei were different (Fig. 16). Scarcely any case of the separation of the daughter chromatids of the univalents in the 1st meiotic division was observed, but they passed into one pole without any actual separation (Fig. 16, *u*).

It was observed in some PMCs that the equatorial region of the cell was depressed, probably due to the rash intrusion of the fixing fluid, and the atractosome was also depressed at the equatorial region, while the poles remained in the original position; consequently the atractosome assumed a crescent shape, the convex side being opposite to the depression (Fig. 17).

In some PMCs fibers radiating from the poles and having no connection with any chromosomes were found, partly belonging probably to the central fibers (Fig. 18). The number of the central fibers was numerous when the fibers were finer, while they were smaller when the fibers were thicker. The difference may be due to an external condition such as the fixing fluid.

After the 1st meiotic division cytokinesis generally occurred in the PMCs, though the divisions were often incomplete and irregular, resulting in the formation of irregular shaped interkinetic cells. Extranuclear chromosomes, miniature nuclei formation, and reconstruction nuclei were observed (Fig. 19).

The second meiotic division was rather regular, but due to the irregularities of the 1st one the tetrad formation was not regular (Figs. 19–22). Such irregular behaviour, however, occurred in different degrees in the different PMCs in the different individuals as well as in the different flowers of the same plant, and even in the different anthers of one and the same flower. Thus very few fertile pollen grains were produced, so that the formation of healthy seed was rare, even when some good healthy ovaries were pollinated with these pollen grains. I have not examined yet the meiosis in the macrospore mother cells, but probably the same conditions occur in them, because we could also scarcely get seeds even when the F_1 plant was back-crossed with healthy pollen grains of the parent plants.

Figs. 19-22. Photomicrographs of PMCs of the F_1 plants. 19, 3 PMCs of interphase; one having 2, one larger and another smaller, nuclei and an extra-nuclear chromosome, one containing a large reconstructed nucleus and a miniature nucleus, the third in which complete cytokinesis occurred after the 1st division; 20, PMCs in interkinesis and in the 2nd telophase; 21, PMCs in which irregular cytokinesis occurred; 22, PMCs in tetrad formation, almost all of them having miniature grains beside larger grains. $\times 1000$.

Discussion

1. *The homology of chromosomes and syndesis in PMC of F_1 plants.* The chromosomes of the PMCs of *Papaver bracteata* are rather uniform in size, but those of *P lateritium* are

various; and the chromosomes in the PMCs of F_1 plants show conspicuous differences both in their size and structure. In spite of such differences there are generally more than 3, sometimes 5 pairs of chromosomes found associated to form bivalents in the prophase. At least 2 pairs of these bivalents showed clearly inequality of components. Such unequal associations were reported already in other plants by several investigators (KUWADA 1919, NAVASHIN 1927, 1934, AVERY 1930, ONO and SATÔ 1935 etc.).

Since BELLING and BLAKESLEE (1926) reported the association of the non-homologous chromosomes, the relationship between the homologous parts in the bivalents has been much discussed and was used for the criterion of the affinity between the parent plants. The association of the non-homologous chromosomes in the case of *Datura* does not mean the association of the non-homologous parts of the chromosomes, but the association of the chromosomes of different sets, between the homologous parts of the chromosomes translocated from a chromosome of a different set. Sometimes the association of true non-homologous parts of chromosomes was observed as reported by MCCLINTOCK (1933) in *Zea Mays*, but such an association seems to be temporary. Moreover, the recent studies of the salivary chromosomes in *Diptera* show us that the association of chromosomes is really a strong indication of the homology of those associated parts of the chromosomes. The unequal association now in question would seem to give some evidence for the view that the association may occur between different kinds of chromosomes, but the difference in size is not necessarily a sign of the non-homology of the internal constituents of the chromosomes, because 1stly the larger chromosomes may have more heterochromatin than the smaller, though they have the same genic constituents, 2ndly they may have homologous parts between them, namely the smaller one may represent parts of the larger chromosomes, 3rdly they may be homologous in their genic qualities but different in their quantities which may cause the difference in size. These question must be settled by further research.

From the study of the components of the chromosome sets in *Papaver somniferum* and its hybrids with other species, I came to the conclusion that 7 gametic chromosomes in *Papaver* might have been derived from two different ancestors, one having 3 and the other 4 gametic chromosome sets, i.e. *Papaver* having 14 $2n$ -chromosomes are probably amphidiploid (YASUI 1937, 1940). SUGIURA (1940) confirmed the latter view from the study of the chromosome numbers in various sections of this family. Such amphidiploid derived generally from hybrids or their derivatives whose parents

in either cases are rather remote, and the chromosomes in the F_1 plants scarcely associate as we see in the tetraploid *Primula kewensis*, a well known amphidiploid derived by the chromosome duplication from an artificially raised interspecific hybrid whose parents were remote. From this and also the presence of the unequal association of bivalents we can consider that these parent plants, *P. bracteata* and *P. lateritium*, have the gametic chromosome sets in which no autosyndesis could occur. So that the bivalents in the F_1 plants are to be considered as the result of the allosyndesis, in other words *P. bracteata* has more than 3 chromosomes which more or less resemble those chromosomes of *P. lateritium*. To this extent these plants should be allied to each other. Taxonomic relationship after FEDDE (1936) is also in accordance with this conclusion.

2. *Traction fibers and univalent chromosomes.* As described above, there were observed in a PMC most frequently 3 bivalents and 8 univalents, but sometimes 5 bivalents and 4 univalents. In these cases the smaller number of the bivalents was due to the fact of the earlier separation of their association components in an early prophase, and the fact that such separated partners do not show much difference from the true univalents having no partners from the first.

The behaviour of the univalents in the first meiotic division studied by several investigators may be divided into two types, first the case when the univalents divide into two and separate at the equator of the attractosome after the bivalents have passed into the anaphase and followed the preceeding separated bivalent components to the poles (KIHARA in *Triticum* 1919, YASUI in *Papaver* 1921 etc.), secondly the case when the univalents do not divide in the first division and migrate as such into the poles after the bivalent components have passed into the anaphase (ROSENBERG in *Drosera*, 1904 etc.). In these cases the univalents do not move towards the poles before the separation of the bivalent components.

It seems peculiar in the present case that many univalents passed into the poles before the bivalent components began to move from the equator to the poles, and also that the movements of the univalents were effected by traction fibers connected with one pole. This peculiar behaviour may be due to the earlier differentiation of the attachment points in those univalents and to the fact that most of these univalents are not true univalents but represent the separated bivalent partners.

Simultaneously with the differentiation of the attractosome in the nucleus of the PMC, the chromosomes which were hitherto distributed at the surface region of the nuclear cavity began to move

toward the center of the nucleus. At that stage the attachment points in the bivalent partners were generally differentiated, so that the attachment point of each of them was attached to the free end of the traction fibers stretching out from each pole. According to SCHMIDT's consideration (1939) the traction fibers, in which the molecules are arranged lengthwise, are stretched out at the beginning of the spindle formation, but the fibers contract in the anaphase to telophase. This process probably resembles that of the contraction of tendrils; the free end of the tendrils stretch out until they come to be attached to something, but as they reach something they begin to contract; traction fibers may also stretch out when one end is free, but they will begin to contract when the ends come in contact with the attachment point of the chromosomes owing to some physico-chemical changes occurring in it. At the beginning of the contraction of the fibers, when the tractive forces from both poles and the resistance of the paired chromosomes to those forces are the same and balanced, the bivalents may stay at the equator to form the metaphase. On the contrary those univalents above described which have only one attachment point and offer no resistance as in the separation of the paired chromosomes, and also are subjected to no tractive force from the other pole, may proceed toward the pole earlier than the bivalents.

We cannot agree with BĚLAŘ's view (1929) of "Stemmkörper" which is considered to develop between the daughter- or separated chromosome groups and push them toward the poles. Because we see that the polar movement of the univalents here described has not been effected by the aid of such a body as "Stemmkörper", but they were pulled only by the traction fibers.

Summary

1. In the F_1 plants of *P. bracteata* $\times$ *P. lateritium* some characters were intermediate between those of the parent plants, but many other characters of the male parent, *lateritium*, dominated over those of the female parent, *bracteata*.

2. The chromosome number of the parent plants was the same, but their sizes were different; the chromosomes in *lateritium* being generally smaller than those of *bracteata*. The chromosome number in the F_1 plants was $2n=14$, just the sum of the gametic chromosome number in the parent plants. The variation in chromosome sizes was greater than either of those of the parent plants.

3. In the meiosis of the F_1 plants there were found more than 3 pairs, sometimes 5 pairs of gemini, some of them being unequal

and others separating early in the later prophase and behaving like univalents. Many univalents, probably the just mentioned, proceeded earlier than the bivalents into the poles, though their distribution was at random as we see usually in the non-splitting univalents in the 1st meiotic division. Generally 3, sometimes 2, bivalents formed an equatorial plate after many univalents had gone into the poles, and followed them after separation or without separation of partners. Simultaneously when the bivalents were at the equator few univalents were found in the periphery of the equatorial region; the latter has passed into the poles without splitting.

4. The cytokinesis generally occurred after the 1st meiotic division, though often it was incomplete. Several irregularities in the division were observed. Second division was rather regular, but due to the irregular behaviours in the 1st division very few good pollen grains were produced. The F_1 plants were highly sterile even when they were back-crossed with healthy pollen grains of the parent plants.

5. Traction fibers were observed clearly especially in the aceto-carminic smear materials. The univalents were connected with one pole by the traction fibers which may play the role of the movement of the univalents without aid from any other additional forces, e.g. the "Stemmkörper", which was considered by BĚLAŘ as a separator of the daughter chromosome groups toward the poles.

The writer's sincere thanks are due to Prof. K. FUJII for his kind advice in the course of this study. The expense of carrying out the present work was partly defrayed out of a grant from the Science Research Fund of the Department of Education to which her thanks are also due.

Bibliography

- Avery, P. 1930. Cytological Studies of Five Interspecific Hybrids of *Crepis Leon-tonides*. Univ. Calif. Publ. Agr. Sci. 6: 135-167.
- BĚLAŘ, K. 1929. Beiträge zur Kausalanalyse der Mitose III. Untersuchungen an den Staubfaden, Haarzellen und Blattmeristemzellen von *Tradescantia virginica*. Zts. Zellf. mikr. Anat. 10: 73-137.
- Belling, J. and Blakeslee, A. F. 1926. On the Attachment of Non-Homologous Chromosomes at the Reduction Division in Certain 25-Chromosome Daturas. Proc. Nat. Acad. Sci. 12: 7-11.
- Fedde, F. 1936. Papaveraceae. Engler-Prantl, Die natürlichen Pflanzenfamilien. 2. Aufl. Bd. 17b: 5-145.
- Kihara, H. 1919. Über cytologische Studien bei einigen Getreidearten. I. Spezies-Bastarde des Weizens und Weizenroggen-Bastard. Bot. Mag. (Tokyo) 33: 17-38.
- Kuwada, Y. 1919. Die Chromosomenzahl von *Zea Mays* L. Ein Beitrag zur Hypothese der Individualität der Chromosomen und zur Frage über die Herkunft von *Zea Mays* L. Jour. Coll. Sci., Imp. Univ. Tokyo, 39, Art. 6.

- McClintock, B. 1933. The association of Non-Homologous Parts of Chromosomes in the Mid-prophase of Meiosis in *Zea Mays*. Zts. Zellf. mikr. Anat. 19: 191-237.
- Navashin, M. 1927. Über die Veränderungen von Zahl und Form der Chromosomen infolge der Hybridisation. Zts. Zellf. mikr. Anat. 6: 195-233.
- , 1934. Chromosome Alterations caused by Hybridization and Their Bearing upon Certain General Genetic Problem. Cytologia, 5: 169-203.
- Ono, F. and Satô, D. 1935. Intergenra Hibridigi en Cichorieae, II. Hibridoj de *Crepidiastrum lanceolatum* var. *latifolium* kaj *Paraixeris denticulata*. Jap. Jour. Genet. 11: 169-179.
- Rosenberg, O. 1904. Über die Tetradenbildung eines *Drosera* Bastard. Ber. deut. Bot. Ges. 22: 47-53.
- Schmidt, W. J. 1939. Doppelbrechung der Kernspindel und Zugfasertheorie der Chromosomenbewegung. Chromosoma, 1: 235-264.
- Sugiura, T. 1940. Chromosome Studies on Papaveraceae with Special Reference to the Phylogeny. Cytologia, 10: 558-576.
- Yasui, K. 1921. On the Behaviour of Chromosomes in the Meiotic Phase of Some Artificially Raised *Papaver* Hybrids. Bot. Mag. (Tokyo), 35: 154-167.
- , 1927. Further Studies on Genetics and Cytology of Artificially Raised Interspecific Hybrids of *Papaver*. Bot. Mag. (Tokyo), 41: 235-261.
- , 1937. Cytogenetic Studies in Artificially Raised Interspecific Hybrids of *Papaver*. VII. *P. somniferum* L. $\times$ *P. bracteata* Lindl. Cytologia, 8: 331-342.
- , 1940. Cytogenetic Studies in Artificially Raised Interspecific Hybrids of *Papaver*. IX. On the bivalent-association in the meiosis of the PMC of *Papaver somniferum*. Cytologia, 10: 551-554.