**Cytogenetic Studies in Artificially Raised Interspecific Hybrids
of *Papaver*. IV.**

Interspecific hybrids of *P. orientale* L. and *P. bracteata* Lindl.¹⁾

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(With one Plate and 12 Text-figures)

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Introduction

In *Papaver* on which I have been carrying on cytogenetic studies for many years, polyploid relations were found among some species having seven (x) as the basic number in their chromosome sets or the genom (Winkler 1920). *P. orientale* is a hexaploid having 42 somatic chromosomes, while *P. bracteata* is a diploid plant having 14 somatic chromosomes. Besides, I have found a plant (pedigree No. 41-8) among the seedlings raised from seeds, derived from a foreign seed merchant, named *P. bracteata*, which showed external characters intermediate between those of *P. orientale* and *P. bracteata*, except for the fact that it looked somewhat more slender than either of the above mentioned species. The somatic chromosome number in this plant is 28, which equals the sum of the gametic chromosome numbers of *P. orientale* and *P. bracteata*, and the chromosome behaviours in the meiotic phases of PMC also suggest that this plant is a natural hybrid between the two species mentioned above, or a derivative of the hybrid. The following studies were carried out to ascertain whether this view is correct or not, and to determine the relationship between the genomes in the parent plant species.

Material and Method

P. orientale was crossed with the pollen grains of *P. bracteata*; the reciprocal cross was also made, equally with success. The anthers of the parent plants, F₁ plant and No. 41-8 plant were fixed with CARNOY's alcohol-acetic mixture and also with NAVASHIN's solution. McCLINTOCK's aceto-carmines smears were also tried. Almost all figures were made from aceto-carmines smear-material, though HEIDENHAIN's iron-alum haematoxylin was also used for the staining.

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Observation

Meiosis in *P. bracteata* Lindl. This plant shows seven bivalents in the 1st meiotic division of PMC (Pl. 16, Fig. 1) which divide quite regularly (Pl. 16, Figs. 1–9), so that good healthy pollen grains are formed. Though the macrospore formation has not been investigated yet, a regular and healthy embryo sac formation is expected.

This plant shows generally high sterility when selfed, but inter-crossed yield good seeds, and the crossing with the pollen grains of *P. orientale* proves always successful, and the reciprocal too.

Text-figs. 1–11. Meiotic phases of PMC of an artificially raised interspecific hybrid between *P. orientale* and *P. bracteata*. All figures are Camera lucida drawings made with Zeiss 1/12 Fl. objective and compensation ocular 12 ($\times 15$), reduced in reproduction to 7/10. 1, Nucleus in diaphase, nucleolus not shown; 2, 1st metaphase, 13 bivalents (in black) and 2 univalents (blank) shown; 3, the same, side view, black ones are univalents. *a*, loosely joined bivalent; 4, later stage, 13 halves of bivalents at two poles, two dividing univalents at the equator; 5, still later stage, 13 halves of bivalents (black ones at one pole) and 2 halves of univalents (*u*) (the blank one at other pole); 6, 2nd equatorial plate, a polar view, 14 double-chromatid chromosomes at both poles are shown; 7, the same, side view; 8, irregular shaped restitution nucleus shown; 9, 2nd division in a restitution nucleus, 28 dividing chromosomes are seen at the equator; 10, a diad having the nuclei connected by a bridge; 11, a hexad in a PMC of a plant No. 41, 8, magnification being the same. ca $\times 1260$.

Artificially raised hybrids. FOCKE (1881) stated that these two species (*P. orientale* and *P. bracteata*) are so much alike as to be hardly distinguishable from each other; and the hybrid between them is intermediate when certain differences in their characteristics are clear. My materials showed the same results. *P. bracteata* has two or three bracts and one to three more conspicuous sessil leaves which grow close to the latter; while in *P. orientale* such bracts and sessil leaves are entirely absent. The flower colour in the former is deep magenta, but orange red in the latter. The F_1 plants appear intermediate in several characters, but their resemblance is more maternal than paternal, so that the reciprocal F_1 plants are somewhat different from each other.

In the diaphase of PMC of the F_1 plant, 13 bivalents and two univalents are formed (Text-fig. 1). Association of chromosomes in one bivalent is somewhat looser than in other 12 bivalents. In the next stage bivalents make an equatorial plate first (Text-fig. 2; Pl. 16, Figs. 10, 11), though probably the loosely associated one was already disjoined when the other came in to the equator (Text-fig. 3, *a*). When they are in anaphase, the univalents come in to the equator of the spindle and divide longitudinally and follow those which preceded (Text-fig. 4; Pl. 16, Figs. 12, 13). Consequently in the daughter nuclei thus formed, 13 double-chromatid-chromosomes (disjoined halves of bivalents) and two single-chromatid-chromosomes (divided halves of univalents) can be observed (Text-fig. 5).

In the second meiotic phase, however, there were observed very often 14 instead of 15 chromosomes at each equator of the two daughter nuclei (Pl. 16, Fig. 15; Text-fig. 6), which were derived probably from those PMC in which two univalents one by one have gone without dividing into the opposite poles, namely, there must have occurred in some PMC the first division in meiosis of the *Drosera* type.

Beside the two main nuclei in the interphase, lagging chromosomes, extra nuclear chromosomes and small extra nuclei were also observed, which suggest some irregular behaviour of the chromosomes in the 1st meiotic division. There were observed many PMC having nuclei which seemed to be restitution nuclei, and also those with irregular shaped large nuclei (Text-fig. 8).

The second division is rather regular (Pl. 16, Figs. 16, 18), though the velocity of the movement of different chromosomes is not the same (Pl. 16, Fig. 17), while in some PMC division is irregular, and as a result we find lagging chromosomes, chromosome bridges, extranuclear chromosomes (Pl. 16, Fig. 19) and also restitution nuclei. In some PMC, there was observed the division of small extra

nuclei beside the division of the ordinary interphase daughter nuclei. Some PMC had a large nucleus in which as many as some 28 chromosomes (Text-fig. 9) were counted. From such cells there may be derived those cells in which 28 chromosomes are found at equator of the spindle (Text-fig. 9), and which probably result in diads. In some diads, 2 nuclei which are connected by a chromosome bridge were observed (Text-fig. 10).

In one case, in a random count of 660 PMC, 35 diads (about 6 per cent) were mixed among regular tetrads and few triads (Text-fig. 12). In some other cases about 25 per cent. of diads were found. In some diads small extra nuclei were observed.

Text-fig. 12, A and B. Photomicrographs of PMC of the F_1 plant between *P. orientale* and *P. bracteata*, taken with ZEISS 1/12 F_1 . obj. and LEITZ periplane ocular $\times 8$, and reduced in reproduction to 7/10, tetrad, diad (a) and diad with miniature cell (b) are shown. ca $\times 560$

No. 41-8, a presumed natural hybrid or its derivative. Chromosome behaviours in meiosis and the methods of pollen grain formation in this plant were found quite similar to those corresponding phases of artificially raised hybrids mentioned above (Pl. 16, Figs. 20-25), though in some PMC of the former there was found a small fragment chromosome, which was not found in the latter.

Artificially raised F_1 plants, whose mother was *P. orientale* gave a few seeds, while the hybrid whose mother was *P. bracteata* gave no seeds during the two years.

Though No. 41-8 is highly sterile when selfed, and many sterile pollen grains, even hexad, (Text-fig. 11) were formed in some PMC, sometimes many of its pollen grains as well as egg cells were functional; so that we got seeds by crossing this plant with the pollen grains of either *P. orientale* or *P. bracteata*, and also in their reciprocal crosses. From such crossings auto- or allo-triploids and pentaploids may be derived; octoploid plants also can be expected.

The crossing between No. 41-8 and *P. somniferum* was successful.

Discussion

TAHARA (1915) determined the haploid chromosome number 21, in *P. orientale*, and the writer's material also showed the same number (1921). The basic chromosome number is seven in *Papaver* with some exceptions (SUGIURA 1936), so that *P. orientale* contains 3 genomes in a haploid chromosome compound, as the writer reported this spring at the Tokyo Congress of the Botanical Society of Japan (1936).

In the meiotic phase of PMC of the F_1 plant between this species and *P. somniferum* which has 11 haploid chromosomes, appeared 11 bivalents and 10 univalents (YASUI 1921). When my first paper on *Papaver* appeared (1921) I believed the one chromosome of each pair of bivalents was derived from *orientale* and the other from *somniferum*, though some doubt on the homology among them was pointed out in that paper. LJUNGDAHL also stated the same idea in her paper (1922). SANSOME and PHILP (1933) quoted LJUNGDAHL's paper in their work and concluded "*P. orientale* as an allopolyploid, one of whose ancestors is closely related phylogenetically to *P. somniferum*". But now I have come to an entirely different view on the composition of these bivalents, due to the following reasons.

As I have already reported (1927) in the description of the first meiotic division in the PMC of the F_1 plant of *P. somniferum* $\times$ *P. lateritium*,¹⁾ there were found 4, sometimes 3, bivalents and 10 or 12 univalents. The F_1 plant was backcrossed with pollen grains of *P. somniferum* and then selfed through some generations. After one or two generations almost all the offspring were greatly reversed to *P. somniferum* not only morphologically but also cytologically; that is to say, after three generations they entirely reversed, paternal chromosomes having been entirely excluded. From this fact we may conclude that there are no male chromosomes in the bivalents of PMC of the F_1 plant, in other words there exist no homologous chromosomes between both parental chromosome compounds: consequently the four bivalents in the first meiotic phase are to be considered as derived from *P. somniferum* only, that is, it has four entirely or at least partially homologous pairs in its haploid chromosome complex.

An elimination of maternal chromosomes also occurred in later generations of the hybrid of *P. somniferum* and *P. orientale*. However, it was necessary to backcross with the pollen grains of *P. orientale* through 2 generations to get seeds. This fact suggests that the chromosome relationship in this hybrid is not so simple as I and the three authors mentioned above have previously thought. The

1) In my 1927 paper this plant was mistaken for *P. nudicaule*.

fact of elimination of the maternal chromosomes shows that there is no homologous chromosomes among the parental chromosomes, consequently the 11 bivalents in PMC of F_1 plant between *P. somniferum* and *P. orientale* are to be considered as autosyndetic union, 7 of them being derived from *P. orientale* and the remaining 4 from *P. somniferum*.

I have pointed out already (1921) that in some PMC, in the 1st meiotic division, 7 of these 11 bivalents proceeded to the poles earlier than the remaining four, while the latter entered the equator and formed a second equatorial plate afterwards (Pl. III Fig. 12 in that paper). The univalents were found in that stage still scattered around them so that there were three successive equatorial plate formations instead of the two, which are usual in such interspecific hybrids. This fact is probably another proof of the view that they have two different groups of bivalents.

I suggest for the sake of convenience, denoting the two of the three genomes in *P. orientale* whose chromosomes are paired in F_1 plant, by O_1 and O_2 , and the third by O_3 , and that of *P. bracteata* by B. Then the *orientale* haploid chromosome compound will be denoted by $O_1O_2O_3$, and F_1 genom between *P. orientale* and *P. bracteata* by $O_1O_2O_3B$.

Now 7 of the 13 bivalents in PMC of F_1 plant must be considered as derived from O_1O_2 and the other 6 from O_3B , because if this was not the case we must consider that all the members in O_3 and B are homologous; then we can expect on the one hand six homologous pairs, but not seven, in *P. orientale* and on the other hand five pairs of homologous chromosomes in *P. somniferum*. But the latter expectation does not conform with the fact in the case of *P. somniferum* × *P. lateritium*.

It is a question whether O_1 and O_2 and those chromosomes in 6 bivalents are perfectly homologous or not. However, there is another possibility that *P. orientale* is an autopolyploid as *P. nudicaule* var. *striatocarpum* (LJUNGDAHL 1924). If the latter is the case there is no difference among $O_1O_2O_3$, though they cannot form polyvalents in the meiotic phases, unlike the case of *Datura* (BELLING and BLAKESLEE 1923), while bivalents only are formed as other examples (*Solanum*, JØRGENSEN 1925; *Primula sinensis*, DARLINGTON 1931; *Chrysanthemum*, SHIMOTOMAI 1933; *Fragaria*, LILIENFELD, 1933, 1936 etc.). Therefore in the meiotic phases in PMC any two homologous chromosomes may pair making 7 bivalents, and the 6 in the remaining 7 may pair with the homologous ones in the genom of *P. bracteata*. Consequently we could find 13 bivalents and 2 univalents. Now, we might consider that the genomes of *P.*

orientale are the same as that of *P. bracteata*, except for one chromosome, and that *P. orientale* might be the derivative of a species very closely related to *P. bracteata*. However, the decision will be postponed until more experimental data are at hand.

Summary

1. *P. bracteata* LINDL. has 14 diploid chromosomes, which make 7 bivalents in the meiotic phase of the PMC. The meiosis in the PMC occurs quite regularly and good healthy pollen grains are formed. Though it shows high sterility when selfed, the crossing *inter se* results in good seeds. The genom will be denoted by B.

2. Supposed natural hybrid between *P. orientale* and *P. bracteata* or its derivative (No. 41-8) shows similar behaviour of chromosomes in the meiotic phases in PMC to that of artificially raised *P. orientale* $\times$ *P. bracteata* and its reciprocals, but the gametes in the latter are highly sterile while those of the former show a higher fertility.

The somatic chromosome number in these plants is 28, though in some cells of No. 41-8 plant a small fragment chromosome was found in the meiosis. In meiosis of PMC 12, closely jointed, and one loosely jointed, bivalents, and 2 univalents were observed. These univalents divide or not in the 1st meiotic division. Irregular behaviour of the chromosomes in the 1st and 2nd divisions, namely the formation of diads, triads, extranuclear chromosomes, small pollen grains, was observed in addition to good healthy looking tetrads.

3. It is expected that a triploid will be obtained by crossing No. 41-8 with *P. bracteata*, a pentaploid by crossing *P. orientale* with No. 41-8, and an octaploid by selfing No. 41-8.

4. *P. orientale* is a hexaploid plant, and three genomes in its haploid are dissectable, each consisting of 7 chromosomes. Two of them form 7 bivalents in the meiotic phases of the F_1 plants of *P. orientale* and *P. bracteata*, and also in *P. somniferum* $\times$ *P. orientale* and its reciprocal. Six chromosomes in a genom of *P. orientale* make 6 bivalents with 6 chromosomes in the genom B.

5. *P. orientale* may be an autohexaploid, a closely related diploid plant with *P. bracteata*, but the determination has been postponed until more experimental data are at hand.

6. There were no homologous chromosomes between *P. orientale* and *P. somniferum* nor between *P. somniferum* and *P. lateritium*.

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Explanation of Plate 16

All figures are photomicrographs taken with ZEISS objective 1/2 F₁ and LEITZ periplane ocular ×10. ×1000.

Figs. 1-9. Meiotic phases in the PMC of *P. bracteata*.

- Fig. 1. A polar view of an equatorial plate in 1st meiotic division; 7 gemini are seen.
- Fig. 2. The same, a side view.
- Fig. 3. Anaphase of 1st meiotic division, a side view.
- Fig. 4. Later anaphase, a polar view; 7 chromosomes at one pole are shown.
- Fig. 5. Late anaphase, a side view.
- Fig. 6. Interphase.
- Fig. 7. 2nd metaphase; the polar view of one of the nuclei shows 7 chromosomes; the other nucleus in a side view.
- Fig. 8. 2nd later anaphase.
- Fig. 9. A tetrad, one cell not being shown.

Figs. 10-19. Meiotic phases in the PMC of the artificially raised hybrid of *P. orientale* and *P. bracteata*.

- Fig. 10. 1st metaphase, a polar view; *u*, two univalents.
- Fig. 11. The same, a side view.

- Fig.12. Later stage. Bivalents about to divide ; *u*, univalents at the equator.
 Fig.13. Still later stage, the halves of 2 univalents are following other chromosomes toward the poles.
 Fig.14. Interphase.
 Fig.15. 2nd metaphase, a polar view, one of the equatorial plates is not shown.
 Fig.16. The same, side view.
 Fig.17. 2nd anaphase, some chromosomes are preceding the other.
 Fig.18. 2nd telophase.
 Fig.19. The same ; *r* is a retarding chromosome.

Figs. 20-25. Meiotic phases in the PMC of a No. 41, 8 plant.

- Fig.20. 1st metaphase, a polar view.
 Fig.21. The same, side view.
 Fig.22. Later anaphase of 1st meiotic division.
 Fig.23. 2nd meiotic metaphase, a polar view ; 12 chromosomes are seen at the equator of the spindle ; the remaining 2 indicated by *c*, being pushed out by the pressure in the smear, are seen at the periphery of the cell ; *b* in a lower focus, a side view of another nucleus is shown.
 Fig.24. 2nd later anaphase.
 Fig.25. 2nd later telophase.
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