**Cytogenetic Studies in Artificially Raised Interspecific Hybrids
of *Papaver*. IX. On the bivalents-association in the
meiosis of the PMC of *Papaver somniferum*¹⁾**

By

K. Yasui

Tokyo Imperial University

(With 6 figures)

Received April 1, 1940

Introduction

In previous papers (YASUI 1936, 1937a, 1937b) I have presented the view that the chromosome constitution of *P. somniferum* is $2(4_{II}+3_I)$, and that of *P. orientale* is $2(7_{II}+7_I)=2(O_1O_2+O_3)$, and also that there may be homologous chromosomes among those of O_1 and O_3 and of O_2 and O_3 in *P. orientale*.²⁾ Further consideration on these points led me to the reexamination of the chromosome behaviour in these parent plants, and I have found some evidence in the chromosome associations to justify the chromosome constitution mentioned above. In this paper I should like to limit myself to the description of *P. somniferum*.

Material and Method

Meiotic chromosomes in the PMC were investigated in acetocarmine smear materials and also the paraffin sections. The latter were fixed with NAVASHIN's fluid and BENDA's modification of FLEMMING's fluid. HEIDENHAIN's iron-alum haematoxylin was mostly used for the staining of the paraffin sections. Photos were taken with the aid of ZEISS H. I. $\times 100$ and LEITZ periplane ocular $\times 10$. Drawings were made with a camera lucida.

Investigation

1. **The PMC.** *Papaver somniferum* has 22 somatic chromosomes. In one race of this plant, there appeared in the diaphase of the 1st meiotic division of the PMC 11 bivalents. Of the latter, 6 formed rings, 2 made side by side pairings or open rings, one showed end to end union, one showed a small open ring and the remaining,

1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 252.

2) Cf. YASUI 1936, p. 540.

largest one showed side by side or open ring configuration, while in an other race almost all the bivalents showed closed or open rings. The component chromosomes of the largest pair (I) associate at one end with each other and attach with other ends having satellites to the nucleolus (Fig. 3, *a*). Two large ring bivalents (II and III) attach themselves to the same nucleolus to which the bivalent I attaches (Figs. 1c, *b*, *c*, and 5c, *b* and *c*). Other 2 ring bivalents (VII and VIII), which are smaller than the bivalents II and III, always associate with each other in the diaphase (Figs. 1c, 2, 3, 5c and 5d, *f* and *g*). These two pairs of the bivalents are often interlocked with each other though they do not show any chiasma formation between the arms of the companion sets (Figs. 2, *c*, *b*; 3, *f* and *g*; 5c, *b* and *c*; 5d, 5e, *f* and *g*). Another pair of bivalents (IV and V), which show generally the side by side association but sometimes open rings, also associate (Figs. 1c, 2, *d* and *e*). In some cases the latter join at the closed ends of the bivalents (Fig. 4, *d* and *e*). Such may be the result of interlocking. One bivalent (XI) (Fig. 1c, *i*) showing the end to end association almost always appeared very near to a ring bivalent (IX) (Fig. 1c, *h*).

In another race there is no bivalent which associates end to end, but instead an open ring which associates with another open ring bivalent, so that in this race there are 4 pairs of ring bivalents. The remaining 2 bivalents (VI and X) (Figs. 1c, 2, 5d, 5e, *j*, *k*) always behave as solitaires showing no sign of association, not only between themselves but also with other bivalents. Thus we can recognize 3 sets of chromosomes in the haploid complement of *P. somniferum*. Two of them (S_1 and S_2) resemble each other while the third (S_3) has no chromosomes homologous to those in the other two sets.*

In the metaphase of the 1st meiotic division those associated bivalents separated generally from each other, though in some rare cases they came together at the equator showing the pairings. Here I wish to propose the term "bivalents-association" for such union of bivalents without chiasma formation, and it is an association of two bivalents as a whole in the later prophase of the meiotic division.

2. Root tip cell. The karyotype of *P. somniferum* seems not to be simple because *P. somniferum* consisting of a number of races, seems to have several different karyotypes among them. But as we see in the figure 6, it has some sets of similar chromosomes, though they do not show a perfect resemblance. An example of the chromosome diagnosis is shown in Table 1.

Hereafter we wish to use S_1 , S_2 and S_3 to denote chromosome sets of 4, 4, and 3 in *P. somniferum*.

Chromosomes I–X have their homologous partner in the somatic cell, but XI_1 and XI_2 have no exactly homologous partner, while they are like each other except that XI_1 has 2nd constriction in one arm, while XI_2 has none. These two may associate in the meiosis to form a geminus.

Table 1. Chromosome constitution in a race of *P. somniferum*

Kinds of chromosomes	Length	Position of the 1st constriction	Position of satellite and the 2nd constriction
I	Long	Submedian	A satellite at the distal end of the short arm, 2nd constriction in the distal part of the long arm
II	Long	Subterminal	A satellite at the distal end of the short arm, 2nd constriction at the middle part of the long arm
III	„	„	„
IV	Long	Subterminal	
V	„	„	
VI	Median	Subterminal	A satellite at the distal end of the short arm
VII	Median	Submedian	A satellite at the distal end of the short arm
VIII	„	„	„
IX	Median	Subterminal	A satellite at the distal end of the short arm, 2nd constriction at the distal part of the long arm
X	Median	Subterminal	
XI_1	Median	Subterminal	A satellite at the distal end of the long slender arm, 2nd constriction at distal part of the other arm forming a round knob
XI_2	Short	„	A satellite at the distal end of the long slender arm, no 2nd constriction as in the XI_1

We can see in Table 1, that in 3 pairs of chromosomes, i.e. II & III, IV & V, and VII & VIII, the corresponding partners very much resembled each other. The resemblances in their shape and size led me to the idea that these pairs may correspond to those which form bivalents-associations II & III, IV & V, and VII & VIII respectively. The bivalent consisting of XI_1 & XI_2 (XI) may associate with the bivalent IX to form bivalents-association IX & XI.

In the center of the nucleus in Fig. 6 we see 3 chromosomes which associate intimately. They are from left to right IX_1 and IX_2 , and XI_1 . They always appear very near to each other, while XI_2 does not come together with XI_1 in the somatic division. In this somatic association the nucleolus plays a certain role, but in some other somatic associations of the resembling chromosomes which generally occur in this species no clear relation with the nucleolus was found.

Figs. 1-5. PMC in the diaphase of *Papaver somniferum*. 1-4 from aceto-carmin smear, 5 from a paraffin section, 1a and 1b, one and the same material photographed in different levels. $\times 1000$. 1c-4, camera lucida drawing. 1c, one and the same PMC with 1a, 1b, a; a, bivalent I; b and c, ring bivalents II and III; d and e, bivalent IV and V; showing side by side association; f and g, ring bivalents VII and VIII; h, ring bivalent IX; i, bivalent XI, showing end to end association;

Discussion

1. **Bivalents-association.** There are cases of the chromosome association in the meiotic division other than the normal pairing of homologous chromosomes to form gemini. KUWADA (1910) in *Oryza*, TAHARA (1910) in *Morus* and ISHIKAWA (1911) in *Dahlia* have found the association of metaphase chromosomes in the II meiotic division. DARLINGTON (1928) described a similar association in the 1st meiotic metaphase of *Pyrus* and proposed the name "secondary pairing." He has considered it as the pairing of small homologous chromosomes due to some effect of fixation. After that, LAWRENCE (1931) discussed the secondary association thoroughly, and used the latter as the criterion of the homology of the chromosomes. According to DARLINGTON and LAWRENCE the secondary association is the union of the homologous chromosomes of small size in metaphase which failed to form multivalents in the meiotic prophase. The chromosomes in the secondary association do not show any differences either in their size or shape, so it is different from the writer's bivalents-association, which appear in the later prophase of the 1st meiotic division, but does not appear in the metaphase or later. Chromosomes in bivalents-association show clear differences among themselves not only in their size but also in shape. They may be considered as very loosely associated quadrivalents. For the formation of the bivalents-association, on one hand the so-called matrix (hyalonema of FUJII) of chromosome may have a certain role, while on the other hand we have to consider the influence of the nucleolus. In our investigation the bivalents I-III attach to one and the same nucleolus, where they may have not only the opportunity of association but also may absorb the same material from the nucleolus which have some part in the development of the chromosomes. But in this case the bivalents-association occurs not only in the SAT-chromosomes but also between the bivalents which do not show any sign of having the satellites or second constrictions. Consequently the nucleolus may not be the main cause of bivalents-association.

Here I wish to mention that bivalent I in the chromosome set

j, open ring bivalent VI; *k*, smallest open ring bivalent X; 2, a PMC in which *c* and *b* show the interlocking of bivalents II and III; *d* and *e*, open ring IV and V; 3, a PMC. *a*, bivalent I showing satellites very clearly; *f* and *g*, bivalents VII and VIII showing the interlocking; 4, a PMC. *d* and *e*, interlocked bivalents IV and V at the distal ends. ca. $\times 2000$. 5a and 5b, one and the same material, photographed in different level. $\times 1000$. 5c-5e, camera drawings in different levels of the nucleus in one and the same cell (*a*) as 5a and 5b; 5c, shows 3 bivalents associations of ring bivalents; 5d and 5e show interlocked bivalents associations of ring bivalents (*f*, *g*); 5f, a nucleus reconstructed from 5c, 5d and 5e; ca. $\times 1750$;

Fig. 6, a root tip cell showing a polar view of an equatorial plate. ca. $\times 1750$.

S_3 may bear some resemblance to bivalents II and III in other 2 sets, S_1 and S_2 .

2. Derivation of the chromosome complement of *P. somniferum*. In previous papers (YASUI 1936, 1937a, and 1937b) I have stated that *P. somniferum* has 3 sets of chromosomes in its haploid complement. One of them consists of 3 chromosomes, and the two of similar sets of 4 chromosomes. So that the chromosomes in the latter two sets form bivalents when the haploid complement of *P. somniferum* loses its partner in the meiotic division, e.g. in the interspecific hybrids of *P. somniferum*, which was crossed with other species having no homologous chromosomes. The present investigation confirms the above consideration. *P. somniferum* has the haploid complement which consists of 3 sets of chromosomes, two of them (S_1 and S_2) greatly resemble each other and the third (S_3) has no homologous chromosomes in the other 2 sets, except that one chromosome (I) in set S_3 attaches to one and the same nucleolus on which chromosomes II and III in the S_1 and S_2 sets attach.

The following three considerations may be given for the derivation of such a chromosome complement. 1. a hybrid whose haploid chromosome number is 7 was raised as an amphidiploid by the intercrossing of 2 species in which one has 3 haploid chromosomes and the other 4 haploid chromosomes; then this amphidiploid was crossed with another species closely related to one parent having 4 haploid chromosomes, and again all the chromosome sets were doubled forming an amphitriploid, namely $(3+4) \times 2 = 2(7)$, and then $(7+4) \times 2 = 2(4+4+3) = 2(11)$. 2. the chromosome complement in a species having 4 chromosomes as its haploid number was doubled and then crossed with a species having 3 haploid chromosomes, and again all the sets were doubled, namely $2(4+4) = 2(8)$, and then $(8+3)2 = 2(11)$. 3. in an interspecific hybrid one of whose parents has 4 haploid chromosomes and another 3 chromosomes, the sets of 4 chromosomes was doubled and again the whole sets were doubled, namely $4+3$, and then $2[2(4)+3] = 2(11)$.

As I have mentioned above, the members of the bivalents-association are not completely homologous and neither can take the other's place in the meiotic division in the parent species. Such differences in every set of 4 may be difficult to be derived by doubling of one set of 4 chromosomes, so that it looks to me that the first consideration is more possible.

Summary

1. In a race of *Papaver somniferum* are found 11 bivalents in its diaphase of the PMC, which show the kinds of association as 6

rings, 2 side by side union or, rarely, 2 open rings, 1 end to end union, 1 small ring and 1 largest side by side union or open ring. While in another race there were found only ring or open ring configurations instead of side by side or end to end unions.

2. There were observed in the former very loose associations "bivalents-associations," between 2 pairs of ring bivalents or one pair of side by side bivalents, and also between a ring and an end to end bivalent. In the latter race 4 associations of ring bivalents were found.

3. *P. somniferum* is an amphitriploid, which has probably been derived as follows: an interspecific hybrid is raised between 2 species, one having 4 chromosomes and the other 3 chromosomes as their haploid complements, then the chromosome sets were doubled causing an amphidiploid, namely $2(4+3)=2(7)$. This amphidiploid is crossed with a plant which is closely related to one parent having 4 haploid chromosomes, then the whole sets are doubled again, namely $(3+4)2=2(7)$, and then $2(7+4)=2(11)$.

4. The association or pairing of the two bivalents, "the bivalents-association" was discussed.

The writer's hearty thanks are due to Prof. K. Fujii for his kind advice in the course of the investigation. The expence of carrying out the present work was partly defrayed out of a grant from the Japan Society for the Promotion of Scientific Research, to which I tender my thanks.

Botanical Institute, Faculty of Science,
Tokyo Imperial University, and Tokyo
Higher Normal School for Women

Bibliography

- Darlington, B. C. 1928. Studies in *Prunus*, I and II. Jour. Genetics 19: 213-256.
Ishikawa, M. 1911. Cytologische Studien von Dahlien. Bot. Mag. (Tokyo) 25: 1-8.
Kuwada, Y. 1910. A Cytological Study of *Oryza sativa* L. Bot. Mag. (Tokyo) 24: 267-281.
Lawrence, W. J. C. 1931. The Secondary Association of Chromosomes. Cytologia 2: 352-384.
Tahara, M. 1910. Über die Kernteilung bei *Morus*. Bot. Mag. (Tokyo) 24: 281-289.
Yasui, K. 1936. Cytogenetic Studies in Artificially Raised Interspecific Hybrids of *Papaver*. IV. Interspecific Hybrids of *P. orientale* L. and *P. bracteata* Lindl. Cytologia 7: 535-543.
— 1937a. Cytogenetic Studies in Artificially Raised Interspecific Hybrids of *Papaver*. VI. The Trigenomic Hybrids of *Papaver*. Cytologia, Fujii Jubilee Vol. 1101-1112.
— 1937b. Cytogenetic Studies in Artificially Raised Interspecific Hybrids of *Papaver*. VII. *P. somniferum* L. $\times$ *P. bracteata* Lindl. Cytologia 8: 331-342.