Further Studies on Genetics and Cytology of Artificially Raised Interspecific Hybrids of *Papaver*

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With 14 Text-Figures

The artificially raised F_1 plants *Papaver somniferum* L. var. *glabrum* BOIS. $\times$ *P. nudicaule* L. were selfed in some hundred cases, which produced almost no seed. The microscopical examination showed that the pollen grains were sterile, and the female gametes almost entirely sterile, having an extremely small percentage of seed production. This small partial fertility was used for the study of the distribution of paternal characters in the offsprings and the karyological behavior accompanying them.

The present report will briefly deal with some genetical data concerning 3 successive generations, and their cytology with special reference to the chromosome number and its behavior.

MATERIAL AND METHOD

The parent plants used in the experiments were *Papaver somniferum* L. var. *glabrum* BOIS. and *Papaver nudicaule* L., the former being of the same pedigree with the plants used in the experiments of my previous paper on *Papaver* hybrid (YASUI, 1921).

The characters of *P. nudicaule* L. which were taken into consideration in the present experiment are as follows: perennial slender herb, hairy stem and leaf, waxy green pinnately cut leaf; sepals two as a rule, sometimes three; petals generally 4, occasionally 6, salmon orange to grendine; stamens yellow with purplish white filaments; and the capsule elongated with purple stigma.

In autumn 1921 the seeds of *Papaver nudicaule* L. from the Botanic Garden of Tokyo Imperial University were sown. The plants flowered in spring 1922, and were used as male as well as

female parents of the hybrid. They were selfed too, and the S^1 plants flowered in 1922, and were found to show all the characters in consideration constant.

The F_1 plants were selfed and back-crossed with pollen of both parents.

Fixing and staining. Some material for cytological examinations was fixed with BENDA's solution and stained with HEIDENHAIN's iron-alum haematoxylin, while others were treated according to TAYLOR's smear method (TAYLOR, 1924). The latter proved very successful, especially for the study of chromosome numbers.

Text-fig. 1. F_1 plant of *Papaver somniferum* L. var. *glabrum* BOIS. $\times$ *P. nudicaule* L. 1,8 nat.

EXTERNAL CHARACTERS OF F_1 PLANT

In the reciprocal crossing between *Papaver nudicaule* L. and *P. somniferum* L. var. *glabrum* BOIS., *P. somniferum* L. var. *glabrum* BOIS. $\times$ *P. nudicaule* L. only gave seeds, so that the present descriptions are limited to the F_1 plants of the latter crossing. Text-fig. 1 is a photograph of one of F_1 plants. They are characterized by the following points:

1. F_1 plants germinated in autumn 1922 and flowered in the next spring and died away in the summer, i. e. the plant is annual, and the perennial character of the male parent is recessive to the annual character of the female parent, contrary to the case of the hybrid between *P. somniferum* $\times$ *P. nudicaule* (YASUI, 1921).

2. F_1 plants were tall like the female parent, but neither so stout as the latter, nor so slender as the male parent; branched several times as in the male parent. The shape of leaves were intermediate between those of both parents. The stem and leaves were hairy, but not so thick as in the male parent, with somewhat waxy surface like those of the female parent.

3. Flowers were all single, while the doubleness of the flower of the female parent was dominant to the singleness in the case

of the hybrids among the races of *P. somniferum* var. *glabrum*

4. Color of the petals was scarlet in all cases of the hybrids, the white, pink, purple and deep red of the female parents, having no apparent influence for the color of the hybrid. The purple color of the filament and the yellow anther of the male parent were dominated in the hybrids.

5. The capsules of all F_1 plants were roundish somewhat like that of the female parent, though not so large as in the latter.

6. The pollen grains were irregular in sizes, most of them being sterile. Some hundreds of flowers of F_1 plant were selfed during 1922-1926, but no fruits were obtained, but one capsule which was gathered in 1926.

CYTOLOGY OF F_1 PLANTS

Text-fig. 2. Metaphase chromosomes of a vegetative cell of an ovule of F_1 plant.

P. somniferum L. var. *glabrum* BOIS. has 22 diploid chromosomes as TAHARA (1915) has shown, and *P. nudicaule* L. 14 as LJUNGDAHL (1924) has described.

F_1 plants have $11+7=18$ chromosomes in their vegetative cell (Text-fig. 2) and the pollen mother-cell. After second contraction in the meiotic phase of pollen mother-cells 6 or 8 chromosomes formed gemini, so that there were 3 or 4 bivalent chromosomes or bivalents (or bivs.) and 10 or 12 univalent chromosomes or univalents (or univs.) in a pollen mother-cell, and the chromosomes in gemini jointed end to end, and never parallel (Text-fig. 3, a and b).

The bivalent chromosomes formed equatorial plate first, while the univalent chromosomes are scattered around them (Text-fig. 3, b). When the bivalent chromosomes entered into anaphase univalent chromosomes enter into equator and undergo longitudinal division, and each halves of them follow the course of bivalent chromosomes (Text-fig. 3, c). In this case, all univalent chromosomes did not enter into the equator simultaneously, so that some chromosomes are often excluded from the nucleus, forming extra chromosomes or dwarf nuclei (Text-fig. 3, d), when the nuclear membrane formation, after the 1st nuclear division of the meiosis, took place, as is the case with the meiosis of F_1 plants of *P. somniferum* $\times$ *P. orientale*, though in the former the irregularities are greater than in the latter.

The second division of meiosis is also irregular, more than four pollen grains being formed as its result in each pollen mother-cell

Text-fig. 3. Pollen mother-cells in F_1 plant. a, diakinesis, 3 bivalent (black) and 12 univalent (blank) chromosomes are seen; b, metaphase of bivs., 12 univs. being scattered outside of them; c, chromosome garniture with 4 bivs. at each pole and 12 univs. occupying the central region; d, interkinesis, 2 large nuclei, one smaller nucleus and 2 extra nuclear chromosomes are seen; e, pollen mother-cell with 4 large pollen grains, each with an extra nuclear chromosome, and one small pollen grain; f, a pollen mother-cell with 8 pollen grains, some of them with extra nuclear chromosomes.

as in the case of *P. somniferum* $\times$ *P. orientale*, but the irregularities here are greater than in the latter (Text-fig. 3, e and f).

EXTERNAL CHARACTERS OF F_2 PLANTS (BACK-CROSSED F_1 PLANTS)

In 1924 several flowers of F_1 plants were pollinated with the pollen grains of the female parents. Generally only one or two, sometimes no seeds were obtained from each capsule. The seeds were sown in autumn of the year, and about 60 seedlings were raised. They flowered in next spring, and were characterized as follows:

1. Stems were thicker than that of F_1 plant, but not so stout as that of *P. somniferum* var. *glabrum*.
2. The shape of the leaves was very much like that of *P. somniferum* var. *glabrum*.
3. The surface of the stem and leaves was waxy and not so much hairy as that of F_1 plant.
4. The color of the petal in all but one individual (41, 15, 15, 52) was just the same to that of F_1 plant.

5. The color of the petal of the aberrant individuals just referred to was phlox with paler margin and a purple patch at the base, and the shape of the flower was also similar to that of *P. somniferum* L. var. *glabrum* BOIS.

6. There was one individual (41, 15, 15, 3) among all, which had double flowers.

CYTOLOGY OF F₂ PLANTS

Only 4 individuals of the back-crossed F₂ plants were studied cytologically, and they showed the following chromosome behavior ;

1. 41, 15, 15, 57. 11 bivalent and 5 univalent chromosomes were found in the pollen mother-cells (Text-fig. 4, a). In the first division of the meiosis 11 bivalent chromosomes enter into the

Text-fig. 4. a, chromosome in diakinesis of pollen mother-cell of F₂ (back-crossed F₁) plants, 11 bivalent (black) and 5 univalent (blank) chromosomes ; b-g, pollen mother-cells of F₂ plant ; b, polar view of metaphase of bivs. (11 pairs), 5 univs. scattered outside of them ; c, later stage, from the material derived from the same flower ; d, bivs. in metaphase (black) and 6 univs., the 7th is not seen in this figure (blank) ; e, the later stage, observed in the same material ; f, homotypic division, 12 large chromosomes and two smaller chromosomes seen in the polar view of the metaphase ; g, 4 large pollen and two smaller pollen grains in a pollen mother-cell.

equator first, while the univalent chromosomes remain outside (Text-fig. 4, b). As each halves of bivalent chromosomes leave the

equator for the poles, the univalent chromosomes now get into the equator, and they divide longitudinally and each halves of them follow the bivalent chromosomes to pole (Text-fig. 4, c).

2. 41, 15, 15, 1. 11 bivalent and 6 univalent chromosomes were found in the nucleus of the pollen mother-cell. Among the former 4 pairs were ring-shaped, one pair in side by side, and other three in an end to end arrangement in diakinesis.

3. 41, 15, 15, 58. No first division of the meiosis was observed, but in the second division one nucleus showed 14 chromosomes (Text-fig. 4, f), and more than 4 pollen grains, even 6, were found in each pollen mother-cell (Text-fig. 4, g). The latter case suggests the irregular behavior of the meiosis.

4. 41, 15, 15, 3. 11 bivalent and 7 univalent chromosomes were found. The 11 bivalent chromosomes form the equatorial plate first and the course of the univalent chromosomes and the irregular behavior of the chromosomes were similar to the case described above (Text-fig. 4, d-e).

In comparing of these irregularities in F_2 plants with that of F_1 plants, the former were always less irregular than the latter, and many ring-shaped bivalent chromosomes were found in F_2 plants, but never in the case of F_1 plant.

F_3 PLANTS

Among about 60 back-crossed F_2 plants 19 individuals gave seeds by self-pollination. The main points of the external characters and the chromosome behavior in the meiosis of pollen mother-cells are shown in the following 6 divisions I-VI.

I. In this, the female parent of F_1 plants had the single flower, pale purple petal with a deep purple patch at the base. The F_1 plants were back-crossed with the pollen of a plant belonging to the female parent type, and from this three pedigrees (1-3) were derived.

Pedigree 1. F_3 plants 41, 15, 27 (S_1 of F_2 plant 41, 15, 15, 52, which closely resembled the female parent of F_1 plant, but with petals slightly tinged with pink).

The external characters of the flowers of more than 50 individuals were examined and found to agree with each other and similar to the female parent of F_1 plant.

The results of karyological examination in the meiotic phase of pollen mother-cells in 14 individuals are as follows:

Table 1

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	8	10
	Undetermined	2	
Irregular	11 bivs. + 2 univs. ¹⁾	1	4
	11 bivs. + 1 univ.	1	
	Undetermined ²⁾	2	
Mostly irregular	11 bivs. ³⁾	1	1

1) Meiosis irregular, 2-4 or more pollen grains being formed in a pollen mother-cell (Text-fig. 5, c).

2) 2-4 or more pollen grains formed in one pollen mother-cell.

3) In addition to the pollen mother-cells with the normal tetrad division there are some in which homotypic division is omitted, so that only two pollen grains are formed (Text-fig. 5, e); there are also the cases in which only one of the daughter nuclei of the first division, resulting in the formation of 3 pollen grains in one pollen mother-cell.

Text-fig. 5. Pollen mother-cells of F_3 plant (Pedigree 1). a, diakinesis, 11 bivs. seen; b, diakinesis, 11 bivs. and one univs. seen; c, diakinesis, 11 bivs. and 2 univs. seen; d, metaphases of homotypic division, 11 bivs. and one unv. seen; e, pollen mother-cell in which only 2 pollen grains are formed.

Pedigree 2. F_3 plants 41, 15, 29 (S_1 of F_2 plant 41, 15, 15, 1, whose flower was similar to that of F_1 plant).

The external characters of the flowers of F_3 plants were similar to that of the female parent of F_1 plant except 2, one of which the petals tinged slightly with pink, and the other had petals which were in deeper color than the others.

The results of karyological examination in the meiotic phase of pollen mother-cells of 13 individuals are as follows:

Table 2

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	6	10
	Undetermined	4	
Irregular	11 bivs.+1 biv. or 2 univs.	1	3
	11 bivs.+1 univ.	1	
	Undetermined ¹⁾	1	

1) 2-3 pollen grains formed in one pollen mother-cell.

Text-fig. 6. Three pollen mother-cells of F_3 plants (Pedigree 2). a, diakinesis, showing 11 bivs.; b, diakinesis, 11 bivs. and one univ. seen; c, metaphase of 11 bivs., 3 univs. scattered outside of them.

Pedigree 3. F_3 plants 41, 15, 30 (S_1 of F_2 plant 41, 15, 15, 2, whose flower resembled that of F_1 plant).

The external characters of the flowers of F_3 plants were similar to each other and to that of the female parent of F_1 plant, except one which had deep purple petals.

The results of karyological examination in the meiotic phase of pollen mother-cells of 7 individuals were that the division was regular, and 11 bivalent chromosomes were found in all individuals.

II. The female parent of F_1 plant had the double flower, the entire red petal with white patch at the base. The F_1 plant were back-crossed with the pollen of *P. somniferum* var. *glabrum*, which had the single flower and the lacinated red petal with a purple patch at the base; and 6 pedigrees (4-9) were derived from this.

Pedigree 4. F_3 plants 41, 15, 31 (S_1 of F_2 plant 41, 15, 15, 3, which was only plant having double flower among the back-crossed

F₂ plants. The colour of the flower was similar to that of F₁ plant).

The external characters of the flowers of 29 individuals examined are shown in Table 3.

Table 3

Character	Red petal with a purple patch	Red petal with a white patch
Lacinated { single	10	5
{ double	1	2
Entire { single	5	5
{ double	1	0

Purple patch : white patch = 17 : 12

Lacinated : entire = 18 : 11

Single : double = 25 : 4

The results of karyological examination in the meiotic phase of pollen mother-cells in 6 individuals are as follows :

Table 4

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	0	0
Irregular	11 bivs. + 3 univs.	1	6
	11 bivs. + 2 univs.	1	
	11 bivs. + 1 univ.	2	
	Undetermined	2 ¹⁾	

1) One of them had 2-4 pollen grains in one pollen mother-cell.

Text-fig. 7. 2 pollen mother-cells of F₃ plants (Pedigree 4). a, heterotypic anaphase, showing the garniture of 11 chromosomes (black) derived from bivs. together with one chromosome derived from univ. at each pole; b, a divided univ. at the equator, being excluded out of daughter nuclei at the poles.

Pedigree 5. F_3 plant 41,15,32 (S_1 of F_2 plant 41,15,15,4, whose flower resembled that of F_1 plant).

The external characters of the flowers of 24 individuals examined are shown in Table 5.

Table 5

Character	Red petal with a purple patch	Red petal with a white patch
Lacinated { single	1	2
{ double	2	2
Entire { single	9	6
{ double	2	0

Purple patch : white patch = 14 : 10

Single : double = 18 : 6

Lacinated : entire = 7 : 17

The results of karyological examination in the meiotic phase of pollen mother-cells in 9 individuals are as follows :

Table 6

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	4	8
	Undetermined	4	
Irregular	11 bivs.+2 or 3 univs.	1	2
	11 bivs.+1 univ.	1

Text-fig. 8. Pollen mother-cell of F_3 plant (Pedigree 5), showing regular homotypic division.

Pedigree 6. F_3 plants 41,15,38' (S_1 of F_2 plant 41,15,15,10, whose flower resembled that of F_1 plant).

The external characters of the flowers of 25 individuals examined are shown in Table 7 :

Table 7

Character	Red petal with a purple patch	Red petal with a white patch
Laciniated {single	6	3
{double	2	0
Entire {single	6	0
{double	4	2

Purple patch : white patch = 18 : 5

Laciniated : entire = 11 : 12

Single : double = 15 : 8

The results of karyological examination in the meiotic phase of pollen mother-cells in 13 individuals are as follows :

Table 8

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	6	6
Irregular	11 bivs. + 1 univ.	2	8
	11 bivs. + 2 univs.	2	
	11 bivs. + 1 biv. + 1 univ.	1	
	11 bivs. + 3 univs.	1	
	Undetermined	1 ¹⁾	

1) 2-4 pollen grains in one pollen mother-cell.

Text-fig. 9. 3 pollen mother-cells of F_3 plant (Pedigree 5). a, polar view, 11 bivs. (black) at the equator and 2 univs. scattered outside of them; b, side view of a later stage of heterotypic anaphase, chromosome garniture derived from 11 bivs. at each pole, and 2 smaller chromosomes, resulting by the equational division of univs. following them toward each pole; c, diakinesis stage, 11 bivs. (black) and 3 univs. (blank).

Pedigree 7. F_3 plants 41,15,44 (S_1 of F_2 plant 41,15,15.33, whose flower resembled that of F_1 plant).

The external characters of the flowers of 31 individuals examined are shown in Table 9.

Table 9

Character	Red petal with a purple patch	Red petal with a white patch
Laciniated {single	15	4
{double	1	1
Entire {single	7	2
{double	0	1

Purple patch : white patch = 23 : 8

Laciniated : entire = 21 : 10

Single : double = 28 : 3

The results of karyological examination in the meiotic phase of pollen mother-cells in 6 individuals are as follows :

Table 10

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	4	6
	Undetermined	2

Text-fig. 10. Polar view of heterotypic metaphase of pollen mother-cell of F_3 plant (Pedigree 7), 11 bivs. at equator.

Pedigree 8. F_3 plants 41,15,35 (S_1 of F_2 plant 41,15,15,7, whose flower was the same to that of F_1 plant).

The external characters of the flowers of 28 individuals were examined are shown in Table 11.

Table 11

Character	Red petal with a purple patch	Red petal with a white patch
Laciniated {single	13	2
{double	0	0
Entire {single	9	3
{double	1	0

Purple patch : white patch = 23 : 13

Lacinated : entire = 15 : 13

Single : double = 27 : 1

The results of karyological examination in the meiotic phase of pollen mother-cells in 6 individuals are as follows :

Table 12

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	3	3
Irregular	11 bivs. + 2 univs.	1	3
	11 bivs. + 1 or 2 univs.	1	
	11 bivs. + 1 univ.	1	

Pedigree 9. F_3 plants 41,15,37 (S_1 of F_2 plant 41,15,15,9, whose flower was similar to that of F_1 plant).

The external characters of the flowers of 34 individuals examined are shown in Table 13.

Table 13

Character	Red petal with a purple patch	Red petal with a white patch
Lacinated { single	20	4
{ double	1	0
Entire single	8	0

In addition to those showing in Table 13, there is one individual which had the flower resembled that of F_1 plant.

Purple patch : white patch = 29 : 4

Lacinated : entire = 25 : 8

The results of karyological examination in the meiotic phase of pollen mother-cells in 6 individuals are as follows :

Table 14

Division	Chromosome number	Number of individual plant	Total
Regular	11 bivs.	4	5
	Undetermined	1	
Irregular	11 bivs. + 2 univs.	1	1

• **III.** In this, the female parent of F_1 plants had the double flower, the entire, red petal with a white patch at the base. The F_1 plants were back-crossed with the pollen of *P. somniferum* var. *glabrum* which had the single flower, the laciniated pale purple petal with a deep purple patch at the base, and 2 pedigrees (1-11) were derived from them.

Pedigree 10. F_3 plants 41,15,40 (S_1 of F_3 plant 41,15,15,12, whose flower was similar to that of F_1 plant).

The external characters of the flowers of 36 individuals examined are shown in Table 15.

Table 15

Character	Red petal with a purple patch	Red petal with a white patch
Laciniated {single	2	10
{double	1	8
Entire {single	1	7
{double	0	4

In addition to those in the above table there were 3 plants, as follows:

1. The single flower with the deep purple entire petal 2 indivs.
2. The single flower with the laciniated, purple with a deeper purple patch at the base 1 indiv.

Red : deep purple : pale purple = 33 : 2 : 1

Purple patch : white patch = 7 : 29

Laciniated : entire = 22 : 14

Single : double = 23 : 13

The results of karyological examination in the meiotic phase of pollen mother-cells in 8 individuals are as follows:

Table 16

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	4	6
	Undetermined	2	
Irregular	11 bivs. + 2 univs.	1	1
Mostly irregular	11 bivs.	1 ¹⁾	1

- 1) 2-4 pollen grains formed in one pollen mother-cell.

Text-fig. 11. 2 pollen mother-cells of F_3 plant (Pedigree 10). a, one in which only two pollen grains are formed; b, one in which 4 pollen grains are formed, one of them being unusually large.

Pedigree 11. F_3 plants 42,15,45 (S_1 of F_2 plant 41,15,15,40, whose flower was similar to that of F_1 plant).

The external characters of the flowers of 29 individuals examined are shown in Table 17.

Table 17

Character		Red petal with a purple patch	Red petal with a white patch	Purple petal with a deep purple patch	White petal
Lacinated...	single	4	1	1	0
	double	0	0	0	0
Entire.....	single	14	2	2	2
	double	1	1	0	0

In addition to those showing in Table 17, there was one which had the entire single flower, the purple patch at the base of petal.

Red : purple : white = 24 : 3 : 2

Purple patch : white patch = 23 : 6

Lacinated : entire = 7 : 22

Single : double = 26 : 3

The results of karyological examination in the meiotic phase of pollen mother-cells in 8 individuals are as follows:

Table 18

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	2	2
	11 bivs. + 1 univ.	3	
Irregular	11 bivs. + 2 univs.	1	
	11 bivs. + more than 3 univs.	1	6
	Undetermined	1	

IV. In this, the female parent of F_1 plant had the single flower, the laciniated, red petal with a purple patch at the base. The F_1 plant was back-crossed with the pollen of *P. somniferum* var. *glabrum*, which had the double flower, and the entire white petal; and one pedigree (12.) was derived from them.

Pedigree 12. F_3 plants 41,15,24 (S_1 of F_2 plant 41.15,15,6, whose flower resembled that of F_1 plant).

The external characters of 32 individuals examined are shown in Table 19.

Table 19

Character	Red petal with a purple patch	Deep purple petal	Pale purple with a purple patch	White
Laciniated... {single	2	0	0	0
{double	0	1	0	0
Entire..... {single	6	0	1	1
{double	11	4	4	1

In addition to those in Table 19 there was one which had flower resembled that of F_1 plant.

Red : deep purple : pale purple : white = 20 : 5 : 5 : 2

Single : double = 11 : 21

Laciniated : entire = 3 : 29

The results of karyological examination in the meiotic phase of pollen mother-cells in 11 individuals are as follows:

Table 20

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	4	5
	Undetermined	1	
Irregular	11 bivs. + 4 univs.	1	6
	11 bivs. + 2 univs.	2	
	11 bivs. + 1 univ.	1	
	Undetermined	1 ¹⁾	

1) 2-4 or more pollen grains in one pollen mother-cell.

Text-fig. 12. Pollen mother-cell of F_3 plant (Pedigree 12), bivalent chromosome garniture (black) at anaphase and four univs. (blank) in longitudinal division.

V. In this, the female parent of F_1 plants had the single flower, the entire, red petal with a white patch at the base. The F_1 plants were back-crossed with pollen of *P. somniferum* var. *glabrum* which had the single flower and the lacinated red petal with a purple patch at the base. Four (13-16) pedigrees were derived from them.

Pedigree 13. F_3 plants 41,15,33 (S_1 of F_2 plant 41,15,15,5, whose flower was the same as that of F_1 plant).

The external characters of 32 individuals examined are shown in Table 21.

Table 21

Character	Red petal with a purple patch	Red petal with a white patch
Single { Lacinated	14	5
{ Entire	10	1

In addition to those in Table 21 there was one individual which had flower resembled that of F_1 plant.

Purple patch : white patch = 24 : 6

Lacinated : entire = 19 : 11

The results of karyological examination in the meiotic phase of pollen mother-cells in 13 individuals are as follows :

Table 22

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	6	9
	Undetermined	3	
Irregular	11 bivs. + 2 univs.	1	4
	11 bivs. + 1 univ.	1	
	Undetermined	2 ¹⁾	

1) One of them had 2-4 pollen grains in one pollen mother-cell.

Text-fig. 13. Pollen mother-cell of F_3 plant (Pedigree 13) in diakinesis; 11 bivs. are seen.

Pedigree 14. F_3 plants 41,15,39 (S_1 of F_2 plant 41,15,15,11, whose flower was the same as that of F_1 plant).

The external characters of 24 individuals examined are shown in Table 23.

Table 23

Character	Red petal with a purple patch	Red petal with a white patch
Single { laciniated	3	1
entire	16	4

Purple patch : white patch = 19 : 5

Laciniated : entire = 4 : 20

The results of karyological examination in the meiotic phase of pollen mother-cells in 4 individuals are as follows :

Table 24

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	3	4
	Undetermined	1	

Pedigree 15. F_3 plants 41,15,41 (S_1 of F_2 plant 41,15,15,18, whose flower was the same as that of F_1 plant).

The external characters of the flowers of 23 individuals examined are shown in Table 25.

Table 25

Character	Red petal with a purple patch	Red petal with a white patch
Single { laciniated	11	1
entire	7	3

In addition to those which are showing in Table 25, there was one which had the flower resembled that of F_1 .

The results of karyological examination in the meiotic phase of pollen mother-cells in 8 individuals are as follows :

Table 26

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	1	2
	Undetermined	1	
Irregular	11 bivs. + 2 or 3 univs.	1	6
	11 bivs. + 1 univ.	2	
	Undetermined	4 1)	

1) One of them had 2-4 pollen grains in one pollen mother-cell.

Pedigree 16. F_3 plants 41,15,43 (S_1 of F_2 plant 41,15,15,26, whose flower was similar to that of F_1 plant).

The external characters of the flowers of 17 individuals examined are shown in Table 27.

Table 27

Chracter	Number of individuals
Red petal {	with a purple patch 16
	with a white patch 1

The results of karyological examination in the meiotic phase of pollen mother-cells in 6 individuals are as follows:

Table 28

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	2	3
	Undetermined	1	
Irregular	11 bivs. + 3 univs.	1	3
	11 bivs. + 2 univs.	1	
	Undetermined	1 1)	

1) 2-4 or more pollen grains in one pollen mother-cell.

Text-fig. 14. 3 pollen mother-cells of F_3 plants (Pedigree 16). a, diakinesis,

11 bivs. (black) and 3 univs. (blank) seen; b, later stage, 11 bivs. at the equator and 3 univs. scattered outside of them; c, still later stage, chromosome garniture derived from bivs. in anaphase and univs. in metaphase.

VI. In this, the female parent of F_1 plant had the single flower, the laciniated, red petal with a purple patch at the base, but it was hetero-individual which had to segregate into four different kinds of individuals; 1, red petal with a white patch, 2, red petal with purple patch, 3, pale purple petal with a deeper purple patch, 4, white petal. The F_1 plants were back-crossed with the pollen of a *P. somniferum* which had single flower, the laciniated, red petal with purple patch at the base. Three pedigrees (17-19) were derived from them.

Pedigree 17. F_3 plants 41,15,36 (S_1 of F_2 plant 41,15,15,8, whose flower was the same as that of F_1 plant).

The external characters of the flowers of 41 individuals were examined, and found that all individuals had the single flower and the laciniated petals, and the number of individuals which had petals of different colors are shown in Table 29.

Table 29

Character	Number of individuals
1. Red petal with a purple patch	38
2. Red petal with a white patch	1
3. Deep purple patch	2

The results of karyological examination of pollen mother-cells in 13 individuals are as follows:

Table 30

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	6	10
	Undetermined	4	
Irregular	11 bivs.+2 univs.	2	3
	Undetermined	1 ¹⁾	

1) 2-4 or more pollen grains found in one pollen mother-cell.

Pedigree 18. F_3 plants 41,15,46 (S_1 of F_2 plant 41,15,15,46, whose flower was similar to that of F_1 plant).

The external characters of the flowers of 24 individuals examined are shown in Table 31.

Table 31

Character	Number of individuals
1. Red petal with a purple patch, lacinated; single.	22
2. Pale purple petal with a deeper purple patch, lacinated; single.	1
3. Deep purple petal, lacinated; single.	1

The results of karyological examination in the meiotic phase of pollen mother-cells in 7 individuals are as follows:

Table 32

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	5	5
Irregular	11 bivs.+1 univ.	1	2
	Undetermined	1 ¹⁾	

1) 2-4 pollen grains were found in one pollen mother-cell.

Pedigree 19. F₃ plants 41,15,50 (S₁ of F₂ plant 41,15,15,57, whose flower was similar to that of F₁ plant).

The external characters of the flowers of 20 examined individuals of F₃ plants were of the same, and they had the single flower, and the lacinated red petal with a purple patch.

The results of karyological examination in the meiotic phase of pollen mother-cells in 16 individuals are as follows:

Table 33

Division	Chromosome number	Number of individual plants	Total
Regular	11 bivs.	4	4
Irregular	11 bivs.+1 univ.	1	2
	11 bivs.+2 univs.	1	

DISCUSSION

A. Irregular behavior in meiosis

a. The number of gemini and univalent chromosomes.

1. F₁ generation. There were found only three as a rule, but sometimes four gemini and 12 or 10 unpaired chromosomes. This is very different from the case in F₁ plant, *P. somniferum* var. *glabrum* ×

orientale. In the latter the number of gemini formed are equal to the chromosome number of the one parent which had small chromosome number, i. e. 11 in *P. somniferum* var. *glabrum*, while in the former the number of gemini formed are less than the chromosome number of *P. nudicaule*, which has 7 haploid chromosomes, the basic unit number among *Papaver* species.

Many authors believe that the gemini formation in meiotic phase is due to the affinities between the chromosomes from both parents, but some experiments, e. g. BORGSTAM (1922), SAKAMURA and STOW (1925), show that the external factors, e. g. temperature, may induce it. The present writer also observed the similar case in the course of the research; but still she would emphasize that the affinities between chromosomes is the main factor in nature and will determine the gemini number in hybrid plants.

2. F_2 generation. In the three cases examined there were 11 gemini and 7, 6, and 5 univalent chromosomes.

3. F_3 generation. Table 34 gives the frequency distribution of

Table 34

Name of pedigrees	Number of individuals with 11 bivs.	Number of individuals with more than 11 bivs. and additional chromosomes
3	7	0
7	4	0
14	3	0
1	9	2
2	6	2
4	0	4
5	4	2
6	6	7
8	3	3
9	4	1
10	5	1
11	2	5
12	4	4
13	6	3
15	1	3
16	2	2
17	6	2
18	5	1
19	4	2
	81	44

chromosome number among the above 19 mentioned pedigrees of mentioned F_3 generation.

As it is seen in the above table, in 3 among 19 pedigrees all individuals had 11 bivalent chromosomes like *P. somniferum* var. *glabrum*, and 67 out of 111 individuals belonging to the 16 remaining pedigrees had 11 bivalent chromosomes also, and the remaining 44 individuals more than 11 bivalents or more than bivalents, and a number of univalents.

Table 35 shows the frequency distribution of chromosome number in individuals with 11 bivalents and a number of additional chromosomes.

Table 35

Number of chromosomes	Number of individuals
11 bivs.+1 univ.	19
11 bivs.+2 univs.	18
11 bivs.+3 univs.	4
11 bivs.+4 univs.	1
11 bivs.+1 biv.+2 univs.	1
11 bivs.+1 biv.+1 univ.	1
Total	44

By comparing the chromosome numbers in the three generations F_1 , F_2 , F_3 and the structure of gemini we may conclude

1. Successive diminutions of the number of univalent chromosomes.
2. Restitution of 11 bivalent chromosomes in F_2 generation.
3. Fixation of individuals with 11 bivalents in F_3 generation.
4. Absence of parasyndetic figure in F_1 generation and its appearance in F_2 generation.
5. Considerable increase of the number of gemini in F_3 generation.

The simultaneous restitution of the appearance of the number of *somniferum* chromosomes and parasyndetic figures indicate the stronger affinity between the allelochromosomes¹⁾ and their number

1) The term *allelochromosome* has been introduced by Prof. K. FUJII in his course of lectures on genetics for the academic year 1919-20 in the Faculty of Science, Tokyo Imperial University, in place of generally used term "homologous chromosome," especially for the case of hybrid plants, in which this term conveys the idea of chromosomes carrying allelogenes (differential genes concerned in the development of allelomorphous characters). As the antonym or allelochromosome to denote a homozygous pair of chromosomes, he proposed the term *homochromosome*.

may determine the number of gemini in the meiosis. KIHARA (1924) observed the restitution of the chromosome numbers of both parent plants in individuals of F_4 generation and the stableness of meiotic phase in pentaploid hybrid of *Triticum* species. In the present case of my *Papaver* hybrid, there is no restitution of the smaller number of chromosomes characteristic to one of the parents, while individuals with increased number of chromosomes appear in addition to the restitution of the larger number of chromosomes characteristic to the other parent.

b. Irregular cell division. All individuals in F_1 and F_2 generations in these experiments, as far as observed, showed irregular cell division in meiosis of pollen mother-cells, but such individuals diminished in number in F_3 generation with the elimination of univalent chromosomes among them as is shown in the following table:

Table 36 (F_3 individuals)

Name of pedigree	Number of individuals with regular meiotic division	Number of individuals with irreg. meiotic division
1	10	3
2	10	3
3	7	0
4	1	5
5	8	1
6	6	7
7	6	0
8	3	3
9	5	1
10	6	2
11	2	6
12	5	6
13	9	5
14	4	0
15	2	6
16	3	3
17	9	4
18	5	2
19	4	2
Total	106	59

Among these 59 individuals, which show irregular divisions in meiosis, there were about 7 individuals, in which some pollen

mother-cells divided only once in meiosis; in some pollen mother-cells, only one of two daughter nuclei derived from the heterotypic division divided homotypically; and in others normal meiotic division took place. The result is the formation of 2-4 pollen grains in a pollen mother-cell instead of constantly 4 pollen grains. In these cases the pollen mother-cells contained 11 bivalent chromosomes and their behavior in heterotypic division was regular, so that the pollen grains formed through one division only had 22 haploid chromosomes. Some of F_4 individuals which were derived from such as the latter, are expected to be tetraploid or triploid, but unfortunately these F_3 plants are mostly sterile, so that such a tetraploid-plants will be very rare.

B. Cytological findings in relation to genetics. The yellowish orange color in the male parent dominated to all other different colors in the flower of *P. somniferum* var. *glabrum*, namely white, purple, and red, so that all F_1 plants are uniform.

More than 60 back-crossed F_2 individuals had similar flowers to that of F_1 plant except one, and among more than 500 F_3 plants only 6 individuals had similar flower to that of F_1 plant, but other individuals had flower similar to that of *P. somniferum* L. var. *glabrum* BOIS. In their interspecific hybrids of *Nicotiana*, GOODSPEED and CLAUSEN (1922) got individuals identical with each parent in the back-crossed F_1 generation by back-crossing with each parent pollen to F_1 plants, and all their offsprings were found identical to their parents.

In the present case almost of all back-crossed F_2 individuals resembled the F_1 plants, but not *P. somniferum* var. *glabrum*.

These eliminations of male characters in successive generations take place simultaneously with the eliminations of univalent chromosomes among them, which indicates that the genes for the eliminated character of the male plant was represented in some univalent chromosomes which dropped off in the process of meiosis generally in that of F_2 plant, and rarely in F_1 plants. Thus the plants in following generations tend to return to *P. somniferum* L. var. *glabrum* BOIS. generally.

The segregation of the allelomorphs i. e. $\frac{\text{lacinated}}{\text{entire}}$, $\frac{\text{single}}{\text{double}}$, $\frac{\text{purple patch}}{\text{white patch}}$, etc. in F_3 generation is not so regular as may be expected in the Mendelian hybrids among the races in *P. somniferum* L. var. *glabrum* BOIS., e. g. 7 purple patch : 29 white

patch, 6 laciniated : 19 entire, 3 laciniated : 29 entire, etc. The ratios indicate the dominance of entire to laciniated, and of white to purple patch. These F_3 plants are however as phenotype *P. somniferum* L. var. *glabrum* BOIS., in which the character entireness in petal is recessive. F_4 generation which is now in seedling forms, will determine the point in course of the year. It may be conjectured, however, that the presence of supernumeral univalents from either side of parents or interchanges of genes concerning these characters, between *P. somniferum* L. var. *glabrum* BOIS. and *P. nudicaule* L., which might have taken place during the successive generations F_1 - F_3 , may have caused the reversal of dominance in *P. somniferum* L. var. *glabrum* BOIS.

There was one individual whose flower had red spots on the pink petals among F_3 plants. This character was not observed in previous generations as far as we know. Whether it is due to a chromosomal mutant must be left undecided.

SUMMARY

1. In F_1 plant of *Papaver somniferum* L. var. *glabrum* BOIS. $\times$ *P. nudicaule* L. the orange color of the petal of *P. nudicaule* dominated over the color of the petal of *P. somniferum* var. *glabrum*, hairy to glaucous, entire to laciniated, single to double, but perennial was recessive to annual.

2. Such dominant characters were eliminated in almost all individuals of S_4 plants of back-crossed F_2 plants, and the characters of *P. somniferum* var. *glabrum* were restituted among them.

3. The chromosome number of F_1 plants was 18, viz. the sum of the haploid chromosome numbers of both parents. Among these chromosomes 6 or 8 chromosomes only mated to form gemini in the meiosis of the pollen mother-cells and the rest remained as unpaired univalent chromosomes.

3. Out of 3 examined individuals of back-crossed F_2 plant, one had 11 bivalent chromosomes and 7 univalent chromosomes, another had 11 bivalent and 6 univalent chromosomes, and the remaining one 11 bivalent and 5 univalent chromosomes.

4. The univalent chromosomes were eliminated largely in F_3 individuals. Out of 122 individuals, in which the chromosome numbers were determined, 82 individuals showed 11 bivalent chromosomes only.

5. In 42 individuals which had more than 11 bivalent chromosomes, the largest number of univalent chromosomes was 4.

6. The parallel eliminations between the paternal characters and univalent chromosomes, and parallel restitution between the maternal characters and parasynopsis indicate that the univalent chromosomes belonged mostly to *P. nudicaule* L.

7. The large deviation in the segregation of F_3 plants may be ascribed to the irregular behavior of chromosomes in meiosis, but in addition the writer expects interchanges of genes concerning these characters.

8. There was one plant whose petals had red spots which never observed in their previous generations.

In closing the writer wishes to express her cordial thanks to Professor K. FUJII for the valuable materials given her for this experimental study and important advices throughout the course of the work.

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BIBLIOGRAPHY

- BELLING, J. (1921) The origin of chromosomal mutations in *Uvularia*. Journ. Genetics. Vol. 15. pp. 245-266.
- BELLING, J. (1923) The attraction between homologous chromosomes. Eugenics, genetics and the family. Vol. 1. pp. 84-85.
- 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. Vol. 12.
- BORGENSTAM, E. (1922) Zur Zytologie der Gattung *Syringa*. Arch. f. Bot. Bd. 17.
- CLAUSEN, J. (1926) Genetical and cytological investigations on *Viola tricolor* L. and *V. arvensis* MURR. Hereditas. Vol. 8.
- GOODSPEED, T. H. and CLAUSEN, R. E. (1922) Interspecific hybridization in *Nicotiana*. I. On the results of backcrossing the F_1 *sylvestris-Tabacum* hybrids to *sylvestris*. Univ. of Calif. Pub. in Bot. Vol. 2. No. 1. pp. 1-30.
- KIHARA, H. (1924) Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rücksicht auf das Verhalten der Chromosomen und die Sterilität in den Bastarden. Mem. of Coll. Sci. Kyoto Imp. Univ. Ser. B. No. 1. Art. 1.
- IJUNGDAHL, H. (1924) Über die Herkunft der in der Meiosis konjugierenden Chromosomen bei *Papaver*-Hybriden. Sv. Bot. Tidsk. Bd. 18. H. 2. S. 279-291.
- SAKAMURA, T. und STOW, I. (1926) Über die experimentell veranlasste Entstehung von keimfähigen Pollenkörnern mit abweichenden Chromosomenzahlen. Japanese Journ. of Bot. Vol. 3. No. 2.
- TAHARA, M. (1915) The chromosomes of *Papaver* (Japanese). Bot. Mag. Tokyo. Vol. 29. pp. 257.
- TAYLOR, W. R. (1924) The smear method for plant cytology. Bot. Gaz. Vol. 78. pp. 236-238.
- YASUI, K. (1921) On the behavior of chromosomes in the meiotic phase of some artificially raised *Papaver* hybrids. Bot. Mag. Tokyo. Vol. 35. pp. 154-167.
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