Chromosome studies in the genus *Acer* L. II. Meiotic abnormalities in PMCs of *A. japonicum* Thunb. var. *typicum* Schw.[1)]

By

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(With Plate II and 48 Text-figures)

Two maple species, *Acer japonicum* and *A. ornatum*, which are usually called "Japan Maples" as the typical representatives of the maples in Japan, are handy ornamental trees or shrubs with handsome or graceful foliage which shows frequently a remarkable tendency to vary in shape and colouring. They are also closely related with each other in morphological any systematical views, both belonging to the same section *Palmata* (*cf.* Koidzumi, '11; Rehder, '35). From the cytological investigations on these two species, however, it has been already found that the meiosis in PMCs of *A. japonicum* var. *typicum* is rather irregular, whereas *A. ornatum* var. *Matsumurae* shows no meiotic abnormalities (*cf.* Takizawa, '40).

Researches on these meiotic abnormalities in *A. japonicum* var. *typicum* were repeatedly and more accurately undertaken by the writer in the following season, and at this time the writer's attention was concentrated on what kinds of abnormality in what frequency occur and on whether they are of individual characteristics or not.

Flower buds used were made available early in this spring. They were collected directly from growing trees cultivated in the experimental arboretum of the Laboratory in Forestry of our University. The bad scales were first dissected away and the flower mass was immersed into an 1:3 mixture of acetic acid and absolute alcohol, and preserved in this solution. After about 24 hr., or more, suitable flowers were selected from the inflorescences, and soaked in aceto-carmine for about 12 hr., then each flower was taken on the slide and pressed gently with cover glass slightly heating the whole preparation. By this rather simple method satisfactory results have been obtained. In addition to this, the usual paraffin method was also adopted, and in this case the staining procedure of crystal-violet-iodine

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by SMITH was applied.

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Meiotic condition

The maples fall into two classes with respect to their time of flowering, one blooming before the development of the leaves, and the other after they have begun to expand. Excepting a few of the exotic species, *e.g.*, *A. Negundo, A. saccharinum, etc.*, a majority of the maples commonly found in the region of Sapporo belongs to the latter group and blooms successively during the period from late March to late May. *A. japonicum* blooms during the middle of April, and is followed by *A. ornatum, A. mono, A. saccharum, A. diabolicum, etc.*

Without regard to the differences in time of flowering, these native trees show in general the same conditions of reduction division which takes place during the swelling of the bud. The tetrad stage is reached as the anthers appear between the scales at the tips, and by the time when the scales have opened, most of the anthers will contain pollen grains. From this condition in the species with relatively condensed inflorescences it might be expected that in any given bud the stages would be almost simultaneous, but contrary to this expectation it was found that there was regularly a great variation showing every condition from mature archesporium to young pollen grains in the same flower cluster, even in the lobes of a single anther. In the species with more complex inflorescences, as in *A. japonicum*, this variation in a flower bud in the time of development reaches the highest degree. It seems likely that the development of flower buds in a condensed inflorescence would be accompanied with very rapid meiosis, and this rapid progress of meiotic divisions seems to be responsible for meiotic abnormalities. It is already well known in many plants that the normal meiotic procedure is more or less disturbed through the effects of artificial changes of the environmental conditions, especially of those of temperature. If it is assumed therefore that the flower bud in developmental conditions of *A. japonicum* is very sensible to temperature changes (it is usually changeable in spring), the meiotic abnormalities in this species, at least some of them, will be explained as dependent upon the

external or environmental conditions, although it cannot be decided why the occurrence of various meiotic abnormalities in the PMCs is limited only to one species, *A. japonicum,* no abnormalities being found in other species of the same group or in close relatives of the genus.

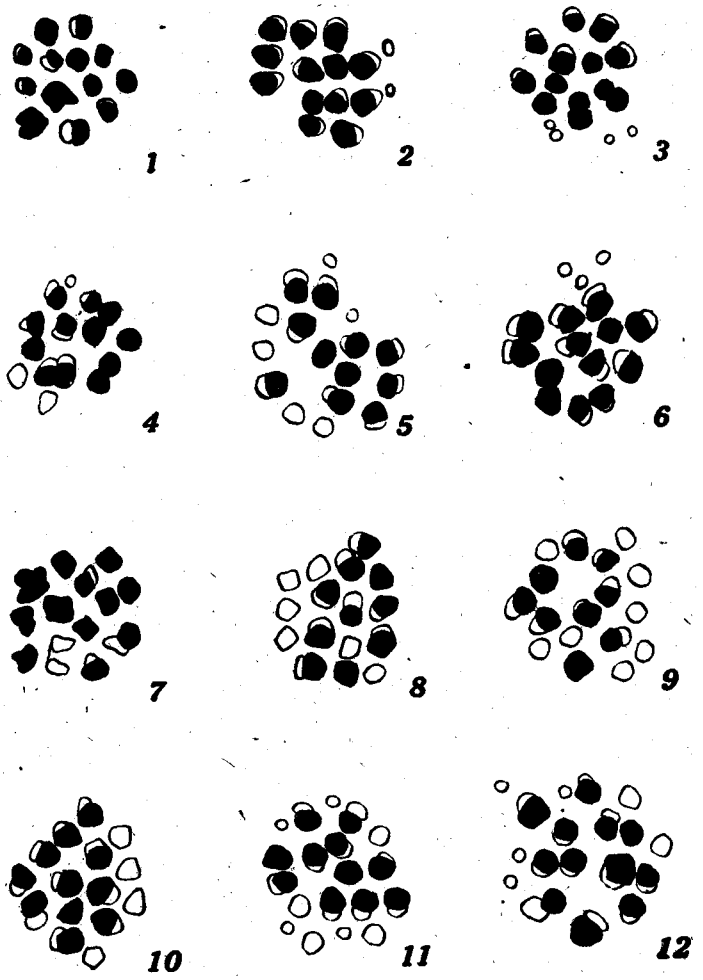
The meiosis in all the plants of *A. japonicum* studied has revealed the occurrence of a number of different types of abnormality, *i.e.,* supernumerary fragments, failure of pairing, multivalent association, irregular separation of chromosomes, syndiploidy, and polyspory. A comparison of the frequencies of abnormalities in the different individuals should shed some light on the underlying causes of them. Therefore, to see what light they throw upon the normal process of meiosis, the abnormalities in various kinds will be described in the following.

Supernumerary fragments

In normal PMCs 13 bivalents form the equatorial plate (fig. 1). The secondary association of bivalents is sometimes seen also in this species, as in other species of *Acer,* showing several groups of two or three bivalents (figs. 2–6). Although various types of secondary association were not analysed in this species, the degree of secondary association seems not to be so great as that observed in *A. ornatum* (*cf.* TAKIZAWA, '40), for the metaphase plates in *A. japonicum* occasionally show no obvious secondary association.

It is an interesting fact that abnormal PMCs containing several supernumerary fragments can be occasionally recognized (figs. 2–6, and 11–12). As given in Table 1, these PMCs with fragments were observed in different frequencies in four different individuals examined, and the number of fragments also varied from one to four in different cells of the same flower: the PMCs with one or two fragments were more frequent than those with three, and those with four were rare. Obviously the fragments in question are not univalents but constitute additional members of the metaphase plates (figs. 2–3, and 6). Where the univalents are found together with the fragments at metaphase, it is easily possible to distinguish them from each other owing to the extremely small size of fragments which is about one-fifth the normal bivalents, while the univalents are nearly the same in size as bivalents (figs. 4–5, and 11–12). The occurrence of these fragments is clearly ascertained also in side view metaphase without univalents (fig. 14).

During the metaphase some fragments appear to enter in the equatorial plate (fig. 14), as the univalents usually do, but the fragments usually remain in the cytoplasm (figs. 20 and 24). It was in majority of cases



Figs. 1–2. Polar view of the first metaphase, showing normal constitution with 13 bivalents (fig. 1), and abnormal constitutions with supernumerary fragments (figs. 2–6, 11 and 12), and with univalents varying in number (figs. 4–5, 7–11 and 12); in fig. 9, eight univalents as the maximum number observed are shown. All the temporary smear preparations: figs. 3 and 8, Plant No. 1; 2, 5, 7 ad 9, Plant No. 2; 10, Plant No. 3; 1, 4, 6, 11, and 12, Plant No. 4. × *ca.* 2400.

impossible to locate them in late anaphase stage or in second metaphase. Sometimes, however, they were observed to be included in the spindle region, having evidently separated after the other complements in PMCs reached to the poles (fig. 33). From these irregular behaviours the inclusion of fragments in the daughter nuclei at telophase seems to depend on

their original position during first metaphase. When they were located nearer to one pole, they may be included in one of the daughter nuclei. When they were situated in the equatorial plate, they may divide later than the other complements in PMC and each of the halves may be included in the daughter nuclei, if the division of them occurs during early anaphase, while if it occurs during late anaphase, after the chromosomes already moved to the poles, the chance of them being included in the telophase nuclei may be rare.

Such behaviours of fragments are expected to occur at second division, but it could not be stated with certainty, because the second division plates showed more frequently various anomalities as the results of other irregularities at first division, such as multivalent association, lagging chromosomes, *etc.* (*v. infra*).

The inclusion of supernumerary fragments in meiotic chromosome complement is not rare in plants, for many species with supernumerary fragments have now been found. However, when compared with the fragments found in other species, it can be pointed out that the present case is characterized by several peculiarities which seem to be very unique and are difficult to explain.

Table 1. Frequency of the abnormal PMCs containing supernumerary fragments (f)

	Chromosome association	No fragment	Cells with fragments					Total
			1f	2f	3f	4f	total	
Plant No. 1	normal(13_{II})	11	2	5	1	1	9	20
	others	15	—	—	—	—	0	15
	total	26 (74.29%)					9 (25.71%)	35
Plant No. 2	normal(13_{II})	18	5	11	6	1	23	41
	others	19	2	5	1	—	8	27
	total	37 (54.41%)					31 (45.59%)	68
Plant No. 3	normal(13_{II})	22	21	23	11	8	63	85
	others	27	3	6	1	1	11	38
	total	49 (39.84%)					74 (60.16%)	123
Plant No. 4	normal(13_{II})	30	22	29	3	5	64	94
	others	32	6	7	2	1	16	48
	total	62 (43.66%)					80 (56.34%)	142
	Total	174					194	368
	Average	47.28%					52.72%	

1) In the first place, it will be pointed out that PMCs with fragments occur in unexpectedly high and variable frequencies, as shown in Table 1, the percentage of PMCs with abnormal constitution, *i.e.*, the cells containing the fragments in addition to the full complement, is not uniform. Plant No. 3 has the highest percentage of these PMCs, *i.e.*, 60.16%, which is followed by Plant No. 4 and Plant No. 2 with 56.34% and 45.59%, respectively. In Plant No. 1, abnormal cells occurred in the lowest percentage, 25.71%.

It will be noticed here that the chromosome association, even if without regard to the fragments, is not regular due to the occurrence of multivalents and univalents, which have been occasionally recognized together with the fragments in the same PMC. With respect to the relation of the chromosome association to the occurrence of fragments, it was noted that the fragments were met with much more frequently in the cells with 13 bivalents than in those with other chromosome associations, and that in Plant No. 1 the fragments occurred only in the cells with normal 13 bivalents, while the cells with other chromosome associations had no fragment. However, there was nothing to indicate any correlation between various chromosome associations and the occurrence of fragments, so that the existence of fragments in *A. japonicum* may be said to be independent of the various chromosome associations. Thus, out of the total number of PMCs, 368 cells, from four different plants, 194 cells (52.72%) contained fragments without regard to the types of chromosome association. Such a high percentage of fragments constitutes one of the most striking characteristics of meiosis in this species.

2) Secondly, there is no numerical stability in the fragments of *A. japonicum,* since, as already described, some cells possess the fragments in various numbers ranging from one to four, while others do not contain any fragment. Such a numerical instability of fragments in this species seems to be unique, no such an instance having been known in other plants.

3) The supernumerary fragment chromosomes are in general believed to be small portions of chromosomes originated by fragmentation of duplicated chromosomes resulted from polyploidy or non-disjunction; it is then usually found that mitotic fragments correspond to meiotic ones, *e.g., Tradescantia virginiana* (DARLINGTON, '30; KOLLER, '32), *T. paludosa* (WHITAKER, '36), *Lilium Henryi* and *L. japonicum* (MATHER, '35), *Secale cereale* (DARLINGTON, '33; HASEGAWA, '34; TAKAGI, '35), *Arachis Rusterio* (HUSTED, '36), etc. Consequently, it must be determined whether mitotic fragments exist or not in *A. japonicum* too. In archesporial cells at pre-

meiotic divisions[1], the mitotic metaphase plates were examined, but the somatic chromosome number did not differ from the usual number ($2n=26$) found in this genus (*v.* fig. 13, in No. 1 of this series).

As a matter of fact, however, there is a doubt because the fragments are so small that they would have been concealed among the major chromosomes and failed to be found out, since in *Acer* the major chromosomes themselves are sometimes difficult to distinguish from each other due to their small size (*cf.* No. 1 of this series). Therefore, to ascertain the above statement that the somatic cells of *A. japonicum* have no additional chromosomes, it requires still further studies of mitotic division.

4) The additional meiotic fragments found in other plants show in several and different types to associate among themselves or with major chromosomes, or their association takes place only among themselves and never with major chromosomes, although in a proportion of cases the fragments fail to pair. This variability in pairing of fragments is generally interpreted on the basis of the assumption of the chiasmatype theory. Thus, the following characteristics of the fragments in one species, or at least in some definite individuals within a species, *i.e.*, the pairing property during meiosis, the constant and numerically stable occurrence, and their maintenance at mitosis, suggest that the fragments are not merely segmental fractions but small chromosomes provided with individuality, since they have obviously their spindle fibre attachments (*cf.* DARLINGTON, '37).

Very unlikely to the fragments in the above named examples, those in *A. japonicum* show no marked indication of pairing, which is the fourth peculiarity to be noted. They are usually found free from the other complements and from each other (figs. 2, 5, 11, 20 and 24), although where even numbered fragments (two or four) occur, sometimes there is a tendency to indicate that two of them lie next to each other (figs. 3, 6, 12 and 14).

5) Furthermore, we know that in some plants expermentally arisen by crossing between individuals with different chromosome sets, or in mutants havings spontaneously occurred, their meiotic chromosome complements happen to contain some fragments, *e.g.*, in trisomic tomato (LESLY and LESLY, '29), in triploid progeny of *Avena* (NISHIYAMA, '34), in triploid selfed of *Petunia* (MATSUDA, '35), in monosomic derivatives of *Nicotiana*

1) Unfortunately the writer failed to raise seedlings from this species, since the seeds, though they were not abundantly sown, did not germinate inspite of repeated undertakings during two years. It was also in practice impossible to obtain the root-tips containing actively dividing cells from grown large trees.

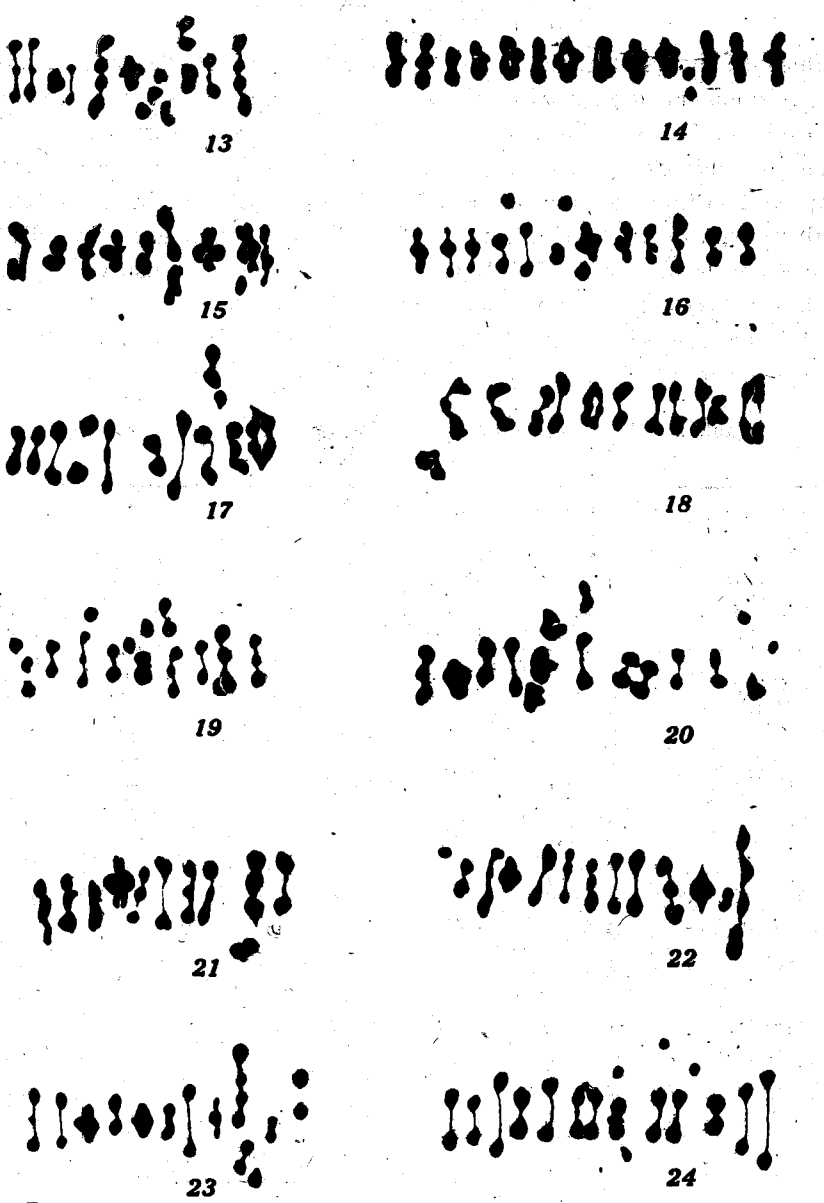
tabacum (OLMO, '36), in trisomics of *N. sylvestris* (GOODSPEED and AVERY, '39), *etc.* In these cases the fragments are usually constant in number, but rarely found to vary from cell to cell even in the same individual, as in *N. tabacum* coral haploid (LAMMERTS, '34), in the plants from irradiated rice seeds (PARTHASARATHY, '38). Evidently the chromosome constitutions themselves of these examples are in an unbalanced condition either due to lack of the complete set of chromosomes, or due to duplication of one or more chromosomes. It is then understandable that such numerical and structural hybridities play an important rôle for the occurrence of fragments.

However, this is not the case of *A. japonicum,* because the present material seems to be a balanced diploid species, although cytologically *Acer* as a whole has been inferred to be secondarily balanced diploid with the primary basic number of five (*cf.* No. 1 of this series), and because there is no evidence suspecting its hybrid or mutant origin. Although *A. japonicum* contains many varieties or sub-species characterized by various morphological differences in leaf shape and colouring, its variety *typicum* here investigated is constant in its external morphology. Therefore, it may be said that the occurrence of fragments in *A. japonicum* is due to some causes different from hybridization in broad sense.

Various chromosome associations

As a rule 13 bivalents are formed at first metaphase in all the plants examined, but occasionally multivalents are found. In the polar views of metaphase plate, bivalents often show secondary association in varying degrees, so that it is difficult to distinguish whether the groups of chromosomes observed are due to true multivalent association or to close secondary association. In favourable side views, however, it is possible to identify real multivalent association.

The multivalents commonly found were quadrivalents (figs. 13, 18–21, and 23–24); but rarely the occurrence of trivalents together with quadrivalents was met with (figs. 15, 17 and 22). Multivalent associations found in *A. japonicum* are characterized by their number and type; *i.e.,* first, there are no more than two quadrivalents, and no other associations higher than quadrivalents per cell; secondly, the chromosomes constituting multivalents are found to associate in varying forms, although they nearly always form the end-to-end connexion, *viz.,* four chromosomes in ring or chain giving quadrivalents, and three in chain giving trivalents. Univalents, as many as eight, have been observed at first metaphase, together



Figs. 13–14. Side views of the first metaphase or early anaphase, showing various chromosome conjugations consisting of uni-, bi-, tri-, and quadrivalents. These various chromosome associations (*q.v.* Table 2) drawn separately in the analysis of selected observations of side views, in which a fair degree of certainty was attained. Note the fragments occupying a random position to the equatorial plate (figs. **14**, **20** and **24**): one or two bivalents being occasionally non-orientated to the equatorial plate (figs. **13**, **15**, **17–21** and **23**). Figs. **13**, **16**, **19** and **23–24** from the permanent preparations (aceto-alcohol: crystal-violet), others from the temporary smear preparations (aceto-alcohol: aceto-carmine): Figs. **15**, **17**, and **22–23**, Plant No. 1; **14** and **18–19**, Plant No. 2; **13**, **21**, and **24**, Plant No. 3; **16** and **20**, Plant No. 4. × *ca.* 2400.

with multivalents or without them (figs. 13, 15, 17, 19–20, and 22–23).

A summary of the various chromosome configurations obtained is given in Table 2. There is nothing to say on the correlation between the chromosome associations and the fragments in question in their causal relationship, and so the grouping of the PMCs in this table has been undertaken without regards to the fragments. The PMCs observed were thus divided into three groups; viz., (1) PMCs with 13 bivalents normally; (2) those with both bivalents and univalents varying in their numbers; and (3) those containing one or two multivalents, correspondingly less bivalents, and univalents in addition also varying in number.

Table 2. Frequency of PMCs with various chromosome associations at first metaphase.

Chromosome association	Plant No. 1 n	Plant No. 1 f	Plant No. 2 n	Plant No. 2 f	Plant No. 3 n	Plant No. 3 f	Plant No. 4 n	Plant No. 4 f	Total
13_{II}	11	9	18	23	22	63	30	64	
total	20 (57.14%)		41 (60.29%)		85 (69.11%)		94 (66.20%)		240 65.23%
$12_{II}+2_{I}$	3	0	8	0	14	7	20	5	
$11_{II}+4_{I}$	1	0	3	1	3	0	7	1	(fig. 16)
$10_{II}+6_{I}$	1	0	0	2	1	0	4	0	
$9_{II}+8_{I}$	1	0	1	0	—	—	1	0	
total	6 (17.14%)		15 (22.06%)		25 (20.33%)		38 (26.76%)		84 22.83%
$1_{IV}+11_{II}$	2	0	3	2	3	1	0	3	(figs. 18 & 24)
$1_{IV}+10_{II}+2_{I}$	1	0	2	0	—	—	0	3	(fig. 20)
$1_{IV}+9_{II}+4_{I}$	1	0	—	—	0	1	0	2	(fig. 23)
$1_{IV}+8_{II}+6_{I}$	—	—	2	1	1	1	0	1	(fig. 19)
$2_{IV}+9_{II}$	—	—	0	2	1	0	0	1	(fig. 21)
$2_{IV}+7_{II}+4_{I}$	—	—			2	0			(fig. 13)
$1_{IV}+1_{III}+5_{II}+1_{I}$	3	0			2	1			(fig. 22)
$1_{IV}+1_{III}+8_{II}+3_{I}$	1	0							(fig. 17)
$2_{IV}+1_{III}+7_{II}+1_{I}$	1	0							(fig. 15)
total	9 (25.71%)		12 (17.65%)		13 (10.57%)		10 (7.04%)		44 11.96%
Total	35		68		123		142		368

N.B. n = PMC without fragment; f = PMC with supernumerary fragments varying from one to four (*q.v.* Table 1).

1) The percentage of the cells belonging to the first group may be taken as a measure of the regularity of meiosis in each plant. Plant No. 3,

in which the normal PMCs are in 69.11%, appears to have the most regular meiosis, and it is closely followed by Plant No. 4 (66.20%). The remaining two plants, No. 2 and No. 1, show somewhat less regularity, having 60.29% and 57.14%, respectively.

2) The cells in the second group, which contain univalents varying from two to eight, could be easily counted in polar view of metaphase, and their frequencies in all the plants examined were similar. Thus the average frequency of this group (22.83%) is not so variable as that of the third group. This rather regular occurrence of univalents could be further confirmed at anaphase, and is significant to its origin (*v. infra*).

3) It must be taken into consideration that the frequency of the cells belonging to the third group will not be apt to show the real frequency of them. Because, only in side views of metaphase the true multivalent association was possible to be identified exactly, and therefore the observations were necessarily subjected to considerable limitation. This may be the reason why the frequency of these cells is so variable and different in each individual ranging from 25.71% to 7.04% (11.96% in average).

Though the frequency of PMCs with multivalents is variable and low in every plant examined, its general occurrence in *A. japonicum* seems to be noteworthy in connexion with the view that the apparent basic number of 13 in *Acer* will be of a secondary balanced nature (*cf.* No. 1 of this series), which may now be evidently supported by this variability of chromosome conjugation, for it indicates by itself the feature of polyploidy in *Acer*. Similar situation of various chromosome associations in "diploids" has been found in the studies on the chromosome constitution of the *Pomoideae*, including apples, peaches, and pears, *Pyrus*, which has the apparent basic number of 17 which should be regarded as the unequal reduplication of the primary number being seven (*cf.* DARLINGTON and MOFFETT, '30; MOFFETT, '31; HEILBORN, '35).

It is also interesing to notice that the differences in the amount of secondary association and of the multivalent formation are found between two closely related species, *A. japonicum* and *A. ornatum*: first, the degree of secondary association appears to be less evident in *A. japonicum* than in *A. ornatum*, such as has been demonstrated to be less pronounced in pears than in apples (MOFFETT, '34); secondly, the general occurence of multivalents in *A. japonicum* is remarkably contrasted to the absence of them in *A. ornatum*, just as the difference between *Prunus domestica* and *P. institia*, though these are both not diploids but hexaploids (MATHER, 37).

Irregular anaphase separation

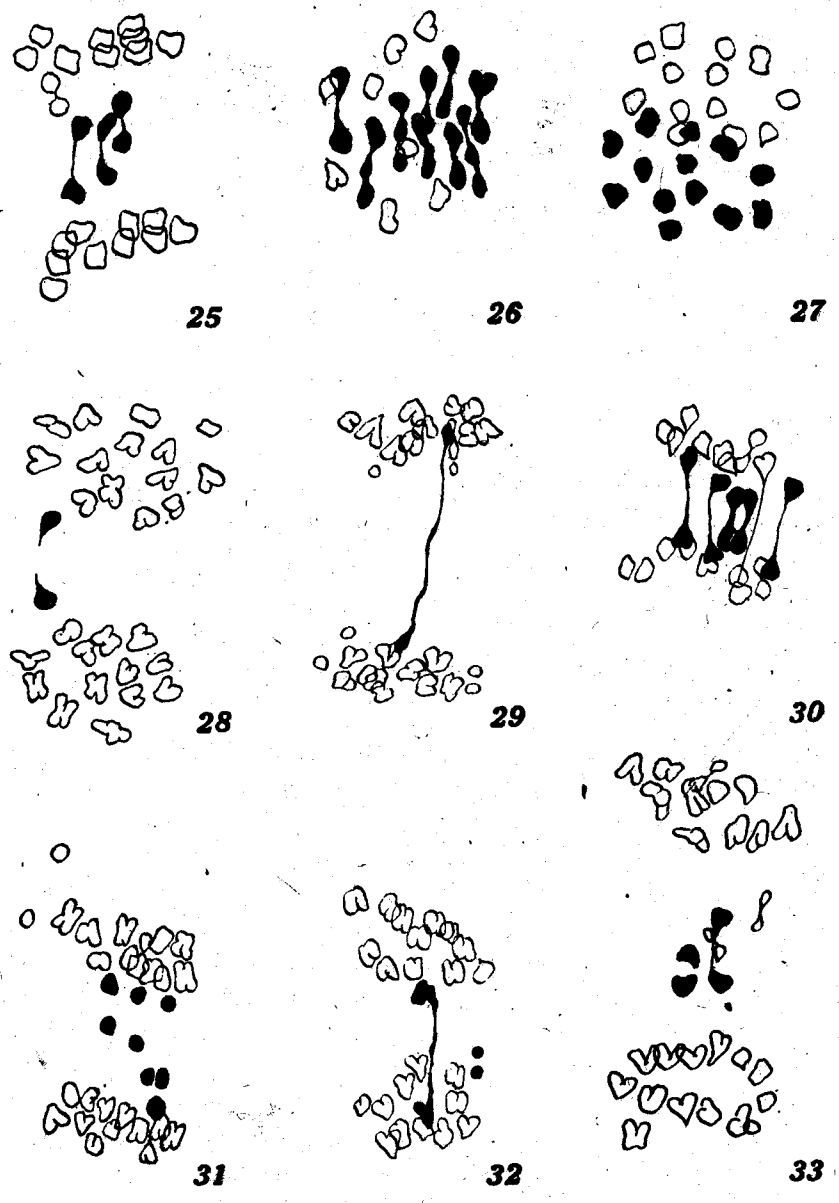
As the consequence of various chromosome associations found at first metaphase, there occur various irregularities in anaphase chromosome separation. Three kinds of irregularity were actually met with: (1) lagging univalents, (2) lagging bivalents, and (3) chromatid bridges.

1) As many as five univalents have been observed lagging to divide on the equatorial plate after the bivalents separated to the poles, and in general the dividing halves of them appeared to reach to the poles and to be included in the daughter nuclei (figs. 28 and 33). Sometimes, however, both of half-univalents move towards one pole (fig. 31), suggesting that the division and inclusion of them in daughter nuclei depend upon their position during first metaphase, in which univalents were occasionally situated at random out of the equatorial plate (figs. 13, 16, 19 and 23). At the second anaphase some chromosomes also lag between the chromosome groups (figs. 37–38, and 41), or lie off in the cytoplasm (fig. 37) after the others passed to the poles. The majority of these laggards at the second division are presumably the halves of univalents which had split at first division, but sometimes the univalents themselves which were undivided and included in one of the nuclei at first division.

The frequency of PMCs with lagging univalents at first anaphase is given in Table 3. The results are parallel to the frequency of univalents occurring at first metaphase (*v.* Table 2), excepting that one individual, Plant No. 2, showed a very high frequency of lagging univalents (over 60%) which does not coincide with the frequency of univalents observed at first metaphase. This exceptional case, however, is assumed to be due to some special conditions to which this material was brought at the time of observations, and not to represent the real difference of this plant from others.

2) It will be noticed that at first anaphase not only univalents but some bivalents, as many as four in one PMC, also lag in dividing (fig. 25), though their frequency is extremely low in all the plants examined, *i.e.*, the highest percentage of the cells with lagging bivalents is only 2.25% in Plant No. 4.

The lagging bivalents are, however, significant in relation to the origin of anomalous anaphase divisions, for in majority of cases they will reach to the poles in time to be included in the daughter nuclei, but in some cases they failed inclusion in the daughter nuclei and remained in the middle of the cell (fig. 7 in Pl. I), then through the very short interkinesis



Figs. 25–33. Anaphase separation of the first division. **27**: Normal separation with 13 chromosomes in each chromosome group at early anaphase. **26** and **30**: Side views of early anaphase, showing the numerically normal but non-simultaneous disjunction of bivalents. **25**: Late anaphase with three lagging and dividing bivalents in centre. **28** and **31**: Anaphases with one and four univalents lagging to divide, respectively. **29**: Chromatid bridge without fragment at late anaphase. **32**: Anaphase with a chromatid bridge and two fragments. **33**: Two univalents lagging and dividing in centre, and in addition two fragments also lagging in centre to divide. All figs. from the aceto-carmine preparations: figs. **25**, **27** and **30** Plant No. 1; **26**, **29** and **31–32**; Plant No. 2; **28** and **33**, Plant No. 4. × *ca.* 2400.

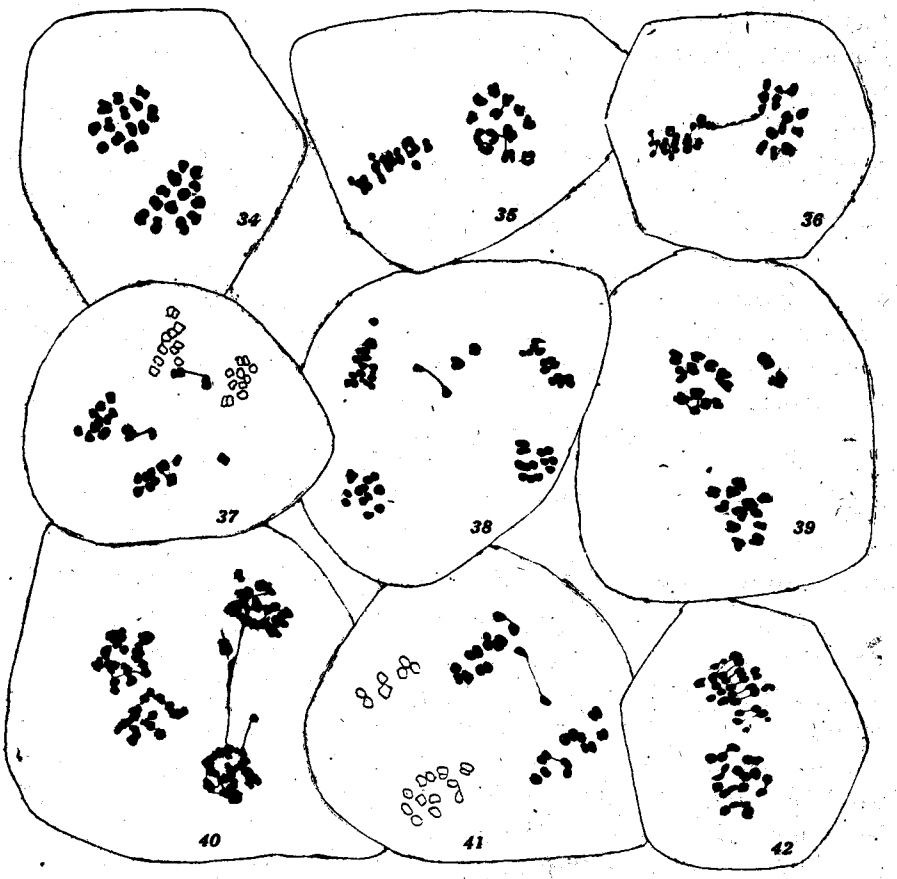
Table 3. Frequency of PMCs with lagging univalents (I), lagging bivalents (II), and chromatid bridges (b) at first anaphase.

	Normal separation	1_I 2_I 3_I 4_I 5_I	1_{II} 2_{II} 3_{II} 4_{II}	b b+f*	Total
Plant No. 1 (7-flowers) total	267 75.21%	40 21 10 2 — 73 205.6%	2 1 1 1 5 1.41%	7 3 10 2.82%	355
Plant No. 2 (3-flowers) total	38 32.48%	27 16 13 4 2 72 61.54%	1 1 — — 2 1.71%	3 2 2 4.27%	117
Plant No. 3 (5-flowers) total	136 74.73%	16 11 6 4 — 37 20.33%	2 1 — — 3 1.65%	5 1 6 3.30%	182
Plant No. 4 (6-flowers) total	127 71.35%	14 16 5 3 1 39 21.91%	2 1 1 — 4 2.25%	5 3 8 4.49%	178
Total	568	221	14	29	832

* The case that both fragments and chromatid bridges are found between two anaphase chromosome groups as in fig. 32.

they presumably resulted to the bridges at second metaphase between two equatorial plates (fig. 30; and fig. 2 in Pl. I). It must be here emphasized that in a large proportion of cells even in the normal PMCs, the end of metaphase or the beginning of anaphase is extremely marked by the division of chromosomes which is *not simultaneous*. As has been found in figures 26 and 30, the anaphase separation has begun for some bivalents but not for all. Therefore, when such a nonsimultaneous chromosome separation takes place in the extreme degree during metaphase-anaphase, it results that some bivalents come late in dividing and lag on the centre. Hence it may be said that the lagging bivalents are at least partly due to the failure of synchronisation in the time of division of the chromosomes.

3) More less frequently than the lagging chromosomes, chromatid bridges were found at both first and second anaphases. As it is given also in Table 3, the frequency of PMCs with them at first anaphase varies from 2.82% to 4.49% in the four plants examined. It is a remarkable fact that the chromatid bridges found in the present study were in the majority of cases without their accompanying fragments (fig. 29, and fig. 3 in Pl. I), or at least these passive fragments (acentric chromatids) could not be recognized with certainty. Where the fragments actually occurred



Figs. 34–42. Metaphase and anaphase of the second division. **34**: Normal second metaphase. **35** and **42**: Metaphases showing irregular distribution of chromosomes in two chromosome groups. **39**: Metaphase with two non-incorporated chromosomes into metaphase groups. **36**: A bridge formed between two metaphase chromosome groups. **38** and **41**: Anaphases with three and one chromosomes lagging in centre of two anaphase chromosome groups on one side, respectively. **37**: Anaphase, showing both plates of two anaphase chromosome groups each with a lagging chromosome in centre, and one chromosome lying off in the cytoplasm. **40**: Late anaphase showing a chromatid bridge formed between two anaphase chromosome groups on one side. All aceto-carmine preparations: figs. **37–39** and **41**, Plant No. 1; **36** and **42**, Plant No. 2; **34** and **35**, Plant No. 3; **40**, Plant No. 4. × *ca.* 2400.

at anaphase (fig. 32), it could not be clearly ascertained in this species whether they are real passive fragments originating from the breaking of chromatids during metaphase-anaphase, or whether they are the supernumerary fragments above mentioned being included at random into the spindle region of anaphase. This fact is at variance with the assumption

that bridges are due to the occurrence of chiasmata between two dislocated chromatids (*cf.* DARLINGTON, '37).

Irregularities of the first anaphase separation result also to the variabilities of the total number of chromosomes of two second metaphases: some PMCs gave the accounts of less than 26 chromosomes (fig. 35 and 36), and some others those of more than 26 (figs. 39 and 42), although their detection was frequently difficult partly due to the earlier splitting of some half-bivalents into two chromatids, and partly due to the persistent grouping of associated chromosomes which may be a continuance of the first division relationships in secondary association. Such variabilities of total chromosome number of two second metaphases are explicable as due to that some multivalents resulted to a certain extent to the non-disjunction, or due to that some univalents isolated in the cytoplasm at first division did not be incorporated in the telophase nuclei, or due to that some univalents have divided twice.

Binucleate PMC and the abnormal sporad[1] formation

The occurrence of giant PMCs with two nuclei may originally differ from those of chromosomal irregularities above described, because the binucleate PMCs could be detected throughout the whole meiotic division regardless to the other abnormalities found, and the frequency of them, as it is given in Table 4, showed to be similar in the four plants examined with the slight differences varying from 4.8.% to 6.41%. The distinction between normal and giant PMCs was easily possible due to the latter being about twice in volume (fig. 43), although in no case it was possible to determine the exact number of chromosomes in these cells. These giant cells are undoubtedly due to two diploid nuclei having been fused either completely or incompletely. When they are fused completely, it results in a tetraploid nucleus and when incompletely the two nuclei may be sometimes subject to independent division. These syndiploid PMCs should necessarily reveal more irregular chromosome behaviours than those in

1) The term *tetrad* has been widely adopted to designate the groups of four cells formed at the tetrad stage. However, where these groups do not always consist of four cells but vary in their number of cells, *viz.* monads, diads, triads, tetrads, *etc.*, the term *sporad* seems more appropriate to use for these varying groups including the tetrads too, in order to avoid its terminological confusion. This term has been proposed by WEBBER ('33), and later supported by IVANOV ('38) and TOMETORP ('39). In agreement with them, the writer uses this term *sporad* for the groups of cells at tetrad stage limiting the term *tetrad* only to the sporad which consists of four cells.

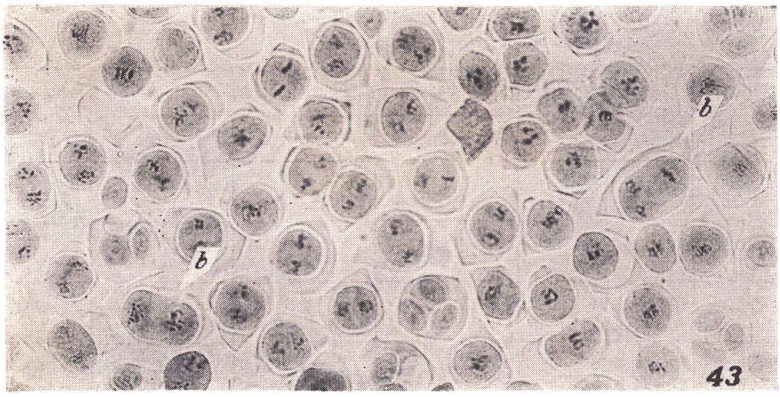
Table 4. Frequency of normal and binucleate PMCs in summing of 5-flowers in each plant.

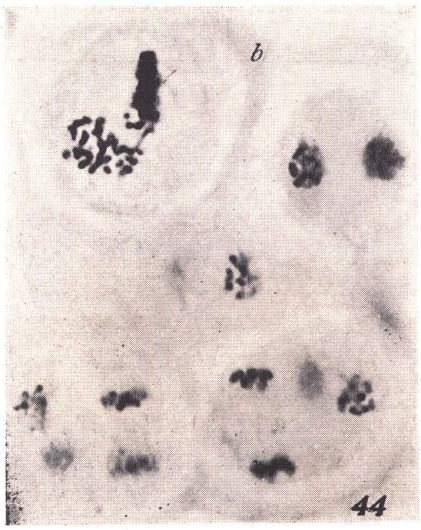
	Single nucleus	Binucleus			Total
		Incomplete	Complete	total	
Plant No. 1	291 (94.17%)	14	4	18 (5.83%)	309
Plant No. 2	295 (95.16%)	11	4	15 (4.84%)	310
Plant No. 3	311 (95.11%)	9	7	16 (4.89%)	327
Plant No. 4	336 (93.59%)	15	8	23 (6.41%)	359
Total Average	1233 94.48%			72 (5.52%)	1305

normals, owing to interferences caused by the presence of two spindles in a single cell. Evidently in these cells the disjunction of bivalents at first anaphase was found to be imperfect.

Where a syndiploid PMC in which two nuclei were completely fused has undergone the first division (fig. 46), we have two second metaphase plates each with the unreduced number of 26 (fig. 44). Where a partially fused syndiploid PMC has independently formed two first anaphase groups giving an appearence of a second anaphase in normal ones (fig. 45), the second anaphase will consist of eight chromosome groups each with the normally reduced number, 13, instead of four in normals. If in one of these two first division groups the division has been suppressed, at second anaphase there are 13 chromosomes in either group of the one side, and 26 chromosomes in another group of the other side (fig. 47), indicating the formation of a hexad, which will contain four microspores each with the normally reduced chromosome number and the remaining two each with the unreduced number. In addition to these comparatively simple types of division in the syndiploid PMCs, many other cases with more complexity have been evidently found, in which their configurations are too irregular to be said about with certainty.

The existence of such binucleate PMCs is of great significance in regard to the production of gametes with the unreduced or even aneuploid number of chromosomes. The two meiotic abnormalities, *viz.* the nullification or suppression of either meiotic divisions and the irregular disjunction and distribution of chromosomes, occurred in binucleate as well as single



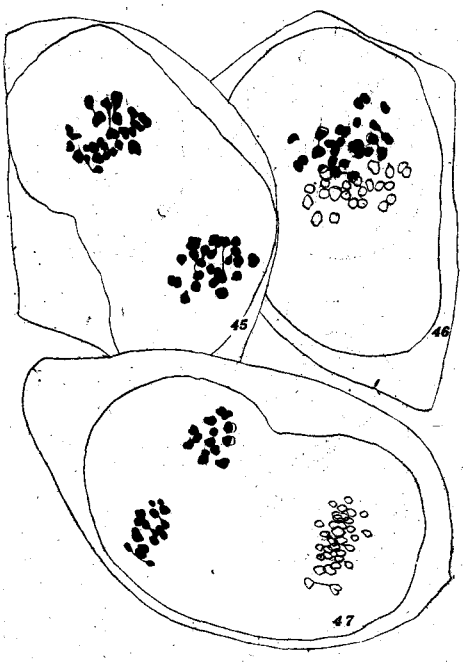


Figs. 43–44. **43**: Illustrating difference of cell size between the binucleate PMCs (two cells marked '*b*') and the normal ones. Note the meiotic stages found in normal size PMCs varying from late diakinesis to tetrad stage. **44**: One binucleate PMC marked '*b*' at second metaphase, in which approximately tetraploid number of chromosomes (26) and a chromatid bridge between two metaphase plates are shown. Note the second anaphase in the other normal diploid cells. Fig. **43** from the aceto-carmine preparation of a flower in Plant No. 3; ×*ca.* 500: **44** from the permanent preparation of Plant No. 2 ×*ca.* 1450.

nucleate PMCs, together with this occurrence of binucleate PMCs itself, may thus be three causes in the present species leading to the formation of abnormal sporads of varying size and number which will be, in corresponding frequencies to those of meiotic abnormalities, formed at tetrad stage, instead of the normal tetrads of equal size.

Evidently the normal tetrads have most frequently occurred, but the number of cells in a sporad was occasionally greater or smaller than four; *i.e.*, frequently large tetrads, octads, and hexads, rarely diads, triads, and pentads, and no monads and septads have been found. In these abnormal sporads, microspores and microcytes of varying size and number are formed occurring usually in pairs, but there is no sharp line of demarcation be-

tween a large microcyte and a small microspore. Therefore, by the account of sporads somewhat arbitrary groupings were made, without regard to the size differences between the microspores and microcytes but only by considerring the total number of them in a sporad. The sporads in figure 48 illustrate this variation which extends from diads to octads. Table 5 shows the different classes which were found in the four plants examined. Two kinds of tetrads are of interest in relation to the occurrence of syndiploid PMCs: one is the normal small tetrad with four equal sized microspores and is present in the majority of cases; the other is the large tetrad which is about twice in volume. Where the meiosis in binucleate PMCs takes place in normal manner, the results may give rise to the formation of octads, in which eight microspores must contain the 13 chromosemes in normal reduced number. Therefore, where in these binucleate PMCs one of the divisions has been prevented, large tetrads, each nucleus being tetraploid, or approximately so, may have resulted.



Figs. 45–47. Three giant syndiploid PMCs. **46**: Binucleate cell showing the complete fusion of two diploid nuclei, in which therefore the number and behaviour of chromosomes are as it were of a tetraploid PMC. **45** and **47**: Binucleate cells showing the incomplete fusion of two nuclei, in which therefore the division of two chromosome groups has more or less independently occurred side by side in a single cell. These two cells are the same as a left-lower cell and a right-upper cell (both marked '*b*') in fig. 43, respectively. All acetocarmine preparations of Plant No. 3, × *ca.* 2400.

It will be seen from Table 5 that the frequency of abnormal sporads seems to indicate that those have chiefly resulted from the binucleate PMCs, but occasionally the formation of them depended on the laggards scattered at random in the cytoplasm, and also on the bridges which prevented the anaphase separation. This is evidenced by the fact that the abnormal sporads occur always more frequently than the binucleate PMCs: *viz.*, Plant No. 1 has the highest percentage of the abnormal sporads, 10.91%, while the highest frequency of binucleate PMCs in Plant No. 4 is 6.41%. It

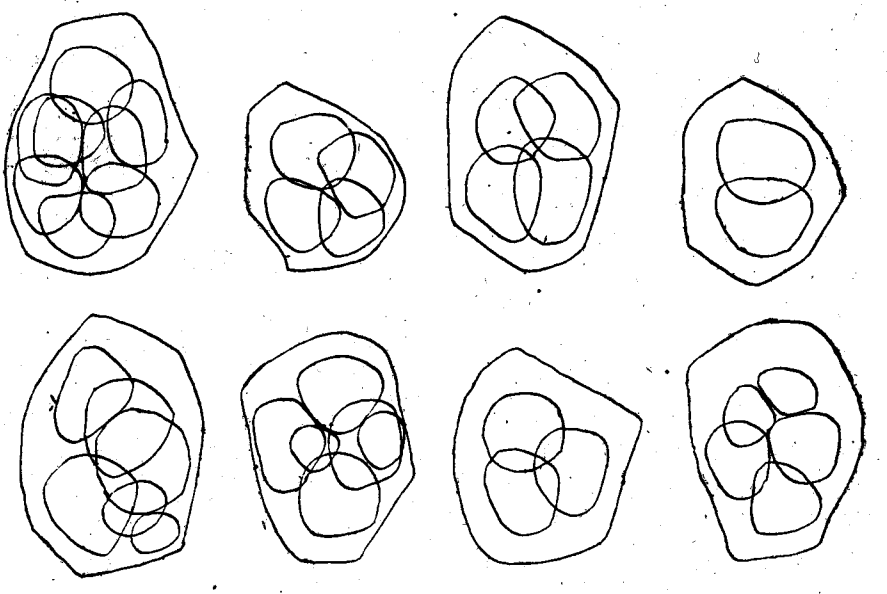


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Fig. 48. Tetrad stage of PMCs showing the various sporad formation. These sporads were obtained in selection from one slide (aceto-carmine preparation) of a flower in Plant No. 3. × *ca.* 1200.

Table 5. Frequency of the various sporads found at tetrad stage.

	Normal tetrad	Diad	Triad	Large tetrad	Pentad	Hexad	Octad	Total
Plant No. 1 (2-flowers)		0	0	6	1	4	5	
total	141			16				157
	89.81%			10.91%				
Plant No. 2 (2-flowers)		2	0	5	1	3	4	
total	179			15				194
	92.27%			7.73%				
Plant No. 3 (5-flowers)		0	2	30	2	6	27	
total	686			67				753
	91.10%			8.90%				
Plant No. 4 (2-flowers)		1	0	2	0	3	3	
total	122			9				131
	93.13%			6.87%				
		3	2	43	4	16	39	
				Total no. of abnormal sporads				
Total	1128			107				1235
Average	91.34%			8.64%				

proves further that the percentages of the abnormal sporads correspond, within the degree of possible divergence, to the percentages of binucleate PMCs plus those of cells with lagging bivalents and chromatid bridges (*v.* Tables 3, 4, and 5): *i.e.*, 10.91, 7.73, 8.90, and 6.87 percentages of abnormal sporads are roughly comparable with 10.06, 10.82, 9.84, and 13.15 percentages in the sum of binucleate PMCs and cells with lagging bivalents and bridges, respectively. The discrepancies are very likely due to the facts that some lagging bivalents become regularly included in the telophase nuclei and are not concerned with the formation of abnormal sporads. From this comparison of the frequency of abnormal sporads with that of binucleate PMCs, we can safely assume that almost all the abnormal sporads result from binucleate PMCs, but sometimes are due to the laggards and the chromatid bridges in the normal PMCs as well as in the binucleate ones.

Pollen abortion may be expected in corresponding frequency to the percentages of the abnormal sporads. However, unexpectedly apparent good-pollen grains were found in nearly 100 percentage in any given ripe anther. However, lacking the estimation of germination power of these morphologically normal and viable grains, it is very difficult to determine only from the cytoplasmic contents whether pollen grains are functional or non-functional.

Interpretation of abnormalities

We see now that certain prior causes may affect the normal course of meiosis, giving rise to so many abnormalities independent from each other, or resulting together into a consequence, therefore certain different consequences can frequently follow the same antecedent, or *vice versa*. Thus the effects of the prior causes leading the meiotic abnormalities cannot be accurately determined by the mere observation of meiosis. Only after a careful study of more different species and their progenies collected from many different conditions of environment, any of the underlying causes responsible for the abnormal meiotic conditions may be definitely confirmed. Evidences obtained in the present observation are far away from such a completeness, however it may not be impossible to suggest at least immediately causal relationships of the meiotic abnormalities to the normal course of meiosis, and in this connexion the abnormalities here observed will be enlightening. As an attempt to consider what matter may be concerned with meiotic abnormalities of *A. japonicum*, it will be possible to include them into the following three heads:—

(1) Spontaneous disturbance of regular mitosis at the pre-meiotic division.

(2) Immediate actions of the occurrence of multivalents and of binucleate PMCs upon the meiotic division.

(3) Failure of synchronisation of chromosome behaviour in the time of division, with the co-operating effects of rapid progress of meiotic division.

The numerically irregular inclusion of fragments in addition to the normal constitution of 13 bivalents in this species is an unexpected and therefore most interesing feature which is difficult to explain. Their behaviours observed indicate that the following peculiarities can be recognized in comparison with the meiotic fragments found in other species; *viz.*, first, the variable frequencies of PMCs containing the fragments in the four plants examined; secondly, the numerical instability in the same plant; thirdly, the normal mitotic constitution with 26 chromosomes at premeiotic division in archesporial cells, which may lead to the assumable regularity of the mitotic constitution in the other somatic tissues of this species; fourthly, the unpaired quality during meiosis that the fragments in question do not associate among themselves nor with the major chromosomes.

However, presumably the occurrence of these fragments must be due to some disturbances at the pre-meiotic divisions giving rise to non-disjunction and fracture of one or more chromosomes. Of course, the interpretation of such a disturbance at the pre-meiotic mitosis is at present a matter for conjecture. However, if it was true in *A. japonicum*, the fragments should have been formed in some archesporial cells due to segmental fracture of one or more duplicated mitotic chromosomes, and should be transmitted to the PMCs in such cases. Thus the results might reach to that no plants of *A. japonicum* possessing fragments regular and constant in number have yet been observed.

The supernumerary fragments of this species, on the other hand, would not be transmitted to the offspring, since they are likely to be disintegrated into the cytoplasm during meiosis. Therefore probably the apparent good-pollen found in this species do not contain the fragments, although the abnormal chromosome constitutions in pollen grains can be expected from the various chromosome associations other than the bivalents.

There is still another possibility to infer the origin of the fragments. They would be not the chromosomal fragments but the so-called persisting nucleoli during meiosis, as in *Fritillaria* (FRANKEL, '37), in *Oenothera* (PATHAK, '40), in *Brassica* (SIKKA, '40), in which some nucleoli being in-

constant in number and size from cell to cell, and therefore recognizably fiddrent from the chromosomes, have remained persistent even until at first telophase two daughter nuclei are re-formed. If it was also the case in *A. japonicum*, the fragments found would have been more frequent in number and more various in size than those known in the present observation. This is, however, not the only satisfactory datum to deny the occurrence of persisting nucleoli in *A. japonicum*. On the contrary, regardless of the comparatively less number and the nearly always constant size of the fragments of *A. japonicum* in comparison with those of the persisting nucleoli found in the examples above named, it would rather tend to support that the fragments of *A. japonicum* might be persisting nucleoli. In order to determine if any of these two interpretations is reasonable, no satisfactory data could be yet obtained.

Cytological observations show that the genus *Acer* can be inferred as a group of secondary polyploids with the apparent basic number of 13 resulting from the unbalanced multiplications of the primary basic set of five (*cf.* No. 1 of this series).

The chromosome conjugations other than the bivalents are always found as a common meiotic feature of polyploids, therefore each valency per cell calculated (Table 6) of the chromosome conjugations varying from univalents to quadrivalents found in meiosis of *A. japonicum* is very significant. This table shows that the number of bivalents per cell being over 12 indicates the almost regular meiosis in each plant; the multivalents are

Table 6. Total (t) and mean (m) numbers of various chromosome conjugations at first metaphase.

	Univalent	Bivalent	Trivalent	Quadrivalent	δ_{II}*	Total no. of PMCs
Plant No. 1 t	37	409	5	10	1.314±0.246	35
Plant No. 1 m	1.057	11.686	0.143	0.286		
Plant No. 2 t	74	819	—	14	0.956±0.179	68
Plant No. 2 m	1.088	12.044	—	0.206		
Plant No. 3 t	87	1519	3	16	0.651±0.122	123
Plant No. 3 m	0.707	12.349	0.024	0.130		
Plant No. 4 t	134	1757	—	11	0.627±0.117	142
Plant No. 4 m	0.944	12.373	—	0.077		

* Deviation of mean bivalent has been calculated from 13_{II} occurring normally in a PMC.

in rare occurrence, *viz.*, in the highest quadrivalency Plant No. 1 show only *ca.* 0.3 quadrivalents and in Plants No. 2 and No. 4 no trivalents are found; and the univalency is also very low, but it is interesting that the number of univalents per cell is rather regular in every plant, containing about one univalent per cell. Consequently, the rare but rather general occurrence of quadrivalents and the somewhat more frequent and regular occurrence of univalents in meiosis of *A. japonicum* appear to be a characteristic of polyploid species, and thus it seems to be a striking evidence to the secondary polyploidy of the genus *Acer* as a whole.

In regard to the variable chromosome conjugations in the secondarily balanced diploid species, however, there exist very complicated situations: first, the occurrence of univalents can be also concluded as the result of the spontaneous failure of pairing between partners due to effects of the various changes of external conditions (*v. infra*); secondly, although the unbalanced multiplications of a basic set of chromosomes in the secondary polyploids mean an evolutionary step of species formation, so that the multivalent associations found may originally differ from those found in the hybrids recently occured, yet the same results would have been brought about in meiosis due to structural hybridity; thirdly, the secondary polyploidy in the genus *Acer* can be supposed as the trebly hexasomic tetraploidy, so the multivalents are able to expect, in the highest number, three sexivalents or five quadrivalents, if the duplicated chromosomes are completely homologous; fourthly, why the multivalent associations have been found only in *A. japonicum* and not in others, even in the closely related species as *A. ornatum?* There is no fulfilled interpretation to solve such complicated questions.

It is well known that the effects of hybridization consist in reducing the chromosome pairing in many hybrids and in their progenies, and also that the actions of genetic factors have defenite influence upon the pairing in the so-called asynaptic strains. These two causes, however, seem hardly probable to be supposed as to *A. japonicum*, or at least cannot be settled without studying on a large scale. The occurrence of univalents in *A. japonicum* that is unstable in various degrees from cell to cell in the same individual, but rather general and regular in all the plants examined, seems to indicate that it is due to different causes from the effects of hybridization, and also that it is more far-reaching than the actions of certain genes.

We have now many instances that the experimentally changed external conditions result to replace the normal course of meiotic division by the failure of pairing (*q.v.* the recent findlings obtained by OEHLKERS and

his colleagues). In these experiments, the temperature treatments have significantly reached to the most clear and definite conclusions. Through the effects of abnormally high temperature, an acceleration of chromosome behaviour in meiosis is definitely demonstrated in *Trillium kamtschaticum* (MATSUURA, '37), as evidenced by the univalent occurrence at metaphase instead of bivalent formation in normals, and by the occurrence of premature splitting of chromosomes. MATSUURA thus comes to the following conclusions: *i.e.,* both the kinetochores and the rest of the chromonemata came to accelerate their behaviour in meiosis through the effects of high temperature, therefore when this acceleration is unbalanced, the various meiotic abnormalities thus resulting are certainly due to the upsets in timing relationships between the behaviour of the kinetochores and that of the chromonema proper, thus the abnormalities are explicable as the results of various "discordant" behaviours in both the parts of chromosomes. And further "the occurrence of non-disjunction or lagging of certain bivalents which is frequently met with in literature, would be (also) explained by assuming the non-uniformity in time co-ordination of the two component parts of the chromosome in individual bivalents within the same cell." (*loc. cit.,* p. 29).

It is therefore possible to suggest in *A. japonicum* that the asynchronisation of bivalent-disjunction which is definitely concerned with the rapidity of the whole meiotic progress may have resulted from the failure of time co-ordination of the behaviours of the kinetochores and of the rest of the chromonemata in each bivalent within a PMC, in which, however, such a discordance may occur to be unequal from chromosome to chromosome, for it results to the occurrences of several univalents at metaphase as well as bivalents lag in dividing at anaphase. This characteristic symptom, *viz.* the asynchronisation of bivalent-disjunction does not always be found only in *A. japonicum* but also in other maples, and thus its localisation is evidently not the symptom of hybridity. Certainly it is, even if not always, depending upon external conditions. Especially the sporadic changes of temperature presumably have brought about in meiosis the asynchronisation of anaphase disjunction of chromosomes.

It will be of interest to quote that the occasional failure of pairing appears even in so-called pure species or inbred strains, *e.g.,* in wheat (HOSONO, '35), in cotton (SKOVSTED, '37), in rice (SAKAI, '40), *etc.* The last example is noteworthy in comparison with the present observation in maple. SAKAI found in three varieties of *Oryza sativa* an unexpected occurrence of unpaired chromosomes, its frequency being *ca.* 40% in

average, and he concluded that "why asynapsis is caused in this case is unknown, but it might have perhaps been caused by some environmental conditions, the genetic effect.....seems not to be applicable in this case." (*loc. cit.*, p. 202).

The existence of binucleate PMCs can be clearly understood as the result due to syndiploidy, which appears rather to be a genetical property of this species and has probably no special connexion with other irregularities found at the same time. The fusion of two nuclei in this species is plausibly more far-reaching at pre-meiotic stage than at meiosis, because the binucleate PMCs have been distinguished from the normals at early prophase, or even at resting stage; besides, the fusion of two nuclei into one cell is not uniform being accomplished completely in some cells or incompletely in others.

The failure of cell wall formation, which is clearly depended upon the degree of the disturbances of spindle mechanism, at premeiotic mitosis may have brought about the aggregation of chromosomes into single PMC, *viz.* the binucleate PMC. DARLINGTON ('30) regards the syndiploidy, accompanied with the irregular orientation of spindles, in *Prunus avium* rather as the first stage of contabescence in an anther related perhaps therefore to male sterility. It occurs also in *P. persica, P. domestica,* and *P. cerasus* (HRUBÝ, '39). Recently, LEBEDEFF ('40) pointed out that the failure of cell wall formation or of cytokinesis during pre-meiotic and meiotic divisions in *Zea* is probably hereditary; this indicates the syndiploidy as a genetical character. Presumably these establishments are also applicable to the conditions in *A. japonicum* here recorded, although the exact genetical nature of this abnormality is a subject for further studies.

The effects of the occurrence of binucleate PMCs, on the other hand, to the later meiotic behaviours are obvious. As it has been above mentioned, the formation of octads and of the large tetrads resulted from these cells, in which the various irregularities of chromosome behaviour produced further the other sporads. The formation of restitution nuclei due to laggards or bridges, and the extrusion of some univalents or bivalents into the cytoplasm during meiosis, these two also must be responsible for the abnormal sporad formation in both binucleate and normal PMCs. By a comparison of the frequencies of abnormal sporads with those of binucleate PMCs in the four plants examined, it is possible to demonstrate the process of the abnormal sporad formation above described. In all the plants investigated the frequency of abnormal sporads exceeds that of binucleate PMCs, and within the probable divergencies the percentage of abnormal

sporads in each plant is corresponding to that of binucleate PMCs plus that of cells with lagging bivalents and with chromatid bridges (*q.v.* Tables 3, 4, and 5).

There are many instances of structural hybridity, in which the structural differences between the corresponding chromosomes are playing the most important rôle of meiotic abnormalities, *e.g.* in *Fritillaria, Tulipa, Tradescantia, Lilium, Paeonia,* etc. In these examples it is to be said that the effects of the "dyscentric structural hybridity" on meiosis are evidenced by the occurrence of chromatid bridges and fragments (dicentric and acentric chromatids) at both first and second anaphases, which is resulting from crossing-over in relatively inverted segments (*cf.* DARLINGTON, '37). The chromatid bridge formation found in *A. japonicum* is in the majority of cases not accompanied with the "acentric chromatids" (*v.* Table 3), and it must be here emphasized that this abnormality in the present material occurs in the functionally balanced diploid species, and that the present material is not the vegetatively propagating plant, in which the unbalanced chromosomal situation due to structural as well as numerical hybridity is possible to servive.

There is no doubt in the present species that all the irregularities of anaphase separation are more or less concerned with the asynchronisation of chromosomes in timing of division, even if in the normal PMCs, that the anaphase-disjunction begins successively from chromosome to chromosome and is not simultaneous in all the chromosomes. Therefore, if such lack of synchronisation accompanied with the rapid progress of meiotic division occurs in the extreme degree, it results to the univalent occurrence due to desynaptic failure of pairing of some bivalents during prophase-metaphase, and to the lagging of some bivalents due to precocity of some bivalents during metaphase-anaphase. Then it is possible to assume that these laggards occasionally result to the bridges between two anaphase nuclei. Such process of bridge formation due to laggards has been observed in *Prunus cerasifera* (MATHER, '37). Directly from the bivalents late in dividing at first anaphase, the bridges between two second metaphases have resulted.

Summary

1) Meiosis in four plants of *A. japonicum* var. *typicum* is described. All the plants are diploid with the meiotic chromosome number of 13.

2) The following meiotic abnormalities are found in all the plants investigated, but in each the frequency of them is different *viz.*, super-

numerary fragments, various chromosome association, irregular anaphase separation, binucleate PMC, and abnormal sporad formation.

3) The numerically irregular inclusion of supernumerary fragments in this species possesses several peculiarities in comparison with those in other plants. Although it is difficult to explain, the occurrence of these fragments is presumably due to a disturbance at pre-meiotic mitosis giving rise to the non-disjunction of chromosomes and then to the fracture of these duplicated chromosomes.

4) Multivalent associations found occasionally are clearly assumed as an evidence of the secondary polyploidy of the genus *Acer* as a whole. The existence of binucleate PMCs is inferred as a genetical character of this species, from which the abnormal sporads have resulted in the majority of cases, and partly they are considered to be due to the irreguralities in chromosome separation at anaphase.

5) The effects of change of the environmental conditions, especially of the temperature, are most probably supposed to be responsible for the irregularities of anaphase disjunction. The occurrence of univalents in this species may be due to the desynaptic failure of pairing of bivalents as a spontaneous irregularity. The asynchronisation of chromosomes in time of division, in correlation with the lagging of bivalents and the formation of chromatid bridges, is one of the most characteristic features of meiosis.

References

BENNETT, E. S., 1938: The origin and behaviour of chiasmata. XIV. *Fritillaria chitralensis*. *Cytologia*, **8**: 343–351.

DARLINGTON, C. D., 1928: Studies in *Prunus*, I and II. *Jour. Genet.*, **19**: 213–256.

——, 1929: Chromosome behaviour and structural hybridity in the *Tradescantiae*. *Ibid.*, **21**: 207–286.

——, 1930: Studies in *Prunus*, III. *Ibid.*, **22**: 65–93.

——, 1930: Chromosome studies in *Fritillaria*, III. Chiasma formation and chromosome pairing in *Fritillaria imperialis*. *Cytologia*, **2**: 37–55.

——, 1933: The origin and behaviour of chiasmata, VII. *Secale cereale* (n, 8). *Ibid.*, **4**: 444–452.

——, 1933: Studies in *Prunus*, IV. *Jour. Genet.*, **28**: 327–328.

——, 1937: *Recent advances in cytology*. 2nd. edit. London.

——, 1937: Chromosome behaviour and structural hybridity in the *Tradescantiae*, II. Jour. Genet., **35**: 259–280.

——, and MOFFETT, A. A., 1930: Primary and secondary chromosome balance in *Pyrus*. *Ibid.*, **22**: 129–151.

FRANKEL, O. H., 1937: The nucleolar cycle in some species of *Fritillaria*. *Cytologia*, **8**: 37–47.

——, 1937: Inversions in *Fritillaria*. *Jour. Genet.*, **34**: 347–362.

GOODSPEED, T. H. and AVERY, P., 1939: Trisomic and other types in *Nicotiana sylvestris*. *Ibid.*, **38**: 381–458.

HASEGAWA, N., 1934: A cytological study on 8-chromosome rye. *Cytologia*, **6**: 68–77.

HELLBORN, O., 1935: Reduction division, pollen lethality and polyploidy in apples. *Acta Horti. Bergiani*, **11**: 129–184.

HICKS, G. C. and STEBBINS, Jr., G. L., 1933: Meiosis in some species and a hybrid of *Paeonia*. *Amer. Jour. Bot.*, **21**: 228–241.

HOSONO, S., 1935: Karyogenetische Studien bei reinen Arten und Bastarden der Emmerreihe. I. Reifungsteilungen. *Jap. Jour. Bot.*, **4**: 301–322.

HRUBY, K., 1939: The cytology of the Duke cherries and their derivatives. *Jour. Genet.*, **38**: 125–131.

HUSTED, L., 1936: Cytological studies on the peanut, Arachis. II. Chromosome number, morphology and behavior, and their application to the problem of the origin of the cultivated forms. *Cytologia*, **7**: 396–423.

IVANOV, M. A., 1938: Experimental production of haploids in *Nicotiana rustica* L. *Genetica*, **20**: 295–397.

KOIDZUMI, G., 1911: Revisio Aceracearum Japonicarum. *Jour. Coll. Sci. Imp. Univ. Tokyo*, **32**, Art. 1.

KOLLER, P. CH., 1932: Further studies in *Tradescantia virginiana* var. *humilis* and *Rhoeo discolor*. *Jour. Genet.*, **26**: 81–96.

LAMMERTS, W. E., 1934: On the nature of chromosome association in *N. tabacum* haploid. *Cytologia*, **6**: 38–50.

LEBEDEFF, G. A., 1940: Failure of cytokinesis during microsporogenesis in *Zea mays* following heat treatment. *Ibid.*, **10**: 434–442.

LESLEY, J. W. and LESLEY, M. M., 1929: Chromosome fragmentation and mutation in tomato. *Genetics*, **14**: 321–336.

MATHER, K., 1935: Meiosis in *Lilium*. *Cytologia*, **6**: 354–380.

——, 1937: Notes on the cytology of some *Prunus* species. *Genetica*, **19**: 143–152.

MATSUDA, H., 1935: Cytological studies in genus *Petunia*. *Cytologia*, **6**: 502–522.

MATSUURA, H., 1935: Chromosome studies on *Trillium kamtschaticum* PALL. V. Abnormal meiotic divisions due to high temperature. *Ibid., Fujii Jub. Vol.*: 20–34.

——, and HAGA, T., 1940: *Do.* VIII. On the mitosis-meiosis relationship. *Ibid.*, **10**: 382–389.

MOFFETT, A. A., 1931: The chromosome constitution of the *Pomoideae*. *Proc. Roy. Soc. London Ser. B*, **108**: 423–446.

——, 1934: Cytological studies in cultivated pears. *Genetica*, **15**: 511–518.

NISIYAMA, I., 1934: The genetics and cytology of certain cereals VI. Chromosome behavior and its bearing on inheritance in triploid *Avena* hybrids. *Mem. Coll. Agr. Kyoto Imp. Univ.*, No. **32**.

OEHLKERS, FR., 1935: Untersuchungen zur Physiologie der Meiosis. *Zts. Bot.*, **29**: 1–53.

——, 1936: *Do.* III. *Ibid.*, **30**: 253–276.

OLMO, H. P., 1936: Cytological studies of monosomic and derivative types of *Nicotiana tabacum*. *Cytologia*, **7**: 143–159.

PATHAK, G. N., 1940: Studies in the cytology of *Oenothera*. *Amer. Jour. Bot.*, **27**: 117–121.

PARTHASARATHY, N., 1938: Cytological studies on *Oryzeae* and *Phalarideae*. I. Cytogenetics of some *x*-ray derivatives in rice (*Oryza sativa* L.). *Jour. Genet.*, **37**: 1–40.

REHDER, A., 1935: *Acer*. BARLEY, "*The Standard Cyclopedia of Horticulture*" Vol. **1**: 195–205.

SAKAI, K., 1940: Chromosome studies in *Oryza sativa* L. II. An unexpected asynapsis of the pollen mother-cell chromosomes. (Jap. with engl. résumé). *Jap. Jour. Genet.*, **16**: 193–202.

SIKKA, S. M., 1940: Cytogenetics of *Brassica* hybrids and species. *Jour. Genet.*, **40**: 441–509.

SKOVSTED, A., 1937: Cytological studies in cotton. IV. Chromosomoe conjugation in interspecific hybrids. *Ibid.*, **34**: 3–44.

STEBBINS, JR., G. L., 1938: Cytogenetic studies in *Paeonia*. II. The cytology of the diploid species and hybrids. *Genetics*, **23**: 83–110.

STRAUB, J., 1936: Untersuchungen zur Physiologie der Meiosis. II. *Zts. Bot.*, **30**: 1–57.

——, 1937: *Do.* VII. Die Abhängigkeit der Chiasmabildung bei *Vicia Faba*, und *Campanula persicifolia* von äusseren Bedingungen. *Ibid.*, **32**: 225–268.

——, 1939: Polyploidieauslösung durch Temperaturwirkungen. (Entwicklungsphysiologische Untersuchungen an der reproduktiven Phase von *Gasteria*). *Ibid.*, **34**: 385–479.

TAKAGI, F., 1935: Karyogenetical studies on rye. I. A trisomic plant. *Cytologia*, **6**: 496–501.

TAKIZAWA, S., 1940: Chromosome studies in the genus *Acer* L. (A prelim. note in Jap. with engl. résumé). *Jap. Jour. Genet.*, **16**: 18–22.

——, (*in preparation*) *Do.* I. Chromosome constitution of the genus *Acer* L.

——, (*in preparation*) *Do.* III. Secondary association in *A. ornatum* CARR. var. *Matsumurae* KOIDZ.

——, (*in preparation*) *Do.* IV. Aberrant pre-meiotic mitosis in the archesporial cells as a cause of meiotic chromosome abnormalities.

TOMETORP, G., 1939: Cytological studies in haploid *Hordeum distichum*. *Hereditas*, **25**: 241–254.

UPCOTT, M., 1937: The genetic structure of *Tulipa*, II. Structural hybridity. *Jour. Genet.*, **34**: 339–398.

——, 1939: The external mechanics of the chromosomes, VII. Abnormal mitosis in the pollen grain. *Chromosoma*, **1**: 178–190.

WEBBER, J. M., 1933: Cytological features of *Nicotiana glutinosa* haplonts. *Jour. Agr. Res.*, **47**: 845–867.

WHITAKER, T. W., 1936: Fragmentation in *Tradescantia*. *Amer. Jour. Bot.*, **23**: 517–519.

Explanation of Plate II.

All the photomicrographs were taken by Professor H. MATSUURA, from the temporary smear preparations (aceto-alcohol: aceto-carmine) of Plant No. 2 (figs. 1–4, and 7), and from the permanent preparations (aceto-alcohol: crystal-violet) of Plant No. 4 (figs. 5–6), giving a magnification of 1450. Note the size difference of cells as well as chromosomes, due to the difference of treatments, being remarkable, but they are surely not the real ones.

Fig. 1. Two PMCs with lagging univalents at first anaphase: left-lower, two univalents lagging to divide on the plate: right-upper, three univalents also becoming late in division between two chromosome groups.

Fig. 2. A PMC at second metaphase, showing a chromatid bridge formed between two metaphase plates.

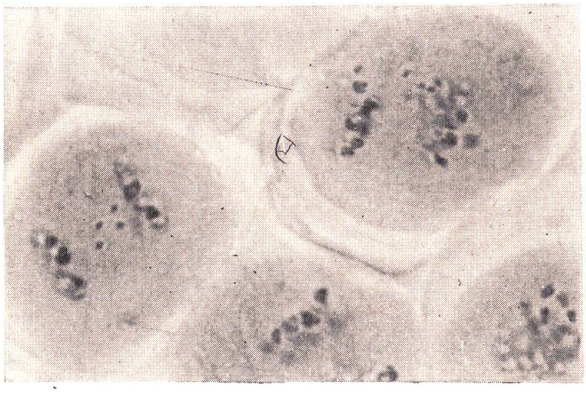
Fig. 3. A cell at first anaphase, showing a chromatid bridge without the accompanied fragments formed between two dividing chromosome groups.

Fig. 4. Abnormal metaphase constitution with four supernumerary fragments. The same cell as Text-fig. **3**. Note that of two of these fragments each has taken a juxtaposition, which is however not the general situation of fragments, and that one fragment is out of sight in this photomicrograph due to its different level from the other three.

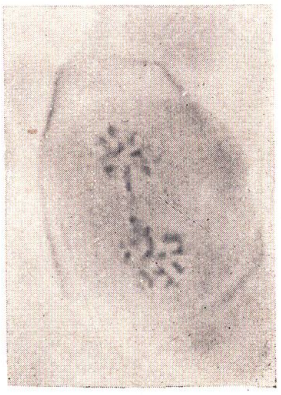
Fig. 5. Two PMCs at first metaphase: left, somewhat oblique polar view of normal PMC; right, side view of metaphase with four fragments, which are situated at random in the cytoplasm (one on upper side and three others on lower side of the equatorial plate), such an irregular position of fragments being their general characteristic.

Fig. 6. Two PMCs at first anaphase: left, normal disjunction of chromosomes; right, formation of two chromatid bridges between two anaphase chromosome groups.

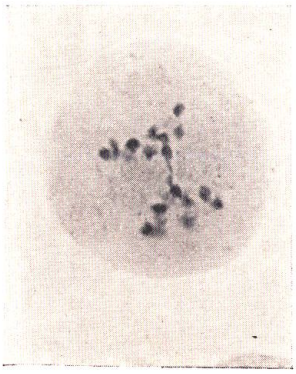
Fig. 7. Late anaphase of the first division, showing a bivalent lagging to divide in the centre. Probably such a bivalent results to a bridge formation between two second metaphase plates as that in Text-fig. **36** or in Fig. 2.



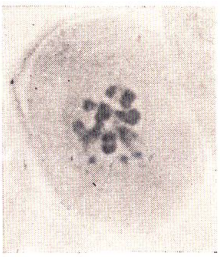
1



2



3



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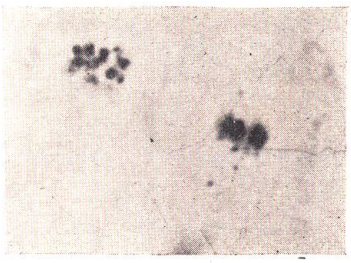

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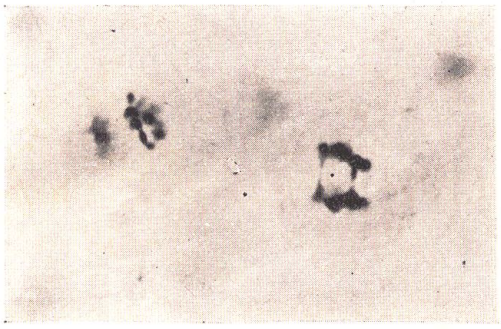

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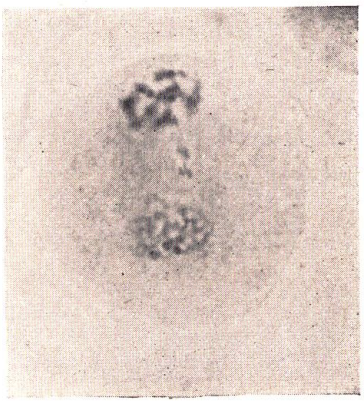

7

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