Chromosome studies in the genus *Acer* L.

I. The chromosome constitution of the genus *Acer*[1)]

By

SENZI TAKIZAWA

(With 31 text-figures and 2 tables)

In a preliminary report (TAKIZAWA, '40), the writer presented an account of somatic chromosome numbers in six species of the maple, but at that time no attention was paid to their chromosome morphology, owing to difficulty due to the extremely small size of the somatic chromosome in the root-tips. During the following season, however, some good preparations of root-tips, enough to make the analysis of the chromosome complements possible, were fortunately obtained in the following five species, *viz. Acer mono* var. *eupictum*, *A. ornatum* var. *Matsumurae*, *A. rufinerve*, *A. cissifolium*, and *A. diabolicum*.

During the course of the investigation of meiosis in PMCs, the author was able to find the archesporial cells at pre-meiotic mitosis. Such mitoses preceding meiosis without prolonged interval will be of interest with regard to their effects on meiosis immediately following. Particular attention, therefore, has been paid to the chromosome behaviours at pre-meiotic mitosis, though the archesporial cells available are from only four species, *viz. A. japonicum* var. *typicum*, *A. argutum*, *A. saccharum*, and *A. diabolicum*, because of the technical difficulties in obtaining the suitable archesporium.

Meiosis in PMCs could be observed in the following 11 species, *viz. A. Miyabei*, *A. mono* var. *eupictum*, *A. mono* var. *Mayri*, *A. ornatum* var. *Matsumurae*, *A. japonicum* var. *typicum*, *A. ginnala*, *A. crataegifolium*, *A. argutum*, *A. saccharum*, *A. diabolicum*, and *A. Negundo*. These are all diploid species with 13 bivalents. They showed a high degree of regularity of meiosis, excepting *A. japonicum*, in which different and variable kinds of meiotic abnormality were found. The secondary association of bivalents occurred in variable degrees among the species,

1) Aided by a grant from the Scientific Research Fund of the Department of Education.

but its general occurrence in all the species examined is of great significance in respect to the secondary polyploidy of the genus *Acer*.

In the present paper, the writer attempts to give a detailed description of mitotic chromosome complements of the five species above named, together with brief descriptions of the pre-meiotic mitosis in archesporial cells and of the meiosis in PMCs, and to discuss the chromosome constitution of the genus *Acer* as a whole.

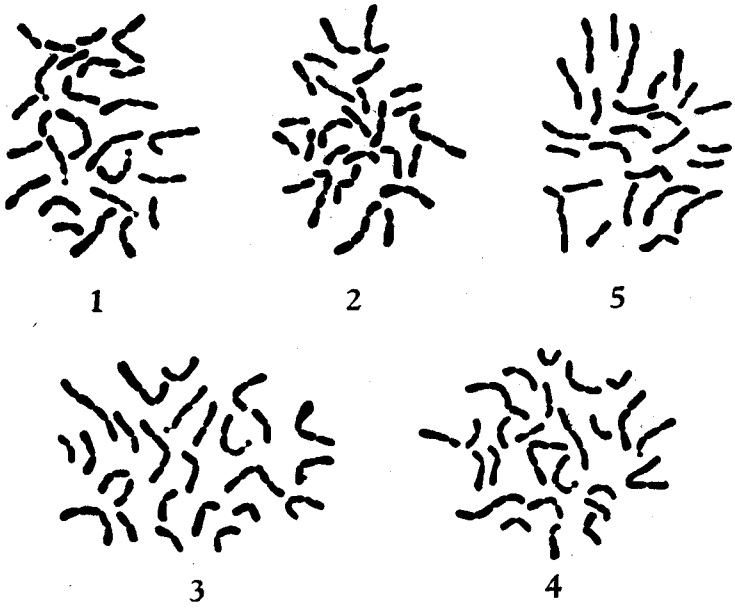
Root-tips used were taken from young trees grown in pots and form plants in the Botanical Garden of University. The fixations were made with LA COUR's 2BE; the stain used was SMITH's ('33) modification of the crystal-violet-iodine method. Very satisfactory results could be obtained by this procedure. Available flower-buds were collected directly from large grown trees and fixed in aceto-alcohol. The aceto-carmine smear method was employed for the stain, which made it possible to get satisfactory results with the pre-meiotic mitosis in archesporial cells as well as with the meiosis in PMCs.

The present study belongs to a series of investigations started under the direction of Professor H. MATSUURA, to whom the writer wishes to express his best thanks, for the original suggestions and valuable criticisms offered throughout the course of the investigation. The writer's thanks are also due to Dr. T. HAGA, who has, by contributions of material and otherwise, assisted him in this work.

Somatic chromosome complements

Mitoses in root-tips revealed metaphase plates in which 26 chromosome could be easily ascertained in all the five species examined (figs. 1-5). Although considerably many root-tips of many different individuals within a species were observed, no aberrant numbes from 26 in any metaphase plate could be counted, showing no such a somatic duplication, of chromosomes as found in the roots of *A. platanoides* (*cf.* MEURMAN, 33).

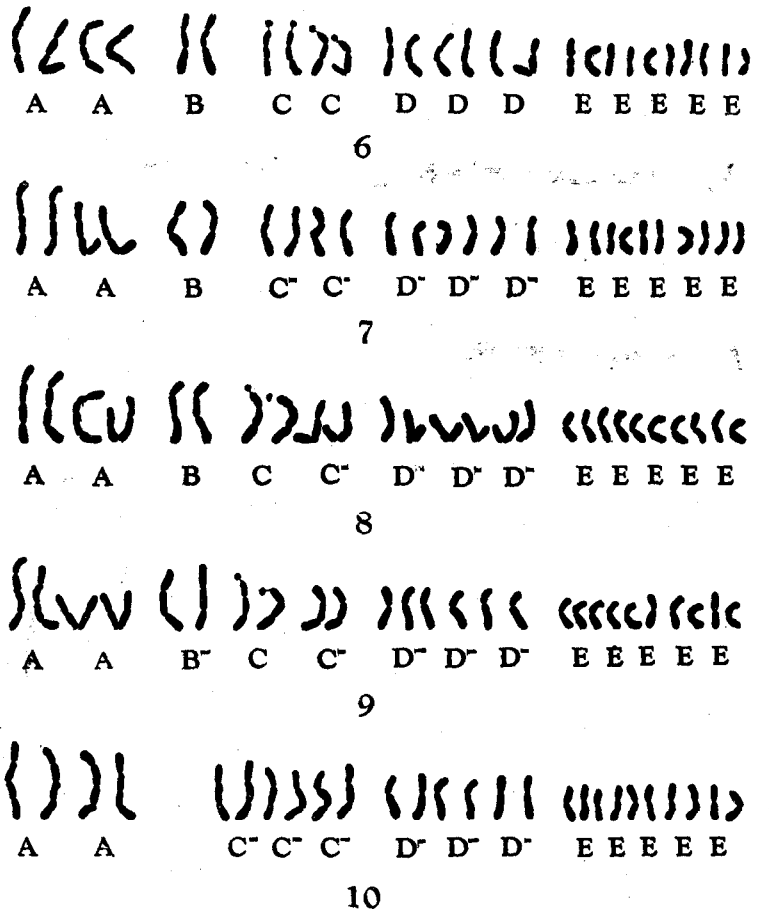
Throughout all the species examined, the morphology of the chromosomes shows great similarity, *i.e.*, they are quite small in size, the longest being about 2 micra long, and the smallest less than 1 micron long, so that an exact measurement of all the 26 units in a plate is impossible. However, some plates in well fixed material are sufficiently good to make possible a classification of the chromosomes into some distinct groups by means of the morphological points of identification.



Figs. 1–5. Somatic metaphase plates of root-tip cells.
1: *Acer mono* var. *eupictum.* 2: *A. ornatum* var. *Matsumurae.*
3: *A. rufinerve.* 4: *A. cissifolium.*
5: *A. diabolicum.* × *ca.* 3400.

Mitotic chromosomes can thus be classified into several types (figs. 6–10). These types designated with the capital letters A, B, C, D, and E, and those with a dash added on the shoulder of the letters B, C, and D, respectively are describable as follows:

Type A: The longest chromosomes with three constrictions evenly distributed, so that the four segments appear to be of nearly the same length.
Type B: Chromosomes similar to type A in respect to length, but possessing two constrictions, one submedian and another subterminal.
Type B⁻: Type B in which two constrictions are evenly distributed, so that the three segments are almost equal.
Type C: Chromosomes of medium length, with two constrictions and one small satellite.
Type C⁻: Type C without satellite.
Type D: Somewhat shorter chromosomes than type C⁻, three-segmented and without satellite.
Type D⁻: Short chromosomes having almost the same length as type D, but possessing only one subterminal constriction.
Type E: The shortest chromosomes, with one constriction either median or submedian.



Figs. 6–10. Somatic chromosome complements of the five species. Chromosomes separetely drawn from a different metaphase plate from that of figs. 1–5 in each species.
6: *A. mono* var. *eupictum.* 7: *A. ornatum* var. *Matsumuare.*
8: *A. rufinerve.* 9: *A. cissifolium.* 10: *A. diabolicum.* × *ca.* 3400.

From figures 6–10, it seems that types C, C⁻, D, D⁻, and E, cannot be distinguished from each other with sufficient certainty, while the distinction of A or B from the others is easily possible. Consequently, it must be particularly remarked that the classification of some types is to be regarded as rather tentative.

1) Indeed, it seems very probable that the five pairs of type E comprise two different types, the chromosomes of one type being some

what longer than those of the other. Such dissimilarity in length, however, is too insignificant to afford a sufficient basis for a further detailed grouping of these pairs. Therefore, they have been indiscriminately regarded as belonging to the one type E with median or submedian constriction.

2) Among the four similar types, C, C$^-$, D, and D$^-$, type C alone can be easily distinguished from the others due to its characteristically small satellites.

3) Between the two type C$^-$ and D, no difference exists in morphological features, and they can both be considered as type C without satellite. However, only in *A. mono*, could type D be distinguished from type C, so the type C without satelite in others was conveniently classified as type C$^-$, taking the chromosome constitution of *Acer* as a whole into consideration (*v. infra*).

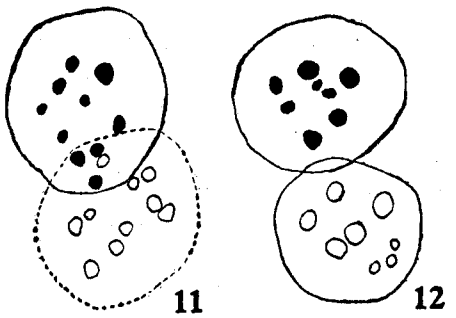
4) The distinction of type D$^-$ from E, with a submedian constriction, may not always be possible, this type D$^-$ offering the most confused case. Only by slight diversities in length, they could be distinguished from each other, therefore the distinction between them in *sensu stricto* may be impossible.

These inaccuracies may bring certain limitations to which the present analysis of karyotypes is subjected: yet it may be said with safety that the karyotypes in the five species examined differ from each other with respect to the members of particular chromosome types. Thus it can be here emphasized that the chromosomes of types A, C, and C$^-$ occur in duplicate, those of C$^-$, D, and D$^-$ in triplicate, and the type E chromosomes in quintuplicate. Such a condition of unequal multiplication of chromosomes is necessarily found in the secondary polyploid species, and it has been considered as an evidence to ascertain the secondary polyploidy in *Acer*, in addition to the secondary association of bivalents.

As one of the important morphological characteristics of the somatic complements in *Acer*, there is a large number of chromosomes with two or three constrictions. One of the constrictions of a chromosome must be regarded as the kinetochore, or the primary constriction, even if it is not to be determined; so the remaining ones including the satellites will be assumed as secondary constrictions. The total number of these secondary constrictions in each species examined is remarkably high, as many as 24 in *A. mono*. Such an occurrence of exceedingly many secondary constrictions in *Acer* is an interesting fact in respect

to the HEITZ' theory of SAT-chromosomes (*cf.* HEITZ, '30; '31: RESENDE, '37; '40), because this theory claims that the number of nucleoli in the somatic telophase of any species given should correspond to the number of satellites and secondary constrictions there present.

The total number of nucleoli would thus be expected to be 24 in *A. mono*, 16 in *A. rufinerve* and *A. cissifolium*, and 14 in *A. ornatum* and in *A. diabolicum*. In the actual counting of nucleoi in two succesive nuclei at telophase, however, no clear agreement with this expectation was shown in each and every of the present species; yet, whenever there was an increase of satellites or secondary constrictions, there was a similar increase in the number of nucleoli. The maximum number of ten nucleoli, in so far as they are capable of being distinguished, is found in *A. mono* (fig. 11). In the other four species, the highest number is likewise found to be eight, never reaching as high as ten (fig. 12). Even in *A. mono*, nucleoli as numerous as ten are met with quite rarely. There can be no doubt that a discrepancy from HEITZ' theory exists, the cause of which should be looked for elsewhere.

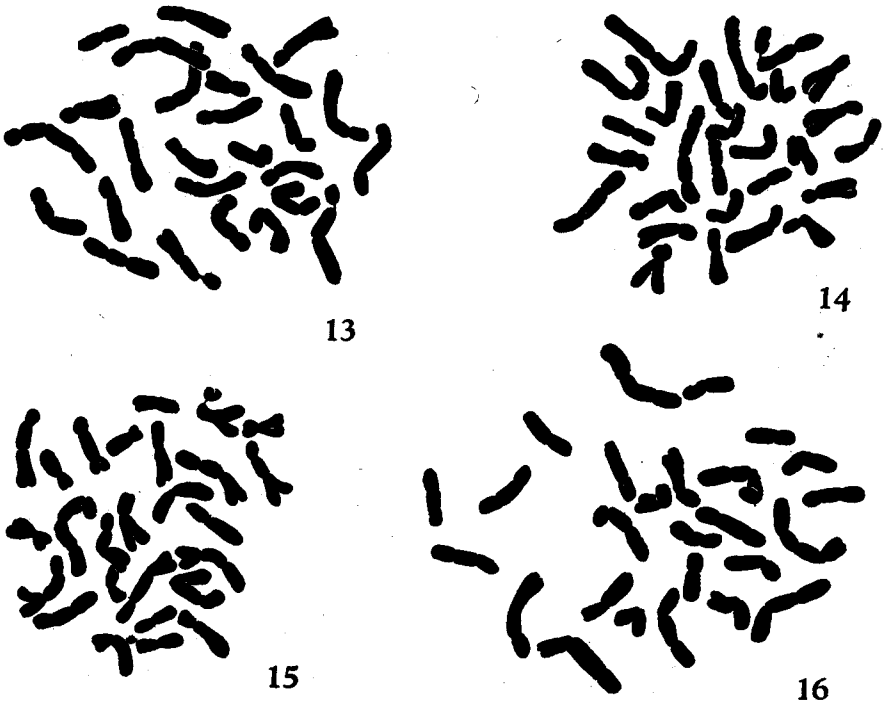


Figs. 11–12. Nucleoli in two successive telophase nuclei of root-tip cells.
11: *A. mono* var. *eupictum*; only in this species 10 nucleoli, as the maximum number of nucleoli found, were verified.
12: *A. rufinerve*. × *ca.* 2000.

Chromosme multiplication in archesporial cells

In the archesporial cells, normal metaphase plates with 26 chromosomes could also be ascertained in the four species examined (fig. 13–16). The preparations were in this case made by acetocarmine smear, so that just such a comparative study of the chromosome complements as that in root-tip cells was impossible in the plates of archesporial cells due to the chromosomes being swollen.

However, it is remarkable to notice that in the plates of archesporial cells the longest chromosomes appeared frequently to be less constricted than those in the root-tip cells. In *A. diabolicum* only, in which both metaphase plates of root-tip cells and of archesporial cells were obtained, the total number of secondary constrictions in a plate

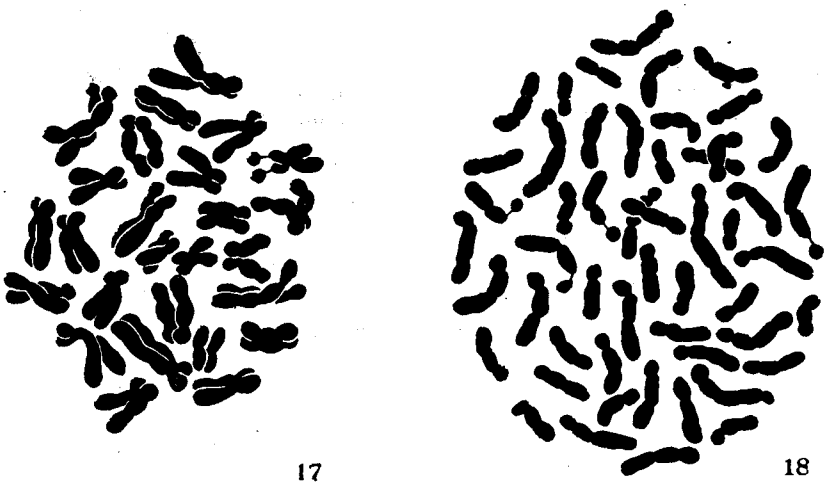


Figs. 13–16. Metaphase plates of archesporial cells. From acetocarmine smear preparations. Note the differences of chromosome size and form in comparison with that of root-tip cells (figs. 1–5).
13: *A. japonicum* var. *typicum*. 14: *A. diabolicum*.
15: *A. argutum*. 16: *A. saccharum*. × *ca.* 3700.

may be counted to be seven or eight at pre-meiotic mitosis (fig. 14); but at the root-tip mitosis it is about twice the number, being 14 (fig. 10). In the other three species (figs. 13, 15, and 16), the total number of secondary constrictions in the archesporial division plates never reached the minimum number of 14 found in the root-tip division plates. This fact, if it is not merely a superficial finding, seems to be of interest, when one takes into consideration the fact that during diakinesis of meiotic division in PMCs of *Acer* species examined, four bivalents are usually connected with the nucleolus (*v. infra*).

Another interesting fact in archesporial cell divisions is the appearance of cells in which the chromosomes are present in the tetraploid number of 52; that is, here is case of the somatic doubling of chromo-

somes or the disomaty. Such disomatic cells in addition to the normal monosomatic ones generally appeared in all the four species examined. Especially, in *A. japonicum*, the disomatic cells were likely to occur very frequently and some cells seemed to be tetrasomatic ones, that is, cells with an octaploid number of chromosomes, though the statistical analysis of the frequency of these polysomatic cells yet remains to be done[1]. In *A. japonicum*, the metaphase plates thus observed showed monosomaty to be most frequent; a fair number of paired disomatic cells occurred, but very few cases of unpaired disomatic and uncertain tetrasomatic cells have been found. Measures of the diameters of monosomatic and disomatic metaphase plates seemed to indicate that the nuclear volume is approximately doubled for a doubling of the chromosome number, but no difference in volume exists between the monosomatic and paired disomatic cells. Such relationships of nuclear volume will be of great importance with regard to the origin of polysomatic cell.



17 18

Figs. 17–18. Disomatic metaphase plates of archesporial cells in *A. japonicum* var. *typicum*. From aceto-carmine smear preparations.
17: Paired disomatic plate. 18: Unpaired. × *ca.* 3700.

1) Polysomaty has come recently to be confirmed as an universal occurrence in various somatic tissues of many species, not merely in various portions of meristematic layers (Stein, '36; Lorz, '38; Gentcheff & Gustafsson, '39; Wulff, '40; Wipf & Coopf, '40; Ervin, '41; Berger, '41), but in certain differentiated tissues in plants (Geitler,' 40; Grafl, '40), also in those in insects (Berger, '38; Geitler, 39'; Oksala, '39). A detailed description of the polysomaty in archesporial cells of *Acer* will be presented later (*cf.* No. 4 of this series).

In regard to the chromosomal feature of the disomatic cells, however, there are no appreciable differences among the four species examined; what follows applies equally, therefore, to all the four. A characteristic feature of polysomatic plates is the frequent close pairing of homologous chromosomes, which is much more intimate than the more or less loose association found in monosomatic plates due to the somatic pairing. In *Acer*, a somatic pairing markedly defined was not recognized in every ordinary metaphase plate of root-tip cells as well as of archesporial cells; however, in the paired disomatic metaphase plates, all the regularly paired chromosomes appeared at the same focal level (fig. 17); the two chromosomes of a pair were either in contact throughout, in contact at some one point, or entirely separate but close and parallel.

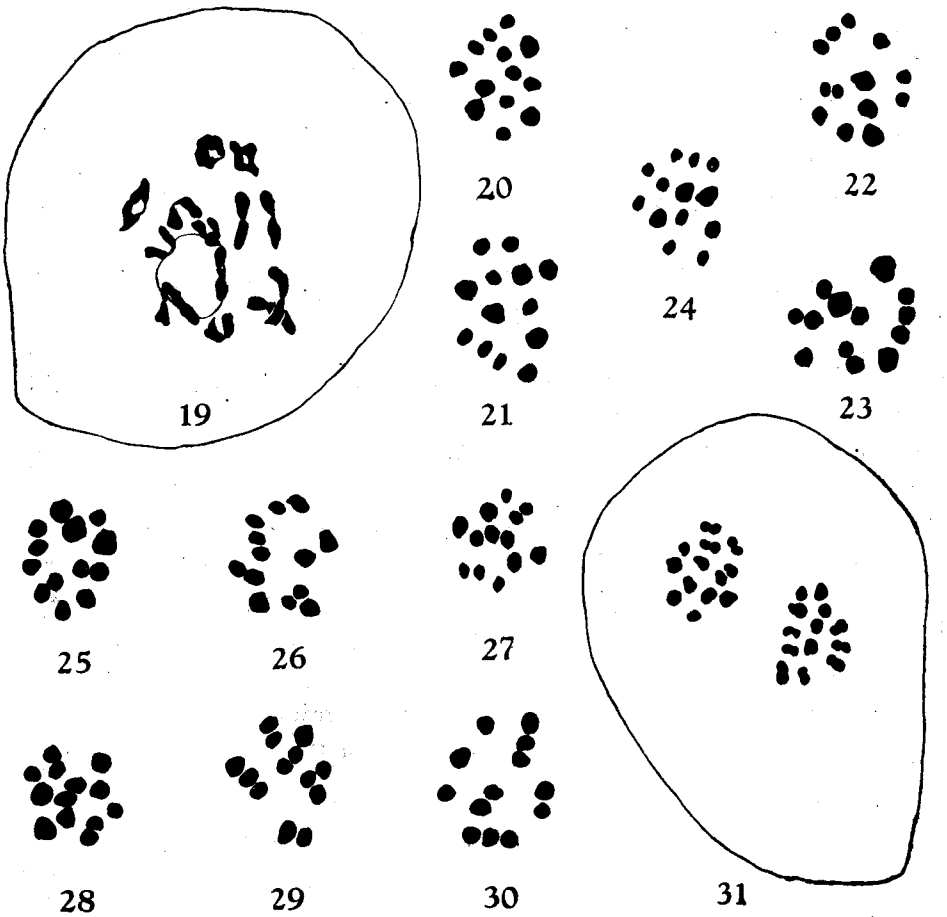
In addition to such paired disomatic cells, unpaired disomatic cells, in which the chromosomes are not paired but lie scattered at random over the equatorial plate in the same manner as in the ordinary monosomatic plate, were less frequently found (fig. 18). This case of polysomatic cells existing in two kinds is another marked characteristic of the polysomatic condition; but only unpaired disomatic cells have been found in the roots of *A. platanoides* (MEURMAN, '33). It must be here noticed that in the unpaired disomatic cells there is a tendency, though not marked but still existing, for holomogous pairs, to lie in the same region, as compared with the monosomatic cells, in which no homologous chromosome manifested a positive somatic pairing in *Acer*.

Meiosis in PMCs

Excepting one species, *A. japonicum*, in which various kinds of meiotic abnormality were met with (*cf.* No. 2 of this series), all the species studied showed complete formation of 13 bivalents during meiosis and no univalent or multivalent were seen; the similar completeness of pairing of bivalents and the same great regularity of meiotic process have been observed on different *Acer* species by previous workers. It strongly suggests that maples are plants which have reached a karyological stability through the long periods of species differentiation.

No attempt was made to observe the prophase stage. At diakinesis, 13 bivalents are evenly scattered throughout the cell and four of them are connected with the nucleolus (fig. 19). At the first metaphase, the exact number of bivalents can be counted with great ease

in polar view of every species examined (figs. 20–30). The 13 bivalents are, however, discernible in only two kinds; *i. e.*, three large bivalents, which are undoubtedly identical with the long chromosomes of types A and B or B⁻ in somatic complement, can be identified in almost every metaphase plate, and the remaining ten bivalents show no clear



Figs. 19–31. Meiosis in PMCs. **19**: Late diakinesis; **20–30**: first metaphase plates; **31**: second metaphase. All figs. from aceto-carmine smear preparations.

19, 24, and 31: *A. japonicum* var. *typicum*. 20: *A. Miyabei*.
21: *A. mono* var. *eupictum*. 22: *A. mono* var. *Mayri*.
23: *A. crataegifolium*. 25: *A. ornatum* var. *Matsumurae*.
26: *A. argutum*. 27: *A. Negundo*. 28: *A. saccharum*.
29: *A. ginnala*. 30: *A. diabolicum*. × *ca.* 1800.

differences among them to enable us to carry out a more detailed grouping.

It is significant that at metaphase the secondary association of bivalents occurs in all the species examined, although its degrees differ from each other. In *A. ornatum*, the degree of secondary associations can be stated to be strongest, and it is least in *A. japonicum*; the remaining species show a range from strong to weak in degree of secondary association between the above two extremities. Such variability in degrees of secondary association among the species seems to be a reason why only the two large bivalents are to be found in juxtaposition while the others never exhibit any tendency of such an affinity as in the metaphase plate of *A. platanoides* (MEURMAN, '33). Particular attention therefore must be paid to the variability of secondary associations among cells within a given species as well as among species within a group.

A statistical analysis made on the frequency of various types of secondary association of *A. ornatum* (TAKIZAWA, '40) shows the most usual type of associations to consist of three groups of three associated bivalents and two groups of two associated ones; thus the five groups of associated bivalents seem to indicate that the apparent basic chromosome number of 13 is derived from an original basic number of five[1]. It must be here emphasized that one large bivalent and two small ones constitute one group of associations. This is one of the strong evidences to support the assertion that the secondary associations not merely mechanical but is grounded upon certain ancestral homogeneities of associated bivalents.

The secondary association in the metaphase plates of the second division was not so intimate as in many of the plates of the first division, due to the more elongated dumb-bell-like appearance of the chromosomes at this stage (fig. 31). The chromosomes are evenly distributed in a majority of cases of well-fixed plates. The second division of meiosis in *Acer* seems thus to be less suitable for a critical study of secondary associations.

1) Various objections raised against the theory of secondary association still exist at present (*e. g.*, SAX, '31; '32: HEILBORN, '26; '37: PROPACH, '37). By a new statistical analysis of the secondary association, the writer has reached to the results that the critical comments disputing this theory have never interfered with the general importance of this phenomenon. The detailed descriptions and a critique will be dealt with later (*cf.* No. 3 of this series).

TABLE 1

List of chromosome numbers examined in the genus *Acer* L.

Section	Species	*n*	2*n*	Author	
Campestria	*Acer campestre* L.	13	—	Foster	'33
	A. platanoides L.	13	26	Taylor	'20
		13	—	Darling	'23
		13	26	Meurman	'33
		—	39	"	
	A. Miyabei Maxim.	13	—	Foster	'33
		13	—	*	
	A. mono Maxim. var. *eupictum* Nakai	13	26	*	
	A. mono Maxim. var. *Mayri* Koidz.	13	—	*	
Palmata	*A. palmatum* Thunb. var. *intermedium* Schw.	13	—	Foster	'33
	A. ornatum Carr. var. *Matsumurae* Koidz.	13	26	*	
	A. japonicum Thunb. var. *typicum* Schw.	13	26	*	
	A. pseudo-sieboldianum Komar.	13	—	Foster	'33
	A. circinatum Pursh.	13	—	"	
Spicata	*A. pseudoplatanus* L.	26	52	Taylor	'20
	A. pseudoplatanus L. var. *euthrocarpum* Carr.	26	—	Foster	'33
	A. ginnala Maxim.	13	—	*	
Indivisa	*A. carpinifolium* Sieb. et Zucc.	—	52	Taylor	'20
Macrantha	*A. rufinerve* Sieb. et Zucc.	13	—	Foster	'33
		—	26	*	
	A. Tschonoskii Maxim.	13	—	Foster	'33
	A. crataegifolium Sieb. et Zucc.	13	—	*	
Arguta	*A. argutum* Maxim.	13	26	*	
Rubra	*A. saccharinum* L.	26	52	Taylor	'20
		— *ca.* 90		"	
	A. rubrum L.	36	—	Mottier	'14
		36	—	Taylor	'20
		40	—	Darling	'12
		52	—	Foster	'33
		ca. 50	—	Toylor	'20
		— *ca.* 90		"	
		ca. 70	—	"	
Saccharina	*A. saccharum* Marsh.	13	—	"	
		13	—	Foster	'33

Section	Species	*n*	*2n*	Author	
Trifoliata		13	26	*	
	A. diabolicum Bl.	13	26	*	
	A. nikoense Maxim.	13	—	Foster	'33
	A. griseum Pax.	13	—	"	
	A. mandshuricum Maxim.	13	—	"	
Negundo	*A. Negundo* L.	13	—	Darling	'09
		13	—	Toolor	'20
		13	—	Sinotô	'29
		13	—	Foster	'33
		13	—	*	
	A. Negundo L. var. *Interius* Sarg.	13	—	Foster	'33
	A. cissifolium Koch.	—	26	*	

N. B. Sections in the classification by Rehder ('35) are for convenience followed in this table. Species names were verified with the aid of Nemoto's "Flora of Japan, Supplement" ('36). Many species names have been recently changed; especially *mono* and *ornatum* are synonyms of previous *pictum* and *palmatum*, respectively. Mottier ('14) has reported $n=12$ or 14 in *A. Negundo*. Darling's two papers ('09; '12) quoted by Foster ('33). * The present writer.

Considerations

i) The basic chromosome number in *Acer*

Data from all the investigations dealing with chromosome numbers in the species and varieties of the genus *Acer*, including also the present study, are summarized in Table 1. From this table, it seems safe to state that maples have 13 as the basic chromosome number and are, for the most species examined, diploids, with a few cases of polyploids. Thirteen species examined by the writer are all diploid species, and they belong to seven different sections, *viz. Campestria, Palmata, Spicata, Macrantha, Arguta, Saccharina,* and *Negundo.* Excepting the section *Spicata,* the other six and the section *Trifoliata* do not include polyploid species, which have been restricted only to the sections *Spicata, Indivisa,* and *Rubra.* The genus *Acer* is a large genus containing approximately 200 species, which is divided into at least 12 sections, and is widely distributed throughout the northern temperate zone; moreover, a great differentiation of species occurs within certain species as a highly polymorphic genus. Therefore, although scanty numbers of the known

species of maples have been cytologically studies, yet their distribution throughout the sections of the genus is sufficiently wide to render the above statement reasonable.

Varying counts within a species made on *A. platanoides*, and *A. saccharinum*, especially on *A. rubrum* are remarkable enough to call attention:—

1) MEURMAN ('33) proved one seedling out of 22 of *A. platanoides* collected from native place to be a triploid having 39 somatic chromosomes. He regarded this seedling as an auto-triploid, which may have arisen from mating between the normal haploid gametes and the unreduced diploid gametes resulting from a somatic doubling of chromosomes in the generative tissues of one parent, and not as a species hybrid arising from the crossing between one tetraploid and one diploid. MEURMAN'S conclusion is based on the facts that no other *Acer* species or tetraploid form of *A. platanoides* are found to grow in the vicinity of the plot where the seedlings were collected, and that the constant occurrence of the disomatic cells is found in all the seedling roots studied. Thus the somatic doubling is undoubtedly not restricted to the root tissues only, but may also take place in the generative tissues. This inference is of great significance in relation to the chromosome reduplication in archesporial cells of the species here investigated and to the occurrence of syndiploid PMCs in *A. japonicum* (*cf.* No. 2 of this series).

2) TAYLOR ('20) gave two different counts of 52 and 91 chromosomes in the roots of *A. saccharinum*. The latter number of 91 apparently is in agreement with the septaploid number; however, it may be due to a counting of disomatic conditions in the root of the ordinary tetraploid species, *A. saccharinum* (2n=52).

3) In *A. rubrum*, TAYLOR (*loc. cit.*) found one batch of plants having meiotic chromosomes, here in the neighbourhood of 50, *viz.* 48, 52, 53, and 54, these numbers approximating to the octaploid number (n=52) found in this species by FOSTER ('33); while another batch had the somatic chromosome number of about 90, *viz.* 88, 90, and 94, which might be due to a faulty counnting of the octaploid number (2n=104). TAYLOR also made the count of 36 in PMCs of this species, which is exactly the same as the number given by MOTTIER ('14); this and the haploid number of 40 given by DARLING ('23) are near to the hexaploid number (n=39). In another case, an exceptional high number of meiotic chromosomes such as 72, 70, 68, and 67, has been found also by TAYLOR.

Such a condition in *A. rubrum* seems to indicate that both hexaploid (n=39) and octaploid (n=52) forms exist within this species and probably the hybrids with the diploid form (n=13) occur.

Thus, in the observations of 27 species and varieties hitherto recorded, only one triploid form of *A. platanoides*, three assured tetraploids, *viz. A. pseudoplatanus*, *A. carpinifolium*, and *A. saccharinum*, and uncertain hexaploid and octaploid forms of *A. rubrum* are all the polyploid species found in the genus *Acer*. This is an interesting fact from the phylogenetic point of view and suggests species differentiation in evolutionary steps in such a large genus of woody plants as *Acer*.

The phenomena of secondary association of bivalents during meiosis may be taken as a criterion of ancestral homology of such secondarily associated bivalents. Consequently, the original or ancestral basic number of the genus *Acer* must be still lower than the numerical basic number of 13. MEURMAN (*loc. cit.*) found in *A. platanoides* the secondary association to occur only in two large bivalents. He states, "it is all the more significant that two of the biggest bivalents ····· are in the majority of cases to be found in close proximity to each other. The third big pair of chromosomes ····· never exhibits any tendency to such an affinity. On the contrary, it lies quite as often at some distance from the two associated ones, with other smaller bivalents between them. ····· Although secondary pairing might be expected to occur also between other pairs, ····· no indication of such a behaviour could be traced. It surely happens that sometimes two bivalents, other than the two big ones, may lie rather near one another, but this is not more common than the cases in which the same thing occurs between bivalents obviously of unequal size." (*q. v., loc. cit.*, pp. 160–162). "The haploid chromosome number of 13 would thus be a secondarily balanced basic set, derived from an ancestral set of 12 units." (*q. v., ibid*, p. 167). From this statement, the haploid constitution of *A. platanoides* might thus be written:

AA, B, C, D, E, F, G, H, I, J, K, L.

As has already been described, all the eleven specise examined by the writer revealed the secondary association in various degrees, with *A. ornatum* indicating the maximum association consisting of five groups of associated bivalents. Therefore, the apparent basic number of 13 can be inferred to have arisen through the unequal multiplication of five basic sets. The haploid chromosome constitution of *A. ornatum*

might be represented by the following formula:

AA, BB, CCC, DDD, EEE.

This dissimilarity of haploid chromosome constitution between *A. platanoides* and *A. ornatum* is probably of no great significance, because difference in degrees of the secondary association has been occasionally recognized between species with identical chromosome complements. It is, however, not clear how the apparent basic number of 13 in *Acer* has arisen, whether through the multiplication of an original basic set of 5 (the writer's opinion), or through that of 12 (MEURMAN's opinion), since there is no direct evidence to ascertain the process of such a chromosome multiplication so far as elucidated merely by the occurrence of secondary associations; therefore the present writer's inference requires the support of other evidences.

The following facts may thus permit an interpretation that the secondary polyploidy in *Acer* is more in agreement with a basic number of 5 rather than of 12:—

1) The multiplication of some types of somatic chromosomes (*v.* Table 2) is too complex to regard *Acer* as an originally simple tetrasomic. With regard to this unequal multiplication of chromosomes in somatic complement which can be recognized also in *A. platanoides*, MEURMAN (*loc. cit.*) states that "the deduced ancestral basic chromosome set 12 for the genus *Acer* is very likely already in its turn a secondary balanced one ..... It can be seen ..... that the lowest possible set would have been eight." (*q.v.*, p. 167).

2) TAYLOR ('20) gave no description of the secondary association, but it may not be mistaken to assume from his illustrations of meiotic metaphase plates (*q. v., op. cit.*, Pl. IX, figs. 51–56, 65–66 and 69–71) that some bivalents are associated at least in *A. Negundo*, *A. pseudoplatanus*, and *A. saccharum*. There it is clearly shown that one or two pairs of two bivalents, or those of three, or both, are found in every PMC. Besides, his misjudging counts of n=11 in *A. platanoides* should be the result of the occurrence of secondary association which is likely to consist of two groups of two associated bivalents (*q. v.*, Pl. IX. figs. 59–62). All the species examined by FOSTER ('33), even polyploids like *A. pseudoplatanus* and *A. rubrum*, revealed the secondary association in various degrees. Such a universal, even if variable, occurrence of secondary associations throughout the genus may thus be taken as an evidence to indicate that the genus *Acer* is a group of secondary polyploids with

an original basic number still lower than 12.

3) Chromosome constitutions of the genus *Aesculus*, related closely to the genus *Acer*, may be of importance in respect to discussing the secondary polyploidy of *Acer*. The lowest haploid number is so far 20 in the genus *Aesculus* (*cf.* HOAR, '27; SKOVSTED, '29; UPCOTT, '36), but LAWRENCE ('31) and DARLINGTON ('32) conclude from SKOVSTED's illustration that the chromosome complement of *Aesculus* may have been originated from a basic set of 10 or probably of 5, since the meiotic metaphase figures published of so-called diploid species in *Aesculus* show obviously the secondary association. UPCOTT (*loc. cit.*) agrees with this assumption of tetraploidy in the apparent diploid species of *Aesculus*. This situation in the genus *Aesculus* lends support to the hypothosis of the ancestral basic number of 5 in the genus *Acer*. Despite a clear morphological difference and a phylogenetical distance of *Aesculus* from *Acer*, the assumption of a basic chromosome set of five may make it possible to believe in a common origin for these two related genera.

ii) The somatic chromoseme complements

The 26 units of the somatic complement in root-tips can be classified into several types, although the classification is sometimes difficult due to smallness in chromosome dimensions. In spite of these limitations, the following characteristic features will be recognized in the somatic chromosome complements:—

1) There are no morphological changes in the type A chromosomes in every species examined, in which uniformly four of them are included. They should be also regarded as corresponding to two of three large bivalents distinctively differing from the others, which are generally found at meiosis.

2) It is remarkable that in *A. diabolicum* the chromosome of type B does not occur, although three other species include one pair of type B chromosomes, and *A. cissifolium* has one pair of type B^-, which is slightly different from type B chromosome. Such a variability of occurrence of the second chromosomes in length at mitosis should correspond to the fact that at meiosis the third big bivalent is in some species difficult to distinguish from others.

3) In relation to type C, the five chromosome constitutions are clearly distinguishable from each other. In both *A. rufinerve* and *A. cissifolium*, similary one pair of types C and C^- respectively has been

found, but the two species are distinguished from each other in relation to type B.

4) Three pairs of type D have been found only in *A. mono*, while the other four possess uniformly six chromosomes of type D⁻.

And thus, where the morphologically similar chromosomes in somatic complement are assumed to be homologous, the following formulae (Table 2) may represent the haploid chromosome constitutions of the five species examined. There exists a dissimilarity between the haploid constitution of the genus as a whole inferred from the secondary association and those deduced from the analysis of somatic chromosome complement. This was, however, to be expected from the assumption that the morphologically similar chromosomes might be not necessarily homologous, nor need the morphologically dissimilar chromosomes be completely non-homologous. In fact, the mitotic chromosomes belonging to one type E in the somatic complement may be divided into two different types. The chromosomes of types B, C, and D, are variable from species to species (*v. supra*); and the morphologically different bivalents show sometimes the secondary association (*v.* figs. 20–30).

How such an alteration of karyotypes has been brought about in the phylogeny of the species is quite difficult to decide in the present material. However, one judges from the results obtained in other examples, in which the detailed analysis of karyotypes is possible, it seems likely that such structural changes as translocation, deletion, reduplication, *etc.*, may have also occurred chiefly in the chromosomes of types B, C, and D in *Acer*.

TABLE 2

Haploid chromosome constitutions

Species	Chromosome constitution
Acer mono var. *eupictum*	A A B C C D D D E E E E E
A. ornatum var. *Matsumurae*	A A B C-C⁻ D-D-D⁻ E E E E E
A. rufinerve	A A B C C⁻ D-D-D⁻ E E E E E
A. cissifolium	A A B⁻ C C⁻ D-D-D⁻ E E E E E
A. diabolicum	A A C-C-C⁻ D-D-D⁻ E E E E E
genus *Acer* as a whole*	A A, B B, C C C, D D D, E E E

* From the maximum number of groups of secondarily associated bivalents during meiosis (*v.* text).

It is interesting that the karyotype of *A. mono* is similar to that of *A. platanoides* (*cf.* MEURMAN, '33), *i.e.*, these two closely related species possess a great similarity in their nuclear constitutions, *viz.* the haploid set of

A A, B, C C, D D D, E E E E E,

as mentioned above; while of the other four species, which are represented by the more separate four sections in so far as they are dealt with in this investigation, each possesses a characteristic karyotype differing from each of the others (*v.* Table 2.)

LANGLET ('32), in his extensive karyosystematic study of the family *Ranunculaceae*, distinguished two karyotypes, *viz.* the R-type and the T-type, and "Innerhalb je einer Gattung kommt immer nur ein Chromosomentypus vor. Ein derartiges Uebereinstimmen muss zweifel los tief begründet sein, ... das wir von den Chromosomenverhältnissen sehr gut begründete Schlüsse auf die natürliche Verwandtschaft der Gattungen zu ziehen berechtigt sind." (*q. v., op. cit.* p. 295). FLOVIK ('36), however, in the study on the arctic *Ranunculus*, recognized morphological differences of chromosomes in a high degree between species belonging to even the same group and divided the R-type into two karyotypes, *viz.* R. l-type and R. p-type. Recent findings in the genus *Clematis* (fam. *Ranunculaceae*: MEURMAN & THERMAN, '39) are also quite at variance to LANGLET's view. Within the genus *Clematis*, the diploid chromosome complements are very uniform in every species examined, and "the karyotypes reveal here no parallelism with the systematic classification, ... species belonging to quite different groups may have very similar sets, whereas in nearly related forms the chromosomes of two types (G and H) may differ". (*q. v., op. cit.* p. 9). These rather confusing questions on the karyosystematic and phylogenetic relationship must therefore be dealt with cautiously; they are more intricate than usually supposed, and demand more comprehensive studies and experiment before any definite conclusion can be reached. The same is to be said in the case of *Acer* above described.

iii) The nucleolus-chromosome relationship

As has already been described, the total number of secondary constrictions within a somatic chromosome complement is in no clear agreement with the number of nucleoli actually counted in the telophase nuclei, in which limited and variable nucleoli are usually found instead

of a high and constant number corresponding to the number of secondary constrictions. Instances of such numerical instability of nucleoli during somatic telophase and disagreement between the expected and actual numbers of them have been reported in *Paeonia* (DERMAN, '33; SINOTÔ '38). To try to solve such a discrepancy from HEITZ's theory of SAT-chromosome, the following possibilites can be taken into considerations:—

1) First, some nucleoli may be difficult to detect due to their extremely small size corresponding to the smallness of the chromosomes, even if the whole expected number of nucleoli is originally formed.

2) Secondly, the decrease in number of nucleoli during mitosis, which is due to their gradual fusion into one as the division proceeds, may occur rather rapidly in *Acer*. If it is so, the lower number of nucleoli usually found in all nuclei at telophase than the expected seems very likely to indicate that the nucleoli are rapidly separated from the chromosomes and that some of them are instantly fused.

3) The third and the most appropriate possibility is that the whole number of nucleoli indirectly expected from that of secondary constriction may be practically impossible to be formed, though there is no direct evidence that some secondary constrictions are independnt from the nucleolar formation, since the secondary constrictions have been now assumed to have arisen by stretching of the chromonemata, for which the developing nucleolus is responsible.

MATSUURA's ('38) postulation of a hypothesis on the origin of SAT-chromosomes is of great significance in connexion to the above statement that the formation of nucleolus is not necessarily correlated with the absence or presence of the trabants. According to MATSUURA, there are two general principles of the nucleolus-chromosome relationship, *viz.*, "first, every chromosome can be referred to as a nucleolar chromosome in the sense that it can produce nucleoli under certain specified circumstances; and secondly, there is a differential rate in the capacity for nucleolus-organizing activity of chromosomes within a complement, thus resulting in the usual occurrence that particular chromosomes alone are apparently related to the formation of nucleoli." Therefore, "normally the chromosomes with the greater valencies for production of a nucleolus are in advance so that the chromosomes with lower valencies have little or no opportunity to function, however under a special condition this competition might be disturbed especially when the difference in valency is small." (*q. v., op. cit.* pp. 71–72). This hypo-

thesis suggests that some secondary constrictions found in *Acer* may possess a lower rate of functional activity for the nucleolus formation than the rest, so it may be concluded that the formation of nucleolus in some secondary constrictions has been suppressed by the others with the greater valencies, and therefore it is very questionable to conclude that the secondary constrictions, especially as in the case of *Acer* in which their numbers are considerably high, are all, everytime and everywhere, responsible for the nucleous formation.

Summary

1) Chromosome numbers of n=13 and 2n=26 were determined in 13 maple species. These counts, together with those in the other species given by previous workers (Table 1), show that in the genus *Acer* a large number of species is diploid and a few polyploid species occur.

2) The somatic chromosome complements could be classified into several groups on the basis of their length and form (Table 2), although the distinction of some types was occasionally difficult due to their extremely small size. This analysis indicates that unequal multiplications of chromosomes occur within a somatic complement.

3) No exact correlation was observed between the number of secondary constrictions in the somatic complements and that of nucleoli actually found at telophase. It was interpreted by the assumption that every secondary constriction does not always express itself as the nucleolar organizer, because the functional activity of the nucleolus formation of some secondary constrictions is sometimes suppressed by the others with higher rates of activity.

4) Pre-meiotic mitosis in archesporial cells was observed in four species, and revealed polysomaty. This is of great significance in relation to meiosis.

5) Meiosis in PMCs was quite regular in the 11 species examined, excepting one species, *A. japonicum*. All the species studied, however, showed secondary association of bivalents in different degree which reached its maximum in the appearance of five groups of associated bivalents (*cf.* TAKIZAWA, '40). It is therefore assumed that an apparent basic set of 13 is derived from the original set of 5 by unequal multiplication, so that the haploid chromosome constitution in the genus *Acer* exists in the proportions

A A, B B, C C C, D D D, E E E,

of 5 sets making up the trebly hexasomic tetraploidy.

References

BERGER, Ch. A., 1938. Multiplication and reduction of somatic chromosome groups as a regular developmental process in the mosquito, *Culex pipiens*. *Carnegie Inst. Wash. Publ.*, No. **496**: 211–232.

— " —, 1941. Reinvestigation of polysomaty in *Spinacia*. *Bot. Gaz.*, **102**: 759–769.

DARLING, C. A., 1923. Chromosome behavior in *Acer platanoides* L. *Amer. Jour. Bot.* **10**: 452–457.

DARLINGTON, C. D., 1932. *Recent advance in cytology*. 1st. ed. London.

DERMEN, H., 1933. Origin and behavior of the nucleolus in plants. *Jour. Arn. Arbor.*, **14**: 282–323.

ERVIN, C. D., 1941. A study of polysomaty in *Cucumis Melo*. *Amer. Jour. Bot.*, **28**: 113–124.

FLOVIK, K., 1936. The somatic chromosomes of certain arctic species of the genus *Ranunculus*. *Soc. Sc. Fenn. Comm. Biol.*, **7**: 1–18.

FOSTER, R., C., 1933. Chromosome number in *Acer* and *Staphylea*. *Jour. Arn. Arbor.*, **14**: 386–393.

GEITLER, L., 1939. Die Entstehung der polyploiden Somakerne der Heteropteren durch Chromosomenteilung ohne Kernteilung. *Chromosoma*, **1**: 1–22.

— " —, 1940. Die Polyploidie der Dauergewebe höherer Pflanzen. *Ber. deut. Bot. Ges.*, **58**: 131–142.

GENTSCHEFF, G., and GUSTAFSSON, A., 1939. The double chromosome reproduction in *Spinacia* and its cuases I. Normal behaviour. *Hereditas*, **25**: 349–358.

— " —, and — " —, 1939. *Do.* II. An X-ray experiment. *Ibid.*, **25**: 371–386.

GRAFL, I., 1939. Kernwachstum durch Chromosomenvermehrung als regelmässiger Vorgang bei der pflanzlichen Gewebedifferenzerung. Chromosoma, **1**: 265–275.

HEILBORN, O., 1936. The mechanics of so-called secondary association between chromosomes. *Hereditas*, **22**: 167–188.

— " —, 1937. Notes on chromosome associations. *Cytologia*, Fujii Jub. Vol.; 9–13.

HEITZ, E., 1931. Die Ursache der gesetzmässingen Zahl, Lage, Form und Grösse pflanzlicher Nucleolen. *Planta*, **12**: 775–844.

— " —, 1931. Nucleolen und Chromosomen in der Gattung *Vicia*. *Ibid.*, **15**: 494–505.

HOAR, C. S., 1927. Chromosome studies in *Aesculus*. *Bot. Gaz*, **84**: 156–170.

LANGLET, O., 1932. Ueber Chromosomenverhältnisse und Systematik der *Ranunculaceae*. *Sv. Bot. Tids.*, **26**: 381–400.

LAWRENCE, W. J. C., 1931. The secondary association of chromosomes. *Cytologia*, **2**: 352–384.

LORZ, A., 1937. Cytological investigations of five chenopodiaceous genera with special emphasis on chromosome morphology and somatic doubling in *Spinacia*. *Ibid.*, **8**: 241–276.

MATSUURA. H., 1938. Chromosome studies on *Trillium kamtschaticum* Pall. VI. On the nucleolus-chromosome relationship. *Ibid.*, **9**: 55–77.

— " —, 1942. *Do.* XVI. Alterations of the nucleolus-chromosome system due to irradiation. *Ibid.*, **12**: 271–288.

MEURMAN, O., 1933. Chromosome morphology, somatic doubling and secondary association in *Acer platanoides* L. *Hereditas*, **18**: 145–173.

— " —, and THERMAN, E., 1939. Studies on the chromosome morphology and structural hybridity in the genus *Clematis*. *Cytologia*, **19**: 1–14.

MOTTIER, D. M., 1914. Mitosis in the pollen-mother-cells of *Acer negundo* L., and *Staphylea*

trifolia L. *Ann. Bot.*, **28**: 115–133.

NEMOTO, K., 1936. *Nippon-Shokubutsu-sôran-hoi* (Flora of Japan, Supplement). Tokyo.

OKSALA, T., 1939. Ueber Tetraploidie der Binde- und Fettgewebe bei den Odonaten. *Hereditas*, **25**: 132–144.

PROPACH, H., 1937. Cytogenetiache Untersuchungen in der Gattung *Solanum*, Sec. Tuberarium. I. Sekundärpaarung. *Zts. ind. Abs. Vererbgsl.*, **72**: 555–563.

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

RESENDE, F., 1937. Ueber die Ubiquität der SAT-Chromosomen bei den Blütenpflanzen. *Planta*, **26**: 757–807.

— " —, 1940. Ueber die Chromosomenstruktur in der Mitoseder Wulzelspitze. II. SAT-Differenzielungen, Spiralbau und Chromonemata. *Chromosoma*, **1**: 486–520.

— " —, 1940. Die Nukleolen bei *Antirrhinum majus* L. *Ber. deut. Bot. Ges.*, **58**: 460–470.

SATÔ, D., 1941. Karyotype alteration and phylogeny in *Liliaceae* and allied families. *Jap. Jour. Bot.*, **12**: 57–161.

SAX, K., 1931. The origin and relationships of the *Pomoideae*. *Jour. Arn. Arb.*, **12**: 3–22.

— " —, 1932. Chromosome relationships in the *Pomoideae*. *Ibid.* **13**: 363–367.

SINOTÔ, Y., 1929. Chromosome studies in some dioecious plants, with special reference to the allosomes. *Cytologia*, **1**: 109–191.

— " —, Karyotype analysis in *Paeonia*, I. *Ibid.*, **9**: 254–271.

SKOVSTED, A., 1929. Cytological investigations of the genus *Aesculus* L. with some observations on *Ae. carnea* Wild., a tetraploid species arisen byhybridization. *Hereditas*, **12**: 64-70.

SMITH, F. H., 1934. The use of picric acid with the gram stain in plant cytology. *Stain Techn.*, **9**: 95–96.

STEIN, E., 1936. Die Doppelchromosomen im Blütenbezirk der durch Radiumbestrahlung erzeugten Mutante "cancroidea" von *Antirrhiuum majus*. *Zts. ind. Abst. Vererbsl.*, **72**: 267–286.

TAKIZAWA, S., 1940. Chromosome studies in the genus *Acer* L. (Prèlim. note in Jap. with Eng. resume). *Jap. Jour. Genet.*, **16** 18-22.

— " —, (*in press*): *Do* II. Meiotic abnormalities in PMCs of *A. japonicum* var. *typicum* Schw.

— " —, (*in preparation*): *Do* III. Secondary association in *A. ornatum* Carr. var. *Matsumerae* Koidz.

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

TAYLOR. W. R., 1920. A morphological and cytological study of reproduction in the genus *Acer*. *Contrib. Bot. Labor. Univ. Penn.* Vol. V, No. 2.

UPCOTT, M., 1936. The parents and progeny of *Aesculus carnea*. *Jour. Genet.*, **33**: 135–149.

WIPF, L., and COOPER, D. C., 1940. Somatic doubling of chromosomes and nodular infection in certain *Legminosae*. *Amer. Jour. Bot.*, **27**: 821–824.

WULFF, H. D., 1936. Die Polysomatie der Chenopodiaceen. *Planta*, **26**: 275–290.

— " —, 1940. Die Polysomatie des Wurzelperiblems der Aisoaceen. *Ber. duet. Bot. Ges.*, **58**: 400 410.

Post scriptum

This paper had already been completed for publication in 1944, together with No. 2 of this series of investigations, but in unavoidable circumstances due to World War II, it was suffered to difficulties and impossible to send to press, and the publication has thus been delayed until now.

Subjects dealing with in this paper without revision or supplement, as it was written, are therefore at present out of date from stand points of recent advances in cytology. In the succeeding papers (Nos. 3 & 4 of this series in preparation), however, recent findings concerning to the subject of No. 1 of this series will be described in detail and fully discussed.

The preceding paper, No. 2 of this series, has fortunately been made a publication in Journal of the Faculty of Science, Kokkaido Imperial University, Ser. V Botany, Vol. V, pp. 263–293, 1944.,