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Meiotic Chromosome Behaviour in *Populus nigra* L. and *Toisusu cardiophylla* KIMURA

By

TIHARU SUTÔ

(With 50 figures and 2 tables)

There are many cytologists who have dealt with *Salicaceae*, namely BLACKBURN & HARRISON (1922, '24), MEURMAN (1925), ERLANSON & HERMANN (1927), SINOTO (1928, '30), MÜNTZING (1936), NILSON-EHLE (1936), NAKAJIMA (1937), TOMETORP (1937), WETTSTEIN (1937), PETO (1938), JOHNSON (1940) and others. Of those, BLACKBURN & HARRISON, ERLANSON & HERMANN, MEURMAN, SINOTO and NAKAJIMA found some evidence for the existence of heterochromosomes which are probably responsible for sex determination in *Salix*. Judging from their figures, the heterochromosomes are, however, different in different studies; for instance those observed by MEURMAN are the largest member of a set, and those described by BLACKBURN & HARRISON are of medium size, whereas, according to SINOTO and NAKAJIMA, the heterochromosome is large in some species and small in others. On the other hand, recent studies (MÜNTZING 1936, PETO 1938 and JOHNSON 1940) have failed to detect any unequal pair.

The present paper deals with microsporogenesis in two dioecious species of *Salicaceae*; *Populus nigra* L. and *Toisusu (Salix) cardiophylla* KIMURA. The latter species was considered before as a primitive species belonging to *Salix*. Prof. KIMURA, a famous Salicologist in Japan, has, in his recent work, separated this species from *Salix* and included it to a new genus *Toisusu*, on the ground of the fact that it is much alike *Chosenia*, rather than *Salix* and *Populus*.

Material of *Populus nigra* was obtained from plants cultivated at the campus of our university, and of *Toisusu cardiophylla* from suburbs of Sapporo, where they are abundantly found.

Methods. PMCs were fixed in aceto-alcohol (a mixture of glacial acetic acid 1 parts and absolute alcohol 3 parts) at about the middle of May. The sections were cut in a thickness of 15μ and stained in HEIDENHAIN'S

iron haematoxylin. Only PMCs of triploid aspens were thoroughly studied in permanent smears, LA COUR 2 BE having been employed as fixative, and stained in gentian violet.

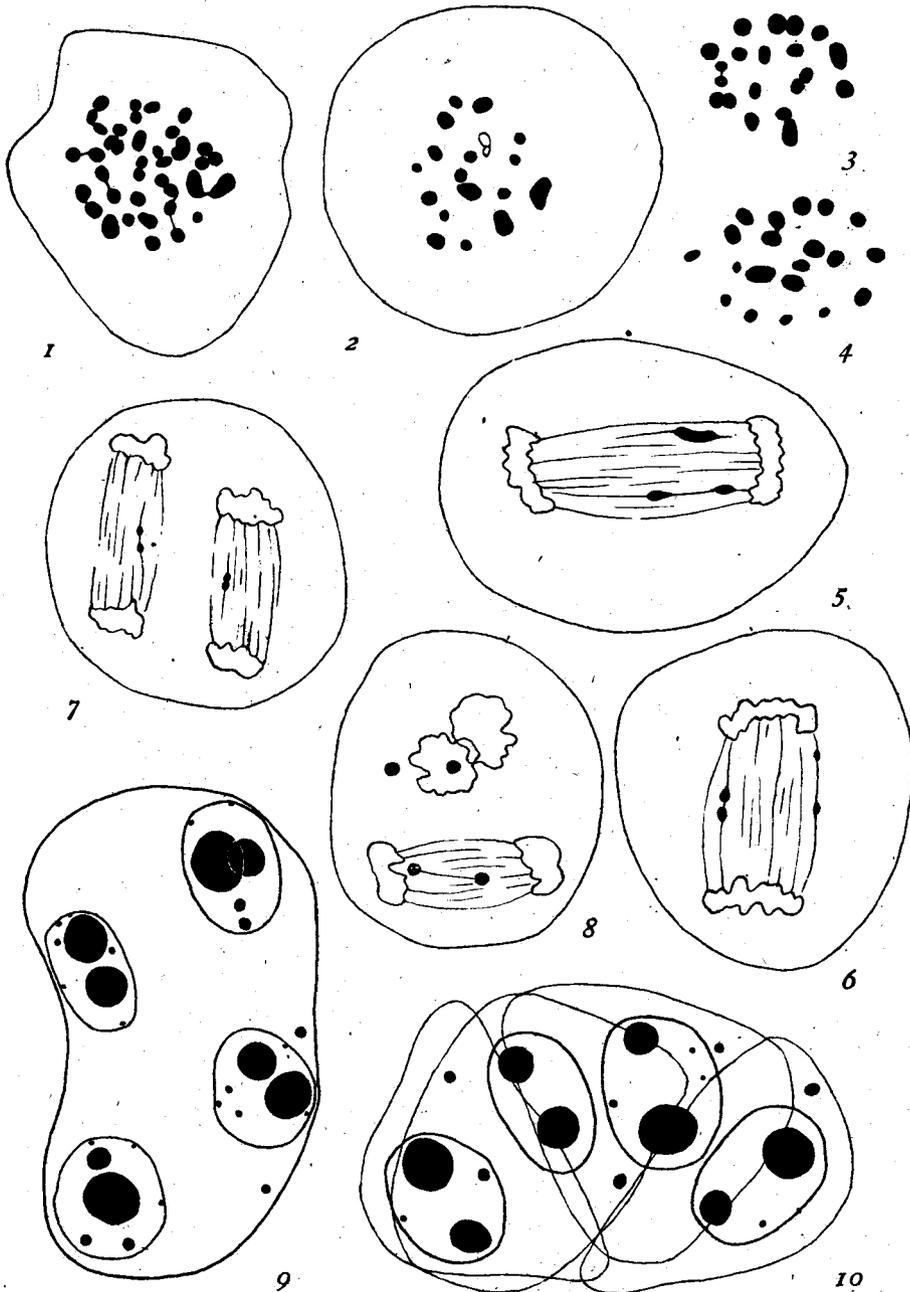
All the figures in this work were drawn with the camera lucida, using a LEITZ objective 1/12 oil immersion and a ZEISS compensating ocular K. 20, at a magnification of about 3500 times.

I. Meiotic chromosomes of diploid plants

Populus nigra. Meiosis was found to be regular, and nineteen chromosomes are always present at the first and second metaphase (Fig. 20). There is no evidence suggesting any abnormality of meiosis. All of bivalents differ in size from one another. In their studies on meiosis of *P. tremula*, BLACKBURN & HARRISON (1924) and MÜNTZING (1936) pointed out that the idiogram comprises bivalents of three different size classes, namely, nine small bivalents of more or less uniform size, nine larger than them, and a remaining one much bigger than the former ones. The present observation seems to be accordant with these statements and differs from that of PETO (1938), in which he reported all of bivalents to be of equal size.

On closer observation, the complement of this plant seems to consist of bivalents which can be grouped into five types: (1) one very large, (2) three large, (3) six medium, the smallest one of those being heteromorphic as will be later described in detail (p. 146-149), (4) six small, and (5) three very small. There is also slight difference in size within members of each class. For making the comparison of those bivalents more clear, see figures 20 and 21. As one can see in those figures, the difference is not so great between the members of the following bivalents: 3-4, 5-6-7, 8-9-10, 12-13-14, 15-16 and 18-19.

Toisusu cardiophylla. The course of meiosis is regular. The idiogram is much similar to that of *P. nigra*, and different from that of *Salix* species in that at least one bivalent is distinctly larger than the others. There is little variation in the chromosome size within a set, as compared with *Populus* and hence it is difficult to clearly identify all the members of a set. The haploid chromosome number is nineteen, and there is nearly always one quadrivalent besides normal seventeen bivalents in the complement (Figs. 2-4). The quadrivalent consists of two bivalents referred as 8 and 9 (Figs. 29-30). The total 100 cases of configurations of these two chromosome pairs were analysed, and they were found to constitute a ring-shaped quadrivalent, a chain-shaped one and two separate bivalents in the pro-



Figs. 1-10. Meiosis in diploid *Toisusu cardiophylla*: 1-4. First metaphase in polar view; 1, a tetraploid PMC. 2-4, normal diploid PMCs (Fig. 2 showing $1_{IV}+17_{II}$, one of which is heteromorphic. 3, $18_{II}+2_{I}$). 5-6. First anaphase in side view. 7-8. Second anaphase in side view. 9-10. Pollen tetrads representing chromatin bodies outside the nucleus.

portion of 80:2:18.

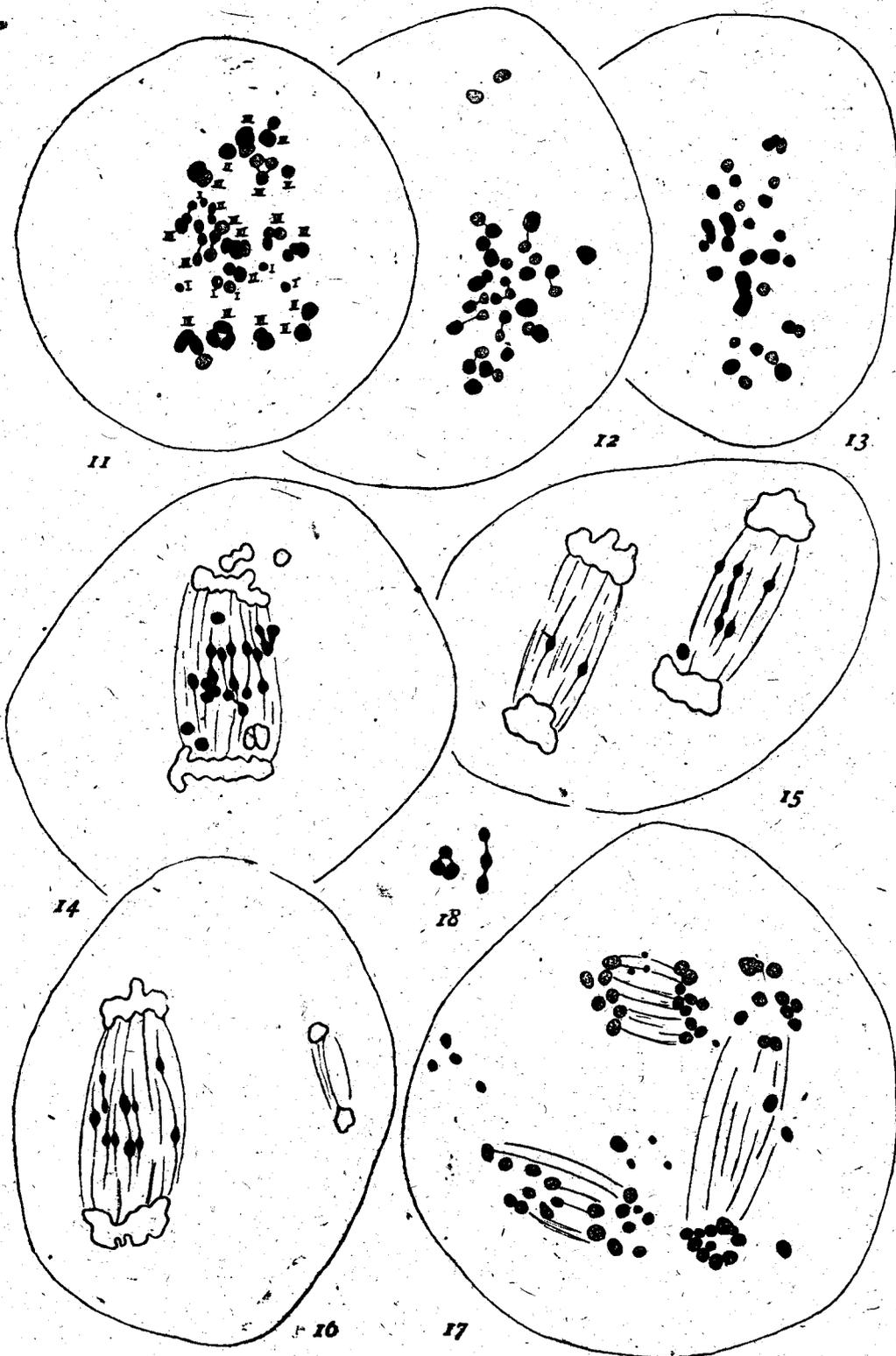
At the first division of meiosis, univalents are rarely met with. The chromosome configuration in Fig. 3 is represented as $18_{II}+2_I$ and two small univalents in it may have possibly come from either 18th or 19th bivalent. At the first anaphase some lagging chromosomes are sometimes observed (Fig. 5). The second division and the formation of tetrads seem to take place rather regularly and the resulting young pollen grains appear uniform in size. Undivided or divided lagging chromosomes remain sometimes at the equatorial plane and form chromatin bodies outside the nucleolus. Figures 6-8 represent these bodies which in the tetrad and young pollen grains (Figs. 9 & 10) express themselves just like nucleoli. Such chromatin bodies have been described by JOHNSON (1940) in a triploid clone of *P. tremula*.

2. Triploid plant of *P. nigra*

A triploid giant plant has been found in our campus. Its leaves and catkins are larger than those of diploid plants, as usual in other autotriploid plants. This plant was pure male. The triploidy of aspens has been found by several authors: MÜNTZING (1936), TOMETORP (1937), WETTSTEIN (1937) and JOHNSON (1940) in *P. tremula*, and PETO (1938) in *P. alba* and *P. canescens*. In their data, the triploid plant is either pure male or pure female, but never intersexual. Such a manner of sexuality agrees with that of polyploid *Melandrium* reported by WESTERGAARD (1940).

In all cases hitherto investigated, meiosis was of the same appearance as studied by MÜNTZING in triploid *P. tremula*. Various irregularities were sometimes found in all course of meiosis. The first metaphase is characterized by varying number of trivalents and univalents in addition to bivalents, as seen by Figs. 11-13. Fig. 11 shows a configuration of which is usually met with. Configurations of trivalents were of either ring or rod shape (Fig. 18). It will, therefore, be inferred that this triploid aspen is of an autopolyploid nature. The members of univalents were frequently less than those of bivalents, so that the multivalent association between non-homologous univalents will occur. This finding, as well as the phenomenon of secondary association of bivalents, may be recognized as an evidence of affinity between some of the bivalents (p. 152). Both the first and second anaphase represent some irregularities, such as lagging bodies, bridges of chromosomes and multipolared divisions (Figs. 14-17).

There are rarely tetraploid PMCs mixed with normal ones in both diploid *Populus* and *Toisusu*, which have thirteen eight bivalents (Fig. 1).



Figs. 11-18. Meiosis in triploid *Toisusu cardiophylla*: 11-13. First metaphase in polar view, 11 showing $13_{III} + 6_{II} + 6_I$. 14. First anaphase with many lagging chromosomes. 15-16. Second anaphase having lagging bodies and a chromosome bridge, one division (in fig. 16) being very poor. 17. Second anaphase showing tripolar division. 18. Two types of trivalents.

The occurrence of tetraploid PMCs will furnish an opportunity for the formation of diploid gametes. Owing to the fact that triploid aspens have been often found in various localities, diploid gametes resulted from tetraploid PMCs will be regarded as not so unusual in natural conditions. Thus, it may be inferred that such an autotriploid clone has been derived from a conjugation between normal gametes and such unreduced ones.

3. Heterochromosomes and sex

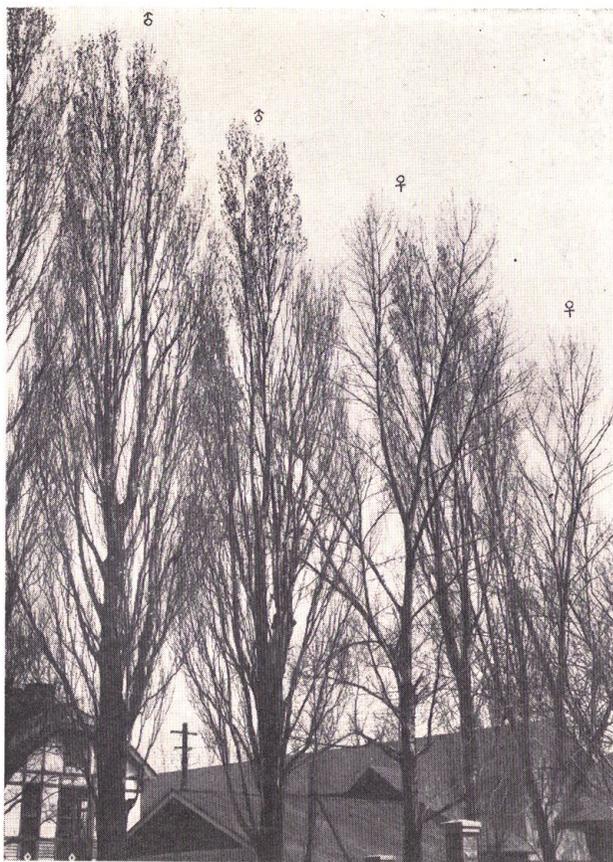


Fig. 19. Male and female trees of *Populus nigra* characterized by the specific crown.

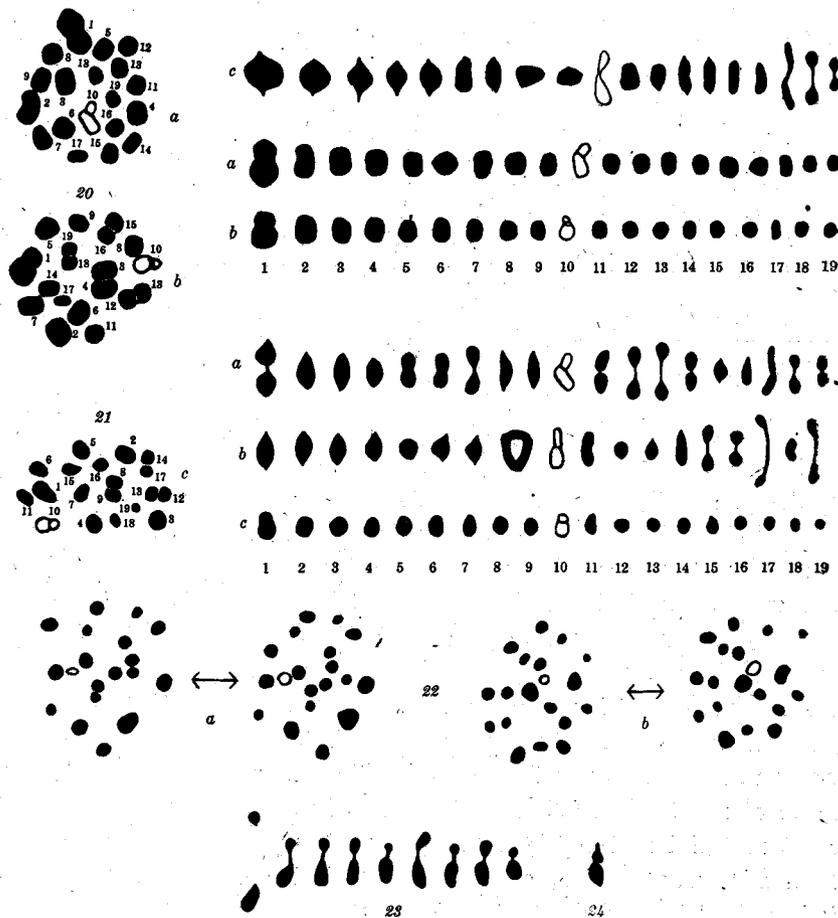
In *P. nigra*, the sex difference accompanies a certain difference in growth habit, that is, the male tree has branches spreading at acute angle and forming a rod crown, whereas the female tree is characterized by branches spreading widely and forming a conical crown (Fig. 19). In *Toisusu*, such difference between male and female trees does not exist at all.

From the alignments of metaphase bivalents which were designated by the numbers from 1 to 19 according to their size (Figs. 20 & 21), it will be seen that there is a distinct similarity

in the chromosomal organisation between *P. nigra* and *T. cardiophylla*, and that there is a pair of heterochromosomes which are numbered as 10 in both species. The existence of the heteromorphic pair was further

ascertained by chromosomes at the first anaphase in *Toisusu* (Fig. 22). This finding agrees with that of BLACKBURN & HARRISON (1924), and differs from that of MEURMAN, SINOTO and NAKAJIMA mentioned already (p. 141).

The heterochromosomes behave themselves regularly as other normal



Figs. 20. Bivalents of *Populus nigra* in serial arrangement; a and b in polar view, c in side view. 10th-bivalent is the heteromorphic pair. 21. Bivalents of *Toisusu cardiophylla*. 22 a. and b. Pairs from anaphasic plates, heterochromosome pair being very conspicuous. 23. Heterochromosome pairs of *Populus nigra* in side view. 24. A heterochromosome pair of *Toisusu cardiophylla* in side view.

bivalents. There is no indication of such special behaviours as heteropycnosis, premature or retarded division etc., as have been often observed in sex chromosomes of other species. Figs. 23 and 24 represent these heterochromosomes, in which the smaller one of the pair seems to be fairly variable in size.

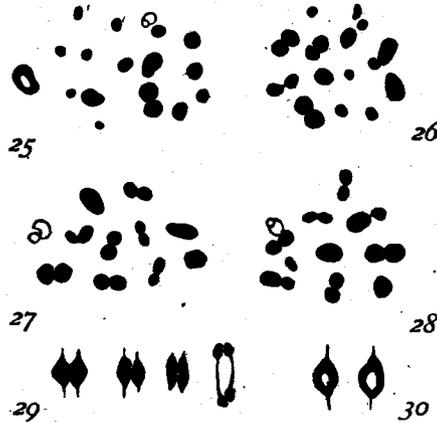
From the data mentioned above, it is assumed that triploid plants as well as diploid plants are strictly dioecious, being either pure male or pure female. According to WESTERGAARD (1940), this type of sex determination is the typical one of "*Vallisneria*" type, in which polyploid plants are dioecious as in diploid ones. He assumed that the Y chromosome contains a very strong element which determines maleness, so that zygotes taking only one of Y chromosomes should all give rise to male plants irrespective of how many X chromosomes they have. In the present case, it is assumed that the smaller half of the pair represents an Y chromosome and the large half an X chromosome; male trees may be of the complement of $2n=36+XY$, and triploid trees with the complement of either $3n=54+XYY$ or XXY may be male and only those with the complement $3n=54+XXX$, female.

4. Secondary association

Although rather intense studies on chromosomes in *Salicaceae* comprising *Salix* and *Populus* have been done by many workers, the phenomenon of secondary association has never been reported. On the basis of the secondary association which has been taken as an evidence of genome complexity, DARLINGTON and MOFFET (1930) concluded that the haploid number of *Pyrus*, seventeen, has been derived from a primary haploid number of seven. It is well known that the species of *Salicaceae* resemble those of *Rosaceae* in both cytological and taxonomical points of view (BLACKBURN & HERRISON 1924). It is then suggested that the haploid number of nineteen in *Salicaceae* may not be the primary number. The present material fortunately serves as one of good examples concerning the secondary association, since such association can be observed fairly well in polar views of the first metaphase.

At the diakinesis nineteen bivalents lie free from each other, and then any association of more two chromosomes is not found. All members of bivalents are united by single terminal chiasma (Figs. 20 & 21). At the premetaphase some of the bivalents come to lie close together, and at the polar views of first metaphase secondary association is more conspicuous.

The secondary association in *P. nigra* is represented in Figs. 31-50, in which 31-38 are of usual types occurring commonly, and the others are of rare types, and that in *T. cardiophylla* in represented in Figs. 25-29. Among these figures, Fig. 35 shows one of the maximum associations of bivalents demonstrated in the aspen, which represents nine groups of two bivalents and one single heteromorphic bivalent. Fig. 28 represents one of the maximum associations of bivalents in *Toisusu*, one group of three, six of two, two singles and one quadrivalent. Frequently, secondary associations consisting of more than two bivalents were found to occur.



Figs. 25-30. Secondary associations in *Toisusu cardiophylla* (first metaphase): 25, 1iv+1(2)+15(1). 26, 5(2)+9(1). 27, 1iv+7(2)+3(1). 28, 1iv+1(3)+6(2)+2(1). 29, Secondary associations occurring between two bivalents in side view, 3-4, 6-7, 8-9 and 15-16 from left. 30, Quadrivalents consisting of 8- and 9-bivalent, in side view.

The frequency of variable types of secondary associations is given in Table 1. Summing up, a total of 1900 bivalents in the aspen were observed,

Table 1. Frequencies of secondary association types at the first metaphase.

Groups of secondary association	Single chromosomes	<i>Populus nigra</i>	<i>Toisusu cardiophylla</i>	Groups of secondary association	Single chromosomes	<i>Populus nigra</i>	<i>Toisusu cardiophylla</i>
0	19	1	0	1(3)+4(2)	8	2	7
1(2)	17	1	1	1(3)+5(2)	6	4	3
2(2)	15	3	15	1(3)+6(2)	4	7	0
3(2)	13	10	29	1(3)+7(2)	2	2	0
4(2)	11	9	42	2(3)+2(2)	9	0	1
5(2)	9	15	28	2(3)+5(2)	3	1	0
6(2)	7	20	15	3(3)+4(2)	2	1	0
7(2)	5	6	8	1(4)+2(2)	11	1	0
8(2)	3	8	1	1(4)+3(2)	9	1	0
9(2)	1	1	0	1(4)+5(2)	5	1	0
1(3)+1(2)	14	1	3	1(4)+1(3)+3(2)	6	2	0
1(3)+2(2)	12	2	3				
1(3)+3(2)	10	1	7				
				Total		100	163

of which 787 (41%) were single, 1112 (59%) associated in groups of two and rarely three or four bivalents. In *Toisusu*, of total 3097 bivalents, 1712 (55%) were single and 1385 (45%) associated in groups. On the average, half the members of nineteen bivalents show thus the secondary association at the first metaphase, although the ratio of association in the aspen is slightly higher than that of *Toisusu*.

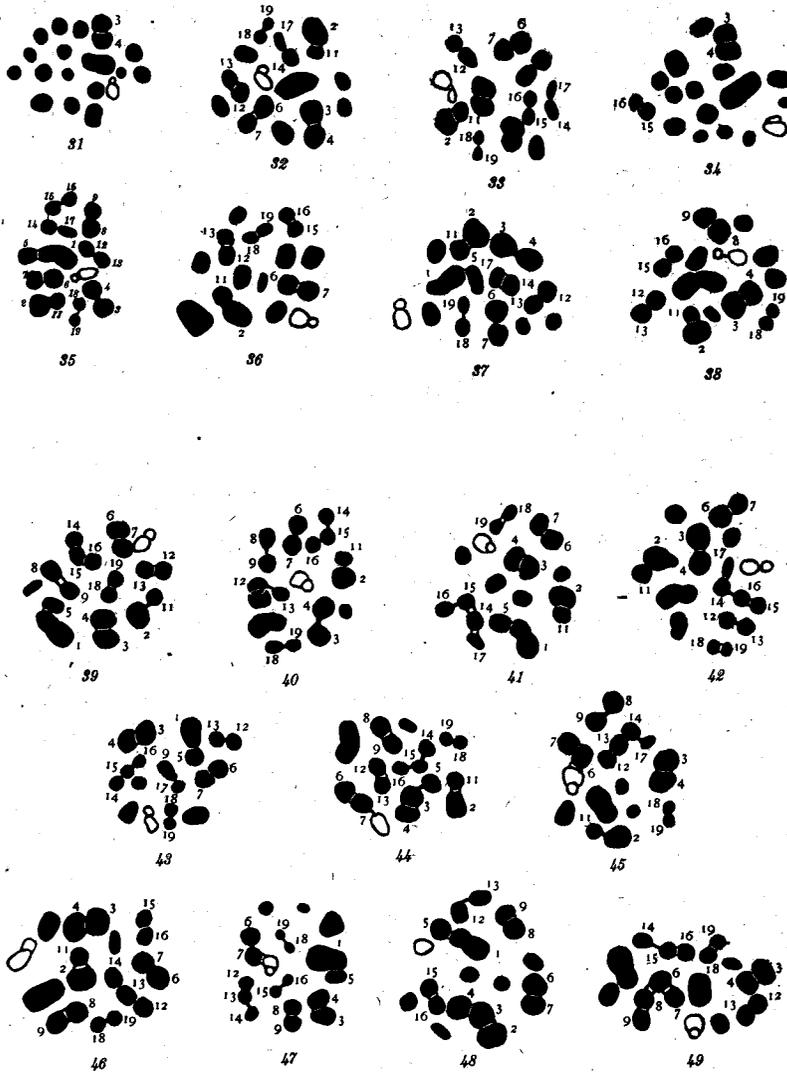
All of the bivalents of a complement can usually be identified, as already mentioned. Thus, it can be detected with certainty between what types of bivalents the secondary association occurs. The results are summarized in Table 2. An important feature of the results is that the associa-

Table 2. Frequencies of the secondary association in *Populus nigra* occurring between each bivalent. (From the observation of 100 PMCs).

Pairing bivalents	Numbers observed	Pairing bivalents	Numbers observed
1-5	32	6-7-8 6-7-8-9	6 } 7 1 } 7
2-11	48	7-10* 6-7-10*	1 } 9 8 } 9
3-4	76	14-15-16	10 } 11 1 } 11
6-7	48	14-17-15-16	
8-9	41	12-13-14 12-13-14-17	2 } 3 1 } 3
12-13	62	3-4-5	2
14-17	39	9-17	2
15-16	65	5-11	1
18-19	73	12-13-14-15	1

* 10-bivalent is the heteromorphic pair.

tion occurs only between specific bivalents and shows no direct connection with the similarity in chromosome size. Accordingly in some cases, similar sized bivalents pair with each other, for instance, 3-4, 6-7, 8-9, 12-13, 15-16 and 18-19, while in the others, different sized bivalents show secondary pairing, for instance, 1-5, 2-11 and 14-17. This fact may suggest that certain partial homologous regions exist between these specific bivalents, but the homology is so weak or limited that those bivalents as a whole cannot form multivalents at the prophase, but secondarily associate at the prometaphase. RICHHARIA (1937) has found in some species of *Brassica* that the secondary association takes place between morphologically different chromosomes. He concluded therefore that "fragmentation, reduplication, translocation, segmental interchange etc. have possibly been



Figs. 31-49. Secondary associations in *Populus nigra* at first metaphase polar-viewed. **31-38.** Usual types of association: **31**, 1(2)+17(1). **32**, 6(2)+7(1). **33**, 8(2)+3(1). **34**, 2(2)+15(1). **35**, 9(2)+1(1) (maximum association !). **36**, 5(2)+9(1). **37**, 7(2)+5(1). **38**, 6(2)+7(1). **39-49.** Rarely occurring types of association: **39**, 2(3)+6(2)+1(1). **40**, 1(3)+6(2)+4(1). **41**, 1(4)+5(2)+5(1). **42**, 1(4)+5(2)+5(1). **43**, 1(3)+6(2)+4(1). **44**, 3(3)+4(2)+2(1). **45**, 1(4)+1(3)+4(2)+4(1). **46**, 1(3)+6(2)+4(1). **47**, 2(3)+5(2)+3(1). **48**, 1(3)+5(2)+6(1). **49**, 1(4)+1(3)+3(2)+6(1).

responsible for the evolution of *Brassica*." MEURMAN (1933) states in *Acer* that at the first metaphase two largest bivalents of a complement, which differ in size, show secondary pairing, although this is doubted by TAKIZAWA (unpublished). From Table 2, it is clear that secondary association with the frequency of more than 30 per cent occurred between the following bivalents; 1-5, 2-11, 3-4, 6-7, 8-9, 12-13, 14-17 and 18-19. Besides them, the associations of (14-17)-(15-16) occur in 11 per cent, those of (6-7)-(8-9) in 7 per cent and those of (6-7)-10 (heteromorphic pair!) in 9 per cent. There are further other types of association which occur very rarely and may be considered as due to bad fixation.

Of course, it is rather premature to draw from these data any conclusion on the primary basic number in these plants. However, if one admits the theory of secondary pairing, *Populus* and *Toisusu* will be regarded as secondary polyploids, and their nuclear organization may be represented as: (1-5), (2-11), (3-4), (8-9)-(6-7)-10, (12-13), (14-17)-(16-15) and (18-19). Thus the primary basic number might be well inferred as seven.

There is nearly always a quadrivalent consisting of 8th and 9th bivalents in *T. cardiophylla*. In most cases (82%) these chromosomes form a quadrivalent and in cases of 18 per cent appear as bivalents. This pairing may not be due to any structural hybridity, but may be more properly considered as due to a high degree of homology between the chromosomes concerned in the same way as the pairing between non homologous chromosomes in the triploid aspen as mentioned (p. 144).

Acknowledgments. The present writer wishes to express his sincere gratitude to Professor H. MATSUURA, with whose careful guidance and criticism this work has been made possible. He is also obliged to Professor KIMURA of the Botanical Institute of Tohoku Imperial University, by whom a clew of this work was given. The present work was aided by a grant from the Scientific Research Fund of the Department of Education, to which the his sincere thanks are also due.

Summary

- 1) A somewhat detailed account is given of the chromosome behaviour during the meiotic divisions in *Populus nigra* and *Toisusu cardiophylla*.
- 2) The bivalents are classified into five distinct classes according to their size. More precisely, all of the bivalents of a complement are identifiable by minor size differences. This distinction is more conspicuous in *P. nigra* than in *T. cardiophylla*.
- 3) The idiogram of *Toisusu* resembles that of *Populus* rather than

Salix.

4) A new triploid clone of *P. nigra* has been found in our campus. The meiosis of this triploid clone is similar to that described by MÜNTZING. It is discussed that tetraploid PMCs observed in diploid clones will furnish some evidence for the occurrence of such an autotriploid clone.

5) Two medium-size chromosomes exhibit an evidently unequal pairing, suggesting sex-chromosomes. In both species, this heterochromosome pair is the 10th in size within a complement.

6) The male and female trees in *P. nigra* are characterized by a certain difference in stature. The determination of sex in both diploid and triploid plants is discussed.

7) In the PMCs at the first metaphase, the specific bivalents exhibit strong secondary associations, their frequencies being summarized by Table 2.

8) The possibility of a still lower primary basic chromosome number is discussed from such secondary associations.

9) An evidence for the secondary association seems also to be gained from a quadrivalent in *T. cardiophylla* and from the univalent behaviour in a triploid clone of *P. nigra*.

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Postscript. After this paper was accomplished, a recent publication by C. VAN DILLEWIJN entitled "Zytologische Studien in der Gattung *Populus* L." (*Genetica*, 22: 131-183, 1940) was received. In it, he reports on a larger scale irregular behaviours of meiosis in seven species of *Populus*, including a triploid race of *P. alba* and secondary associations in *P. nigra* and *P. nigra* var. *italica*. With respect to the last subject, he has attacked essentially the same point as that attacked in the present paper. It is stated by him that eight groups of chromosomes are always found when secondary association reaches its maximum and that *P. nigra* is of a secondary polyploid nature, the original basic number being 8 as shown by the formula: "A'A-BBB-CCC-DDD-EE-FF-GG-HH". From the

assumption that A'-bivalent is probably formed by a fusion of two A-bivalents, he concludes that the original bivalent number in *Populus* is 20 and during the metaphase I bivalents are associated in four groups of two and four groups of three. This statement is different from that made by the present writer. Nevertheless, judging from DILLEWIJN's figures,

each type of bivalents associated secondarily seems to entirely correspond to that observed by the present writer. The writer's designation is therefore applied to DILLEWIJN's figures as Fig. 50.

Thus, it is beyond doubt that there are definite secondary pairings in *Populus*, in spite of minor differences in opinion between DILLEWIJN and the present writer. As to the details, it is impossible to settle this disagreement of opinions, because, with respect to the secondary association, no statistical details are given in the paper of DILLEWIJN.

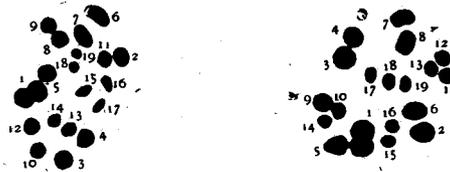


Fig. 50. Figures showing secondary association among bivalents at first metaphase in meiosis, drawn by DILLEWIJN. The designations of chromosomes are given by the present writer.