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**On the Secondary Association of Meiotic
Chromosomes in *Tricyrtis latifolia* MAX.
and *Dicentra spectabilis* MIQ.**

By

HAJIME MATSUURA

(With Plate XVIII and 20 Text-figures)

The theory of secondary association of chromosomes, originally advocated by DARLINGTON ('28) and further systematized by LAWRENCE ('31), is of significance in having presented a clew to the analysis of polyploidy where numerical considerations are not available or fail to elucidate it. It implies that chromosomes displaying this kind of affinity are those closely related with each other, thus enabling us to conjecture the ancestral chromosomal type of an apparent diploid form now existent. The theory has since attracted much attention from cytologists and has been repeatedly substantiated by many observations (*cf.* MEURMAN '33).

It is a generally admitted fact that the secondary association of meiotic chromosomes manifests itself first at pro-metaphase of the first division, and the juxtaposition of the paired bivalents may very often be maintained at metaphase, but they show no association at the preceding diakinesis. DARLINGTON ('32) holds an opinion that the mechanism of chromosome distribution on the metaphase plate is modified by some force of attraction of this kind which is specific to chromosomes, "making it no longer comparable with that given by floating magnets" (p. 339), as advanced by KUWADA ('29) and FUJII ('32). It seems quite difficult however to explain solely in terms of repulsion and attraction why the same affinity does not exert influence at diakinesis in spite of its manifestation at metaphase, because both the diakinetic and metaphasic stages may be considered as essentially the same in the respect of repulsion, save that the former represents a repulsion phase of three dimensions; *i.e.*, within the nuclear cavity, while the latter is one of two dimensions, *i.e.*, the equator, and furthermore the bivalent chromosomes showing association at metaphase are known not to be connected materially with each other.

The present study has been carried out, with the aim to inquire into

this matter, that is, the relationship between chromosome arrangement on the metaphase plate and secondary association.

Material and Methods

The present paper deals with two species, *Tricyrtis latifolia* MAX. and *Dicentra spectabilis* MIQ. Material of the former was obtained from Mt. Zaô in Yamagata Prefecture where the plant is abundantly found. Material of the latter comes from a plant cultivated at the experimental garden of our University.



Text-fig. 1. A portable smear set. ca. 1/8

The PMC was thoroughly studied in permanent smears, TAYLOR'S solution having been employed as the fixative, and stained with iodine gentian-violet in the usual way. The permanent preparations of *Tricyrtis* were made at Mt. Zaô, with the aid of a portable smear set devised by the writer (Text-fig. 1).

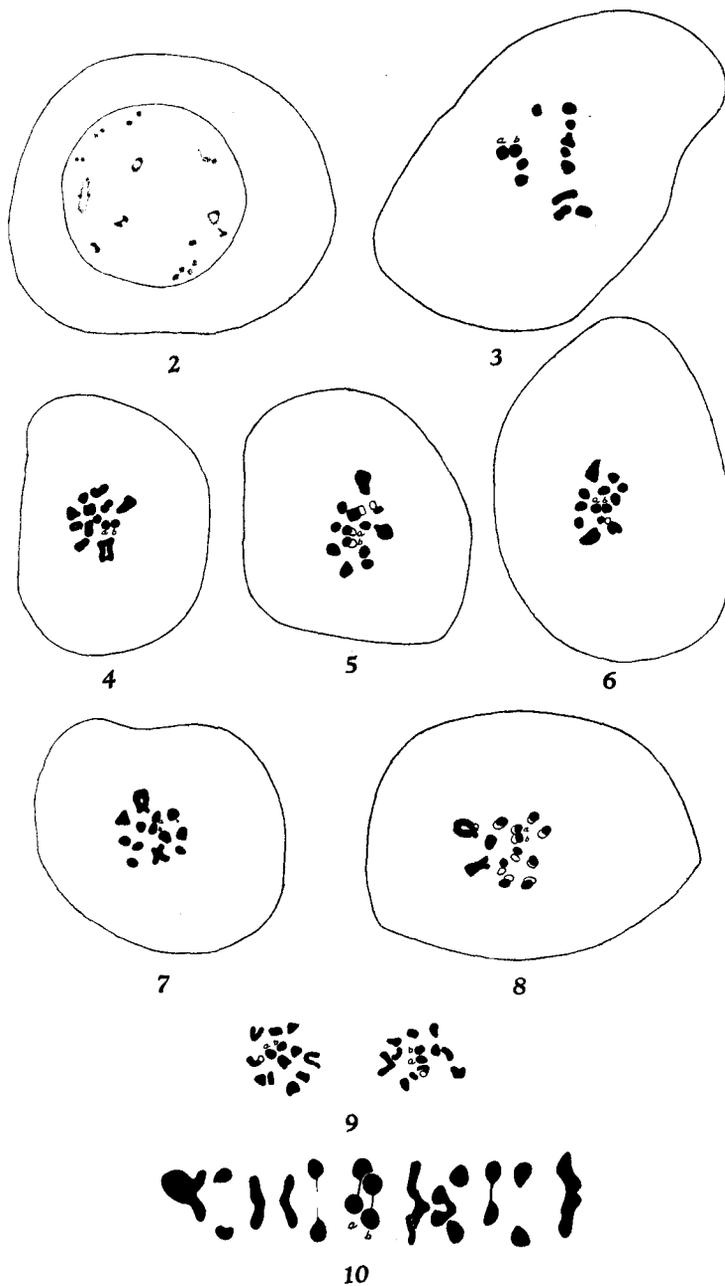
The drawings were made with a Zeiss 2 mm. objective N.A. 1.4 and $15\times$ (for Text-figs. 2-9) or $30\times$ (for Text-figs. 10-19) eyepieces. The magnification reproduced is given under the figure explanations.

In making preparations the writer has been assisted by Mr. T. SUTÔ, to whom he wishes here to express his cordial thanks.

Observations

i) *Tricyrtis latifolia* MAX.

No attempt was made to study the early prohasic stages. Text-fig. 2 represents a nucleus at diakinesis. Though the chromosomes at this stage are very faintly stained and of vague contour, they are easily found to consist of 13 gemini which are scattered enarly equidistantly from one



Text-figs. 2-10. Division I in pollen mother-cells of *Tricyrtis latifolia*. The associated bivalents are indicated by letters, *a* and *b*. 2, diakinesis. 3, pro-metaphase. 4-8, mid-metaphase. 9, anaphase. 10, side view of metaphase; the chromosomes were spaced out in drawing. 2-9, ca. $\times 1200$; 10, ca. 2400.

another throughout the nuclear cavity. At the onset of pro-metaphase (Text-fig. 3), it can be seen that two pairs of chromosomes which are of equally medium size come in close proximity with each other. Thus the association of hitherto separated bivalents appears to be a characteristic feature of pro-metaphase, as pointed out by LAWRENCE ('31) and others. These secondarily paired bivalents are also clearly noticeable in both polar and side views of every metaphasic plate (Text-figs. 4-10; Plate XVIII, Figs. 1-7). It seems to be of frequent occurrence that the chromosomes at metaphase orientate themselves in such a way that the two big bivalents take their position at the periphery of the plate and the associated bivalents lie near its center.

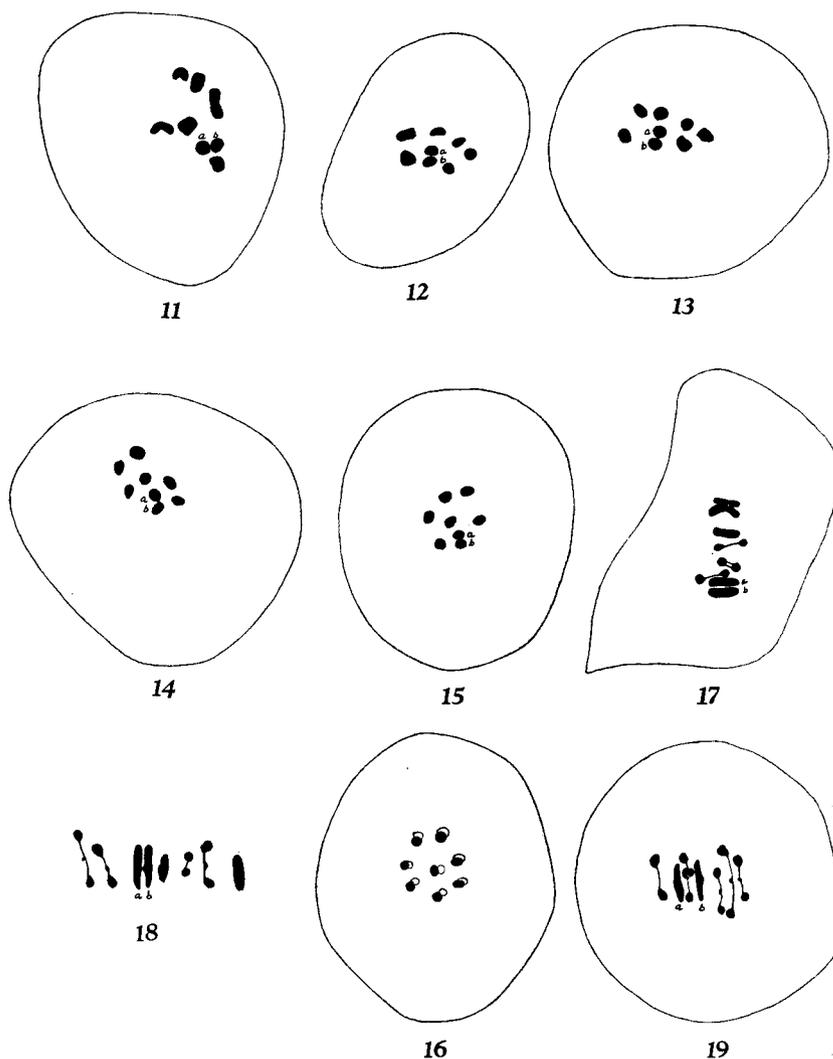
ii) *Dicentra spectabilis* Mrg.

At pro-metaphase (Text-fig. 11), eight bivalents are easily distinguishable, two of which usually associate very closely. In the majority of metaphase plates, the associated bivalents are also clearly observable (Text-figs. 12-15, 17-18; Plate XVIII, Figs. 8-9, 11-13). Sometimes the paired bivalents lie in such close juxtaposition that the observer is easily liable to take them as a single bivalent (see Text-fig. 15; Plate XVIII, Fig. 11). As in the previous case, the paired bivalents are entirely similar in size and configuration.

It is remarkable however that a few plates represent no obvious secondary pairing, eight bivalents being scattered almost nearly regularly and equidistantly (Text-fig. 16; Plate XVIII, Fig. 10). In these cases, otherwise associated bivalents seem to lie next to each other, as indicated by the side-view analysis of the plate (Text-fig. 19). Analogous cases have been reported by MEURMAN ('33) in *Acer* and by MOFFETT ('34) in pears. This so-called variability in the amount of secondary association seems apparently to make a contrast with the case in *Tricyrtis* where the association appears to take place regularly in every case, but this is based on too few observations to be considered as significant.

Discussion

For the critical study of secondary association, the effects of fixation must be taken into consideration in the first place, as already pointed out especially by LAWRENCE ('31). The present materials, *Tricyrtis latifolia* and *Dicentra spectabilis*, are very suitable in this respect, because they have a relatively low number of chromosomes, namely, 13 and 8, respectively, as the haploid set. It is quite probable that if the chromosome



Text-figs. 11-19. Division I in pollen mother-cells of *Dicentra spectabilis*. The associated bivalents (or the bivalents to be otherwise associated when they are free from each other) are indicated by letters *a* and *b*. 11, pro-metaphase. 12-16, polar views of metaphase. 17-19, side views of metaphase. In Fig. 18, the chromosomes were spaced out in drawing. ca. 2250.

number were greater the chance of false association or "clumping" induced by poor fixation would be correspondingly increased.

The theory of secondary association of chromosomes at meiosis implies that the paired bivalents are originally related to each other. It is insisted

by several authors (especially LAWRENCE '31) that in the absence of multivalents, the secondary association provides the only available criterion of chromosome homology. In the present materials, as described on the previous pages, only two bivalents show a marked secondary association at pro- and mid-metaphases of the first division. In accordance with the demands made by the theory, *Tricyrtis latifolia* should be a secondarily balanced diploid, derived from an ancestral form having a haploid set of 12 units, and similarly *Dicentra spectabilis* should have been derived from a form in which the haploid number is 7.

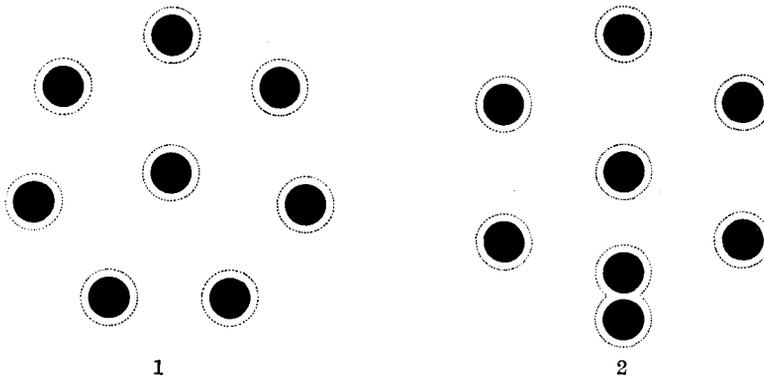
The genus *Tricyrtis* has been karyologically dealt with by several investigators. NAWA ('28) studied somatic as well as meiotic chromosomes in four species, *T. hirta*, *T. formosana*, *T. stolonifera* and *T. macropoda*; MILLER ('30) described somatic chromosomes in *T. macropoda*, *T. pilosa*, and *T. stolonifera*; more recently SINOTÔ & KIKKAWA ('32) gave a brief account, confirming the results by NAWA and also determining the haploid number in *T. macrantha*, *T. Yatabeana*, *T. japonica* and *T. dilatata*. All these investigators agree with $n=13$ in every species. No account has been given however as to such association of two bivalents at meiosis, as seen in the present case, *T. latifolia*. In this connection, it may be noteworthy that ISHIKAWA ('16) described in his chromosome list the haploid number of *T. hirta* as "13 often 12." This may most probably have been due to the close association of two bivalent chromosomes. Indeed his figure (Fig. 32) of meiotic polar view is strongly suggestive of this. Similar configuration is also suggested by some figures of meiotic metaphase in *T. hirta* given by NAWA ('28), e.g. Pl. I, Fig. 4. It will then constitute a further interesting problem to determine whether such secondary association as in the present case is a property common throughout the genus *Tricyrtis*.

To the genus *Dicentra*, little attention has been paid from a karyological point of view, save that SAKAI ('34) reports that the somatic chromosome number of *D. peregrina* var. *pusilla*, an alpine plant, is 16. A study on meiotic chromosomes in this plant is then desirable in comparison with the present material, *D. spectabilis*.

The occurrence of non-association of the otherwise associated bivalent chromosomes, such as found in *D. spectabilis*, seems to be of theoretical importance in connection with the relationship of the mechanism of chromosome arrangement on the metaphase plate to secondary pairing. The writer attempted to attack the problem from two angles, namely, (i) the behavior of the associated bivalents at metaphase and (ii) the structure

of chromosomes.

i) If one takes for granted the floating magnet theory of chromosome arrangement, the secondary pairing should naturally interfere with the free and independent behavior of the chromosomes. Since the meiotic chromosomes of *D. spectabilis* are of nearly equal size, it should offer favorable material for the inquiry into the subject. To the writer's regret however he could not obtain a sufficiently large number of metaphase figures to admit making a statistical study on this point, but the following statements seem evidently to be possible: (a) that in cases where no association takes place, the eight bivalents arrange themselves on the metaphase plate in a form suggested by eight floating magnets (*cf.* Text-fig. 16; Plate XVIII, Fig. 10), and (b) that in cases where the association is complete, they behave as if they were *seven* independent units (*cf.* Text-fig. 15; Plate XVIII, Fig. 11). These two modes of arrangement are diagrammatically represented in Text-fig. 20.



Text-fig. 20. Diagrams showing two possible modes of arrangement of the 8 bivalents on the metaphase plate in *Dicentra spectabilis*. The karyomere is represented by outer circles. Diagram 1 shows the chromosomes free from association; Diagram 2 represents a case in which two bivalents are completely associated. The possible effects on the arrangement to be expected from the change in size of the 'unit' owing to association was neglected in Diagram 2.

ii) The chromosomes do not come directly in touch with the plasm, except the so-called polar granule or the real point of attachment. They lie in the "karyomere cavity" or surrounded by the "chromosome sheath." The clearest evidence for this has been obtained by the writer in materials having large chromosomes, such as *Trillium*, *Rhoeo*, etc. (unpublished). This has been observed only in good smear preparations in which a chromonema structure is evidently presented (*cf.* MATSUURA, '35, Plate I and II).

In the present material, no evidence of spiral structure of meiotic chromosomes was obtained, probably owing to their minute size, but in some favorable cases (*cf.* Plate XVIII, Fig. 13) the karyomere surrounding each bivalent was evidently visible and *the paired bivalents were found to lie in the same cavity.*

It may be then inferred that the attraction force acting on the particular chromosomes at pro-metaphase, though we have no knowledge about its real nature, must result in the fusion of their karyomere cavities during their migration and on account of the retention of the fused cavity, the chromosomes are subject to remain associated in the subsequent stage and as the consequence act as a single unit on the equator. When the mechanism of karyomere fusion fails at pro-metaphase, the chromosomes remain free from each other in their behavior and act as two units at metaphase.

The conclusions here drawn appear to be also suggested from some figures given by previous observers who dealt with secondary association in plants having chromosomes of rather low number and nearly equal size, permitting an easy understanding of the nature of their arrangement on the plate. Several microphotos of metaphase plates in the hybrid between two species of *Lycopersicum* given by AFIFY ('33) and those in diploid varieties of pears given by MOFFETT ('34) are especially of interest in the present connection. Also *cf.* LA COUR's Fig. 6 ('31) and DARLINGTON's Pl. V, Fig. 3 ('32) concerning metaphase plates in *Sorghum* and *Dahlia* respectively. Figures drawn by MEURMAN ('33) representing free and associated arrangements in *Acer* are also strongly suggestive.

It will not be doubted, as pointed out by METZ ('34), that the karyomere plays an important rôle in the activities of chromosomes. The writer's work on the chromosome structure in *Trillium kamtschaticum* also reveals its important bearing on the chromosome behavior during mitotic divisions. Details on these points will be treated in another paper. The present case may be taken as offering an additional evidence favoring the view that the karyomere takes an active part in causing the association of chromosomes.

Summary

1) The present paper deals with secondary association of meiotic chromosomes in two plants, *Tricyrtis latifolia* MAX. and *Dicentra spectabilis* MIQ.

2) The haploid chromosome number of *Tricyrtis latifolia* is 13. At meiotic metaphase two of the bivalents associate with each other.

3) The haploid chromosome number of *Dicentra spectabilis* is 8. At meiotic metaphase two bivalents usually associate with each other, but in some cases they show no obvious association.

4) The paired bivalents lie in the same karyomere cavity at metaphase.

5) The finding stated in (4) is considered in connection with the mechanism of chromosome arrangement at meiotic metaphase.

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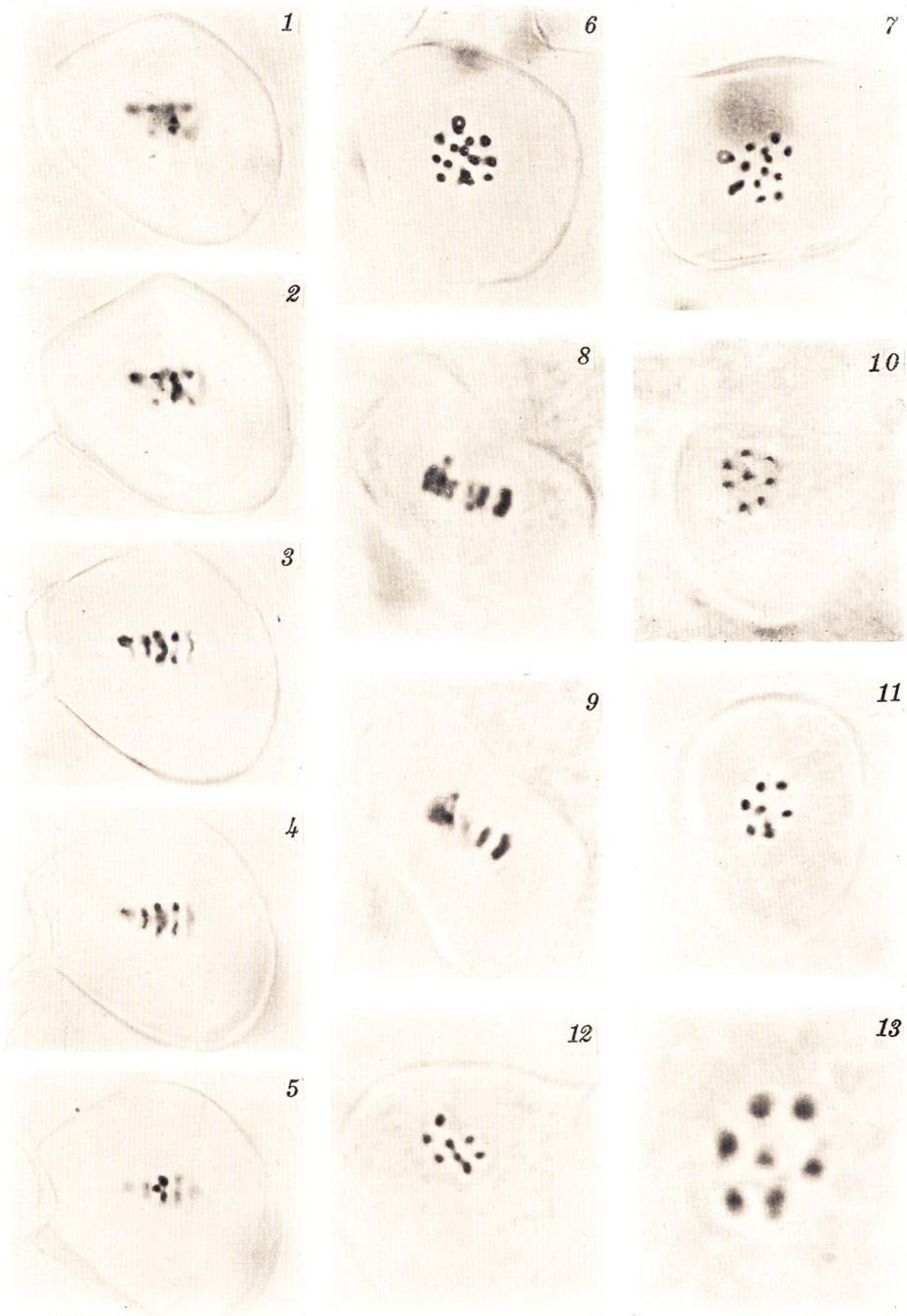
Explanation of Plate

All the microphotos were taken by the writer, with the aid of a Leitz microphotographic apparatus MA II. The lens combinations were: Zeiss apoch. obj. 60× (N.A. 1.3) and Homal IV for Figs. 1-7, Zeiss apoch. obj. 120× (N.A. 1.3) and Homal IV for Figs. 8-12, and the same obj. and Zeiss comp. oc. 30× for Fig. 13.

Figs. 1-7, *Tricyrtis latifolia*.

Figs. 8-13, *Dicentra spectabilis*.

- Figs. 1-5. A PMC at metaphase, taken at five successive foci; same as Text-fig. 10. \times ca. 1000.
- Figs. 6 and 7. Polar views of metaphase; same as Text-figs. 7 and 8 respectively. \times ca. 1000.
- Figs. 8 and 9. A PMC taken at two successive foci; same as Text-fig. 17. \times ca. 2000.
- Figs. 10-12. Polar views of metaphase; same as Text-figs. 16, 15 and 14 respectively. \times ca. 2000.
- Fig. 13. Same as Figs. 10, representing the karyomere surrounding each bivalent and the associated ones. \times ca. 4000.



H. Matsuura photo.

H. MATSUURA: On the Secondary Association of Meiotic Chromosomes.