Cyto-genetical Studies on *Tricyrtis*, IV. Basikaryotype analysis in hybrids of *T. hirta* and *T. formosana*¹

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Introduction

Various karyotypes in *Tricyrtis*, *Brachycyrtis* and their hybrids were analyzed in detail by the junior author (cf. Satô 1939) and differential amphiplasty in hybrids was also observed. According to Sinotô and Kikkawa (1932) the hybrids were divided into two groups, that is, the first group of hybrids was all fertile and their external characteristics were entirely maternal, while the second group was sterile and had intermediate external characteristics. In order to know the process of differentiation of various karyotypes, basikaryotype analysis in the hybrids may be one of the causal methods to be done.

T. hirta and *T. formosana* have each a number of varieties and races, so their hybrids are most important to know the basikaryotypical difference between these plants. The basikaryotype analysis or the study of chromosome behaviour in meiosis of these hybrids was carried out and the results will be described in the present paper.

Material and methods

Two different hybrids of *T. hirta* Hook. (*S* 32.4) and *T. formosana* Bak. (*S* 32.13) used in this study were raised previously by the senior author. One hybrid (*S* 32.26) of these two species has twenty-six somatic chromosomes and another (*S* 32.15) has twenty-five chromosomes showing absence of one short chromosome. Recently an amphidibasic plant was induced by the colchicine method, i.e., by covering young sprouts of the 26-chromosome hybrid with cotton immersed in the 0.1% colchicine solution.

The root-tips were fixed with Navashin's fluid and the paraffin sections were cut 15–20 micra in thickness and Newton's gentian violet method was used for staining. The pollen mother cells were fixed with Bouin's fluid by the ordinary paraffin method and the later procedures were the same as those followed in the case of the root-tips. The aceto-

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carmine smear method and the permanent smear method were also used, the fixative being either the Bonn modification of Flemming's solution or Navashin's fluid, while the gentian violet method was used for staining in the latter case. The pollen grains were observed exclusively by employing the aceto-carmine smear method.

Observation

Morphological description of hybrid and amphidibasic plants

Fig. 1. Flowers of one hybrid ($2n=26$) of *Tricyrtis hirta* and *T. formosana* and a colchicine-induced amphidibasic plant ($2n=52$). a, c, dibasic hybrid (2b). b, d, amphidibasic plant (4b). $\times 1$.

The 26-chromosome hybrid was vigorous, while the 25-chromosome hybrid was less vigorous and especially had wrinkled leaves. The former had twenty-six somatic chromosomes, while the latter only twenty-five clearly showing absence of one short chromosome. The amphidibasic plant is more vigorous and robust than the dibasic hybrids. The hairiness is remarkably more conspicuous and rough; the leaves are stiffer, thicker and more scabrous to the touch and their shade is deeper green. The individual organs such as the flowers (fig. 1), the mature anthers and the styles and also stoma cells (fig. 2) are bigger

in the amphidibasic than in the dibasic hybrids. The difference between the dibasic and amphidibasic plants seems to be larger than that between the dibasic and autotetraploid plants in *T. formosana* (cf. Sinotô and Satô 1942).

Fig. 2. Stoma cells in lower surface of leaves. a, 26-chromosome hybrid. b, amphidibasic plant ($2n=52$). $\times 140$.

Cytological observation in the hybrids

A. Karyotype analysis (chromosome morphology in mitosis)

Three short chromosomes of the 26-chromosome hybrid have satellites at their proximal ends and three corresponding nucleoli are found in the telophase. *T. hirta* has six SAT-chromosomes ($2L_1^t + L_2^t + 3S^t$) and *T. formosana* has only one SAT-chromosome (S^t), so the presence of only three SAT-chromosomes in their hybrid suggests the differential amphiplasty. This hybrid clearly shows the disappearance of the satellite of the long chromosomes derived from *T. hirta* (fig. 4). The terminal type of nucleolar chromosomes derived from *T. formosana* seems to become inert in this hybrid judging from the nucleolus number in the telophase.

Figs 3-5. Karyotypes of hybrids of *T. hirta* and *T. formosana* and the amphidibasic plant, especially showing the relation between SAT-chromosomes and nucleoli in the telophase. 3, 25-chromosome hybrid ($2n=25=2L^t+2L+3S^t+18S$). 4, 26-chromosome hybrid ($2n=26=4L+3S^t+19S$). 5, amphidibasic plant ($2n=52=8L+6S^t+38S$). $\times 1900$.

The amphidibasic plant has fifty-two somatic chromosomes of which six short chromosomes have satellites at their proximal ends and six corresponding nucleoli are present in the telophase (fig. 5). The balance of SAT-chromosomes and nucleoli remains unaffected by chromosome doubling and the differential amphiplasty found in the hybrid is also maintained in the doubled state.

In the 25-chromosome hybrid two long chromosomes have satellites at their distal ends and three short chromosomes have satellites at their proximal ends. So there are five SAT-chromosomes corresponding to five nucleoli found in the telophase (fig. 3).

B. Basikaryotype analysis (chromosome behaviour in meiosis)

The 26-chromosome hybrid was expected to have thirteen bivalents in the first meiotic division (fig. 6), but sometimes two, four or six univalents were also found (figs. 7-10). The chromosome configurations are shown in table 1. The univalent chromosomes were consisted of short chromosomes in most cases, but rarely of long chromosomes (fig. 8). On account of the

Figs. 6-11. Chromosome behaviour in the first meiotic division of PMC's in the 26-chromosome hybrid. 6, thirteen bivalents. 7, twelve bivalents and two univalents of short chromosomes. 8, twelve bivalents and two univalents of long chromosomes. 9, eleven bivalents and four univalents of short chromosomes. 10, ten bivalents and six univalents of short chromosomes. 11, chromosome distribution at anaphase, fourteen chromosomes in one pole and twelve in the opposite pole. $\times 1080$.

univalent formation anaphase distributions of chromosomes are expected to be 13-13, 12-14, 11-15 and 10-16 (cf. table 2). This expectation was fulfilled by the observation in the pollen grain divisions (cf. table 3).

Table 1. Chromosome configurations in the first meiotic metaphase of the 26-chromosome hybrid of *T. hirta* and *T. formosana*

Chromosome configuration	13 _{II}	12 _{II} + 2 _I	11 _{II} + 4 _I	10 _{II} + 6 _I
Frequency	17	7	7	1

Table 2. Chromosome distribution in the first meiotic anaphase of the 26-chromosome hybrid of *T. hirta* and *T. formosana*

Chromosome distribution	13-13	12-14	11-15
Frequency	12	12	2

Table 3. Distribution of chromosomes in the pollen grains of the 26-chromosome hybrid of *T. hirta* and *T. farinosana*

No. of chromosomes per nucleus	11	12	13	14	15	16
Frequency	3	16	17	12	2	1

Univalent chromosomes are usually situated outside the equatorial plate and distributed at random in the first meiotic anaphase and splitted longitudinally at the second division; this inference was supported by the chromosome distribution in the anaphase (cf. table 2). Very rarely univalent chromosomes were arranged at the equatorial plate and splitted

Figs. 12-23. Chromatin bridges and fragments in the first and second meiotic divisions of the 26-chromosome hybrid. 12, one fragment and chromatin bridge of long chromosomes. 13, chromatin bridge of long chromosomes. 14, chromatin bridge of short chromosomes. 15, small fragment and chromatin bridge in the first division. 16, ring-formed chromosome which will show chromatin bridge in the second division. 18, 19, chromatin bridges of long chromosomes in the first division. 21, chromatin bridges of long chromosomes in both the first and second divisions. 22, chromatin bridge and fragments in the second division. 23, chromatin bridge in the first division. $\times 1080$.

longitudinally in the first meiotic division. By these observations at least each two members of one long and three short chromosomes are mutually partially homologous in this hybrid and some of these chromosomes each have small inversion at the distal arm as shown in figs. 12-23. A long chromosome pair has a small inversion at the distal arm and forms a chromatid bridge

and a small fragment in the first or second meiotic divisions (figs. 12, 13, 16-19, 21-23). A short chromosome pair has also a small inversion and form a chromatid bridge (figs. 14, 20).

The 25-chromosome hybrid was expected to have twelve bivalents and one univalent in the first meiotic division (fig. 24), but sometimes three or five univalents were also expected to be found (figs. 25, 26) judging from the basic karyotype analysis of the 26-chromosome hybrid in which two, four and six univalents were rarely observed.

The chromosome configurations in the first meiotic metaphase are shown in table 4. Univalent chromosomes are distributed at random in the first meiotic division and split longitudinally in the second division, but sometimes longitudinally split in the first meiotic anaphase (figs. 27, 28) after the separation of bivalents. The chromatid bridge of the long chromosome

Figs. 24-32. Chromosome behaviour in meiosis of PMC's in the 25-chromosome hybrid. 24, twelve bivalents with one univalent of short chromosome. 25, eleven bivalents with three univalents of short chromosomes. 26, ten bivalents with five univalents of short chromosomes. 27, longitudinal split of a long univalent chromosome in the first meiotic anaphase. 28, longitudinal split of two long univalent chromosomes and one short univalent. 29, chromatid bridge of a long chromosome, and lagging chromosomes in the second division. 31, fragments and chromatid bridge in the first meiotic division. 32, lagging chromosomes perhaps derived from longitudinal split of a univalent chromosome. $\times 1450$.

pair is clearly observed in the first and second meiotic anaphases (figs. 29-31). By comparison of the chromosome behaviour in meiosis in the two hybrids it may be concluded that one absent chromosome of the latter hybrid ($2n=25$) is a short chromosome which is partially homologous with its mate.

Table 4. Chromosome configurations in the first meiotic metaphase of the 25-chromosome hybrid of *T. hirta* and *T. formosana*

Chromosome configuration	12+1	11+3	10+5
Frequency	24	3	6

Table 5. Chromosome distribution in the first meiotic anaphase of the 25-chromosome hybrid of *T. hirta* and *T. formosana*

Chromosome distribution	12-13	11-14	10-15
Frequency	11	3	1

C. Nucleolar condition in the pollen grain mitosis of hybrid ($2n=26$)

This was previously reported by the junior author (cf. Satô 1939). *T. hirta* has both long and short SAT-chromosomes and *T. formosana* has one short SAT-chromosome and long nucleolar chromosomes (of terminal type), while their hybrid has only three SAT-chromosomes in the root-tip cell. Judging from the karyotypes of both parents we can easily imagine that this hybrid has two or three long SAT- or nucleolar chromosomes besides three short SAT-chromosomes. It was however definitely shown in the present observation that differential amphiplasty or disappearance of the satellites of the long chromosomes occurred. The amphidibasic plant has also six short SAT-chromosomes because its chromosome sets were simply multiplied.

Table 6. Number of nucleoli in the pollen grains of the 26-chromosome hybrid of *T. hirta* and *T. formosana*

No. of nucleoli per nucleus	1	2	3	4
Frequency {resting stage prophase	16 9	34 14	17 2	4 —
	25	48	19	4

Table 7. Number of nucleolar chromosomes in the pollen grains of the 26-chromosome hybrid of *T. hirta* and *T. formosana*

No. of nucleolar chromosomes per nucleus	1	2	3	4	5
Frequency	0	7	10	6	1

Table 8. Various combinations of nucleolar chromosomes in the pollen grains of the 26-chromosome hybrid of *T. hirta* and *T. formosana*

Combination of nucleolar chromosomes	Frequency	No. of nucleolar chromosomes
2L ⁿ +3S ^t	1	5
2L ⁿ +2S ^t	2	4
1L ⁿ +3S ^t	4	4
2L ⁿ +1S ^t	2	3
1L ⁿ +2S ^t	5	3
3S ^t	3	3
1L ⁿ +1S ^t	3	2
2S ^t	2	2

The pollen grain mitosis in this hybrid revealed that one or two long chromosomes are attached to the nucleoli in the prophase besides the short SAT-chromosomes. The nucleoli in the prophase of the pollen grain were found to be one, two, three or four in number. On account of irregularities in meiosis the chromosomes also vary from eleven to sixteen (cf. table 3). Bearing this in mind, we can imagine various combination of nucleolar chromosomes in the pollen grains (cf. tables 7, 8). These observations may support the balance hypothesis of nucleolar chromosomes.

Discussion

(1) Chromosome pairing and homology

By the karyotype analysis we can to some extent infer the occurrence of karyotype alterations such as elimination, fusion, translocation, inversion etc., while the basikaryotype analysis may be one of the most critical cytological methods to verify such inference and to detect further such karyotype alterations. The basikaryotype analysis postulates first that pairing of chromosomes or portions of them implies their homology. But when the chromosomes are paired it does not always imply the homology of the whole lengths of these paired chromosomes. Rather it suggests at least their partial homology, and conversely failure of bivalent formation suggests either partial or non homology of the chromosomes except in the case of 'asynapsis'. In the case of the partial homology the chromosomes may form a bivalent or remain as univalents, while in the case of non homology the chromosomes may regularly remain as univalents. Bearing this in mind, we may easily arrive at a conclusion that each two members of three pairs of the short chromosomes and one pair of the long chromosomes in the 26-chromosome hybrid of *T. hirta* and *T. formosana* are partially homologous and that one short chromosome which is partially homologous with its mate is absent in the 25-chromosome hybrid.

These partially homologous chromosomes have inversion at their distal ends and sometimes form chromatid bridges and fragments. Here

we may think of the technical limit of the basikaryotype analysis. In the case of minute inversion these inverted chromosomes may form regularly bivalents and the detection of this inversion may be practically technically impossible. The same thing is said in the case of minute translocation. In this case regular bivalent formation may result inspite of the presence of translocation, or univalent formation may result in a small percentage and so multipartite formation may not occur. This is however at present only inference and can not be developed by the cytological data obtained by the basikaryotype analysis.

In the case of heteromorphic bivalents we can easily detect the partial homology, but the origin of non homologous segment cannot be determined unless inversion or translocation is proved cytologically by the basikaryotype analysis (which may show chromatid bridge and fragment or multipartite). The same thing is said in the case of partially homologous chromosomes which often form bivalents and sometimes remain as univalents.

This discussion may be a premise of the following hypothesis that in order to pair the chromosomes always need certain lengths of homologous segment, that is, pairing blocks of chromosomes. Consequently inversion or translocation of small chromosome segments within the limit of one pairing block cannot be detected cytologically. The length of one pairing block of chromosomes may be altered by hybridization or other physiological conditions. The conception of pairing block may be applicable in the case of polybasics, and multivalent formation may only occur in the chromosomes with two or more pairing blocks. Accordingly regular formation of bivalents and univalents instead of trivalents in autotribasics and regular formation of bivalents instead of tetravalents in autotetrabasics may easily be explained.

(2) Differential amphiplasty and nucleolar chromosome

Navashin (1934) published the differential amphiplasty in *Crepis* hybrids, that is, certain satellites from one parental chromosomes disappear in some hybrids which have the definite combinations of chromosome sets. The similar fact was also observed in the 26-chromosome hybrid of *T. hirta* and *T. formosana*.

The observation of the nucleolar chromosomes in the pollen grains of the 26-chromosome hybrid suggests the presence of unstable nucleolar chromosomes which have become inert in the root-tip cells. The similar result was obtained by the junior author (cf. Satô 1942) in the pollen grains of *Nerine undulata* in which other chromosomes besides SAT-chromosomes are mobilized for nucleolus formation in certain combinations of the chromosome sets. These observations suggest the relation between terminal and interstitial types of the nucleolar chromosomes.

The disappearance of satellites from SAT-chromosomes may result in either nucleolar chromosomes of terminal type or ordinary chromosomes. This disappearance may be caused either by deletion of the satellite or coiling up of the satellite into the chromonema. The inversion or translocation of chromosome ends (which may have intimate relation for nucleolus formation) may result in the formation of new SAT-chromosomes.

Summary

1) One hybrid ($2n=26$) of *Tricyrtis hirta* and *T. formosana* has three short SAT-chromosomes and an amphidibasic plant ($2n=52$) induced from this hybrid by the colchicine method has six short SAT-chromosomes. Another hybrid ($2n=25$) of the same parental species has two long and three short SAT-chromosomes and clearly shows absence of one short chromosome. The number of SAT-chromosomes in these plants corresponds to the maximum number of nucleoli at telophase.

2) The basikaryotype analysis in the 26-chromosome hybrid clearly showed partial homology in three short and one long chromosomes and these partially homologous chromosomes were sometimes remained as univalents which were six in maximum number (cf. table 1). Small inversions of long and short chromosomes were analyzed by the presence of chromatid bridges.

3) The basikaryotype analysis in the 25-chromosome hybrid clearly showed absence of one partially homologous chromosome found in the 26-chromosome hybrid. Univalent chromosomes varied from one to five in the former hybrid (cf. table 4). Chromatid bridges and fragments were also observed and longitudinal split of the univalent chromosomes was rarely observed in the first meiotic anaphase.

4) The hypothesis of pairing block has been advanced to explain the pairing of partially homologous chromosomes.

5) Differential amphiplasty (disappearance of satellites) and mobilization of the nucleolar chromosomes of terminal type were described.

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