New Types of Meiotic Abnormalities in *Trillium kamtschaticum* Pall.¹

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Various types of meiotic abnormalities were described by Matsuura and his co-workers in *Trillium kamtschaticum* ($2x=10$, $5n$). Of the types described, 'precocious,' 'mitotic' and certain others are the types induced by heat treatment of resting or very early prophase PMCs (Matsuura 1937, Matsuura and Haga 1940). This plant is found growing only in the northern districts of Japan (cf. Haga and Kurabayashi 1954). Meiosis in PMCs takes place early in March at Sapporo, Hokkaido, under the snow (0°C) six or more months after the formation of PMCs in the anther locules.

In the course of transplantation experiments of this plant, from Sapporo to Mt. Hiko-San, near Fukuoka, some sample plants were studied cytologically at Fukuoka, Kyushu. Plants transported early in November 1953 from Sapporo underwent meiosis in PMCs and mitosis in pollen grains from late November of the same year to early January of the next year. The majority of the divisions were abnormal and various types of abnormalities were found mixed even within an anther. The purpose of the present note is to describe new types of abnormal meiosis to supplement the previous reports with some remarks on the problem of distribution of this plant.

Plants from Toyoni population, Hokkaido, were kindly supplied by Dr. M. Kurabayashi of the Hokkaido University, to whom the writers' cordial thanks are due. Observations were carried out with temporary aceto-carminic smears.

1. Upset of spindle polarity in precocious types

In a number of PMCs meiosis was normal (Figs. 1-2). But meiosis in many cells was of the precocious type, showing the first anaphase segregation of 10-10, 9-11, 8-12 and so forth when the spindle bipolarity was maintained normally or nearly so (Figs. 4-5). Segregation in this manner is one of the characteristics of the precocious type of meiosis. For, all four chromatids of a precocious bivalent separate from one another and migrate to the opposite poles at first anaphase with 2-2 or 3-1 segregation (Matsuura 1937). Segregation in six cells analysed was as follows: at least one set of five

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Figs. 1-6. 1. Normal metaphase-I. 2. Normal anaphase-I. 3. Metaphase-I, kinetochores showing synapsed metaphase-I condition and single-spiraled arms anaphase-I separation (cf. Fig. 13). 4. Anaphase-I of single-spiraled precocious bivalents. Note the abolition of polar attraction at the lower pole. 1-4: $\times 1000$. 5a-b. Two cells at anaphase-I with double-spiraled precocious bivalents. a. Bipolar. b. Monopolar. $\times 650$. 6. Abolition of spindle polarity at anaphase-I of double-spiraled precocious bivalents. $\times 300$.

chromosomes, A to E, was found at each pole of the cells. Photos of typical metaphase and anaphase configurations of the precocious type of division were omitted to save printing space, for details of which see the previous papers (Plate I, 7-11 in Matsuura 1937, and Figs. 20-24 in Matsuura 1938).

Precocious types were classified into two categories with regard to the structure of chromonema spirals. One is the type with double spiraled or spiral-in-spiral structure (Fig. 5) and the other the type with single spiraled structure (Fig. 4). That the former does not represent anaphase of the 'supra-mitotic' type division is evident in the fact that both poles include at least one complete set of five chromosomes, A to E (Table 1). It is also evident that the latter is not anaphase of the mitotic type division, though the chromosome structure is mitotic, because two chromosome groupings migrating to the opposite poles are not equal in their constitutions (Table 1).

Table 1. Chromosome segregation at first anaphase of precocious type meiosis

Cell no.	A	B	Segregation*		E	Total	Refer to
			C	D			
1	2	2	2	2	2	10	—
	2	2	2	2	2	10	
2	3	1	2	3	1	10	Fig. 5a
	1	3	2	1	3	10	
3	1	1	3	3	1	9	Fig. 4
	3	3	1	1	3	11	
4	2	3	1	2	1	9	—
	2	1	3	2	3	11	
5	1	2	2	2	2	9	—
	3	2	2	2	2	11	
6	2	2	2	1	1	8	—
	2	2	2	3	3	12	

* Distribution of twenty chromosomes to the opposite poles in each cell is shown in two lines up and down.

In a considerable number of precocious type divisions one of the two poles appeared to have lost its activity at early anaphase. In such a case migration and assembling of chromosomes are normal at one pole, but at the other pole chromosomes are found lying scattered (Figs. 4-5a). Further, monopolar movement of all twenty chromosomes was met with in some cells (Fig. 5b). This finding suggests probably one pole had lost its function before anaphase commenced. These two observations support the view that inactivation of polar activity at one pole may happen at various stages of division. If polar function is lost at both poles prior to the meta-anaphase, all the twenty anaphase chromosomes will be scattered irregularly throughout the cell. This is observed to be the case in many cells (Fig. 6). Furthermore, some cells showed a remnant of polar attraction, which appeared to

Figs. 7-12. 7a-e. Asynchronous conversion into telophase-I of the elements lying scattered within a cell. There is seen a remnant of activity of polar attraction which is mono- (a), bi- (b-c) or apolar (d-e). 8. Later telophase-I following the stage shown in Figure 7. 7-8. $\times 300$. 9-10. Cell d and a in Figure 7. $\times 1000$. 11-12. Unreduced diploid and reduced haploid pollen grains at metaphase. $\times 1000$.

be mono- or bipolar (Fig. 7a-c). In this connection, it is worth mentioning the conspicuous asynchronization, within a cell, in transformation of the individual chromosomes and groupings of chromosomes into the telophase condition (Figs. 7 and 9-10). In such a case chromosomes which still maintain their individualities do not reveal splitting. Thus, there remains no possibility for the interpretation that they are chromosomes appearing from the prophase nucleus of the next mitotic cycle. Later, all the chromosomal elements are converted into the resting stage structure, cell walls being formed irregularly between the micronuclei (Fig. 8).

2. Delay of kinetochore separation in single spiraled bivalents

Bivalents lacking major spirals and hence consisting of only minor or mitotic spirals were already described in *Trillium kamtschaticum* (Matsuura 1949) and in *Paris quadrifolia* (Geitler 1938). According to the latter author cells containing such bivalents are found very rarely and solitarily in the anther locules with rather normal contents and anaphase is not normal, showing tangling of the chromosome ends.

In the present study several cells were found to have single spiraled bivalents of the non-precocious type. But, they were different from the previous ones in showing premature separation of chromatids, not accompanied by the simultaneous separation of the synapsed kinetochores (Figs. 3 and 13). In other words, in this case chromosome arms are already in first

Fig. 13. Abnormal bivalents depicted from a cell presented in Fig. 3, bivalents A to E from left to right. $\times 1400$.

anaphase condition, whereas the kinetochores remain still synapsed prophase condition (cf. Figs. 1-2). In normal meiosis of this plant kinetochores remain synapsed until first anaphase commences, so it may be said that, in this abnormality, behavior of chromosome arms proceeds a step further as compared to the case of normal meiosis.

Relational coiling of the two chromatids of an arm seems to indicate

that single-spiraled chromosomes originated from the dissolution of major spirals of the originally double, major and minor, spiraled ones (Fig. 13; cf. Matsuura 1949).

3. Pollen grain and ovule development

As a consequence of meiotic irregularities, pollen grain development was very abnormal. In a considerable number of pollen tetrads, separation into four isolated pollen grains was incomplete, for example, four pollen grains still adhering in a tetrad form (cf. Kayano 1955). Among 78 pollen grains, which were undergoing mitosis, 15 were unreduced diploid, 1 was tetraploid, and the remaining were normal haploid. These polyploid pollen grains contained multiples of a complete set of five chromosomes. Further, in some triad pollen grain assemblages one cell revealed the diploid and the remaining two the haploid constitution. These circumstances strongly suggest the origin of chromosome doubled pollen grains by restitution at the second meiotic division. The cause of restitution is not clear as yet. But, it is evident that, at least, all the polyploid pollen grains are not derived from the precocious type or mitotic type of divisions (cf. Table 1). For, the tetraploid pollen grain and the chromosome constitution of the triad pollen grain assemblage described above can result only from restitutions.

Despite the formation of normal haploid and chromosome doubled diploid pollen grains, though in limited number, anthers in many plants were found aborted before blooming, though the remaining floral organs remained seemingly healthy. But, generally speaking, flower buds aborted as a whole in more than half of the plants transplanted to Mt. Hiko-San as well as those potted and kept at Fukuoka. Such deterioration in floral organ development was reported to take place in plants which were forced to bloom under greenhouse conditions (20–25°C) at Sapporo (cf. Hiraizumi 1954).

Seven plants transplanted to Mt. Hiko-San, which produced apparently normal flowers, showed pollen abortions ranging from 14–42 percent. Transplantation to Mt. Hiko-San was done on 28 November 1953, when some of the plants were undergoing meiosis. Therefore meiotic behavior and pollen grain development at high altitude must be studied further in coming years. Of the 20 plants potted and kept at Fukuoka only one bore a normal appearing flower, the remaining showing death of the plant as a whole, abortion of flower buds involving stamens and ovary, or of stamens only when the deterioration was not so severe.

Therefore, it may be safely concluded that transplantation from Sapporo to Fukuoka is effective in causing severe reproductive inhibition. Since meiosis and pollen grain development are completed underneath the ground, meiotic abnormalities and consequent pollen abortion must have been caused primarily by high temperature. This inference is supported by the cases induced experimentally by high temperature treatment at Sapporo (cf. Matsu-

ura and Haga 1940). In this connection it will be highly suggestive to refer to the meteorological records of the three localities, Sapporo, Fukuoka and Mt. Hiko-San, which are given in the following tabulation.

Locality	Sapporo	Fukuoka	Mt. Hiko-San
Latitude	43°.04' N	33°.35' N	33°.28' N
Longitude	141°.20' E	130°.23' E	130°.54' E
Altitude (m)	17.9	3.8	644
Mean temp. (°C)	{Oct. 9.9 Nov. 3.2	16.3 11.5	13.2 8.6
Mean earth surface temp. (°C)	{Oct. 13.3 Nov. 7.9	20.9 16.4	15.0 9.8

Conclusion and summary

1. *Trillium kamtschaticum* is a plant limited to the northern districts of Japan. Transplantation from the native district Sapporo, 43°.04' N, to Fukuoka, 33°.35' N, caused various meiotic abnormalities which were identical or similar to those induced by high temperature treatment at Sapporo. In many plants, anthers, flower buds or whole plants perished before blooming, resulting in nearly complete reproductive defects.

2. This observation answers, at least partly, why this plant is not distributed to the south, the southern-most boundary of the distribution being located at present at the Mountain-pass Kusakai of Iwate prefecture, 39°.40' N and 750 meters above sea level.

3. Further, new types of meiotic abnormalities such as upset in spindle polarity, delay in kinetochore separation in single spiraled bivalents and others were described.

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