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Some Considerations on the Self-sterility in the triploid *Lilium tigrinum*

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I. Introduction

There have been published a number of cytological studies on *Lilium tigrinum* Ker Gawl which is widely distributed in Japan. The chromosome number first described by Schaffner (1906) was $2n=24$, but later Takenaka and Nagamatsu (1930) reported the number $2n=36$, demonstrating that his specimens were of a triploid nature. Sato (1937) stated that all the forms in Japan are triploid. According to Shimizu (1954), however, a diploid form of this plant, bearing yellow flowers, grows wild in Kyushu, the southern-most big island of Japan. Thus, as described in a book by Woodcock and Stearn (1950), it should be accepted that *L. tigrinum* consists of either clones, diploid or triploid. As the present author has not had access to the diploid form, some details of its characteristic features are yet unknown to him. It is generally said, however, that triploid form of *L. tigrinum* grows bulbils at leaf axils, the plant being self-sterile, while the diploid form is self-fertile and lacks bulbils. Especially the self-sterile nature is very characteristic in the triploid. Even though an enormous collection not only in Japan but also from Korea were investigated, in no case were sucessful the crosses and selfings among the triploids to obtain a fruit set or a seed set (Takenaka, 1958). This clearly indicates that the self-sterility in the triploid is a common phenomenon in this species. To explain this sterility it has been said that an irregular distribution of chromosomes in the spore cells, which must be brought about by the triploidy, will be very responsible (Takenaka and Nagamatsu, 1930). Chandler, Porterfield and Stout (1937) also stated that the triploidy is a major factor in the production of abortive pollens.

Later, some authors paid special attention to the structural hybridity in the triploid form which the earlier workers did not point out (Sato, 1937; Westfall, 1940; Stewart and Bamford, 1943). And Stewart and Bamford (1943) inferred, contrary to earlier opinion, that the triploidy in *L. tigrinum* be of hybrid origin, concluding from the chromosome morphology, the synapsis and the anaphase bridging.

The above mentioned studies have mainly dealt with cytological

outlooks, and the causal analysis of the self-sterility has long been rather neglected. Only a few attempts, however, were successful in getting progenies by the selfing in the triploid *L. tigrinum* (Sato, 1932, 1935, 1937). It was then suggested that "the self-sterity is due principally to self-incompatibility -- rather than to failure of formation of functional gametes" (Westfall, 1940).

There are several apparent examples of the plants that the selfed progenies could be obtained from triploid plants. And it may generally be said that the triploid plants must not always be self-sterile though the triploidy may more or less be a cause of self-sterility. Interested in this problem, the present author has carried out an analysis in the triploid *L. tigrinum*, as will be described presently with some considerations.

II. Material

All the plants used in this work were grown in the experimental garden of the laboratory. They are believed to have propagated vegetatively from a single clone. Their chromosome number is $2n=36$, and all of them are the same in karyotype (Fig. 1). However, the present material seems to differ from the specimens of Stewart and Bamford (1934), who elaborately analyzed the karyotypes in *L. tigrinum* and divided them into three groups. Kumazawa and Kimura (1947) also reported a different karyotype, then more detailed investigations on the variation and distribution of karyotypes may throw some light upon the origin of the species and its differentiation, though the present paper still remains incomplete for that sake. The analysis of the karyotype in this work followed the ordinary squash method: The plant root was immersed in 0.2% colchicine solution at 20°C for 1–2 hr, and was stained with aceto-carmine or aceto-orcein, either fixed beforehand with Carnoy's solution (3:1) or without being fixed.

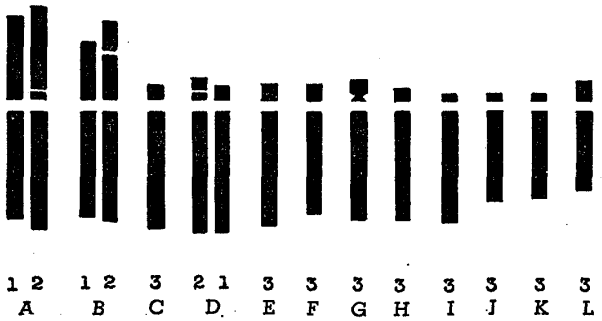


Fig. 1. Karyotype of the triploid *Lilium tigrinum*

III. Observations

1) *Microsporogenesis and Male Gametophyte*

The microsporogenesis and the formation of the male gametophyte in the triploid *L. tigrinum* have already been well described by several authors (Takenaka and Nagamatsu, 1930; Takenaka, 1933. Chandler,

Porterfield and Stout, 1937; Westfall, 1940; Stewart and Bamford, 1943; and etc.), and there are only a few findings to be added here.

The anthers were fixed with Carnoy's solution (3:1), and were stored in refrigerator at 0–5°C. These were stained with aceto-orcein or aceto-carmine or propiono-carmine.

The microspore mother cells always appear normal. The frequency of the univalent formation at MI is 1.88, which the value is not much deviated from those of the previous authors. If twelve trivalents are formed, only the result is an irregular distribution of chromosome in the microspore cells, and there is never seen the elimination of chromosomes. However, the univalents really behave as lagged chromosomes, and these lagged chromosomes are abundantly found at AII. Most of them could not be introduced into the micropore cells.

At MI, when all the bivalents and trivalents are arranged on the equatorial plate, most of the univalents take position outside the plate (Fig. 2). According to Takenaka (1933), the univalents start arrangement on the equatorial plate just after the bivalents and the trivalents have begun the disjunction, and thereafter they split into daughter chromosomes which will be separated toward spindle poles. In the present observations, however, it was found that some univalents behave in this manner and some others move precociously to either pole and are split there. At TI, they are often observed outside the chromosome groups at poles (Fig. 3).

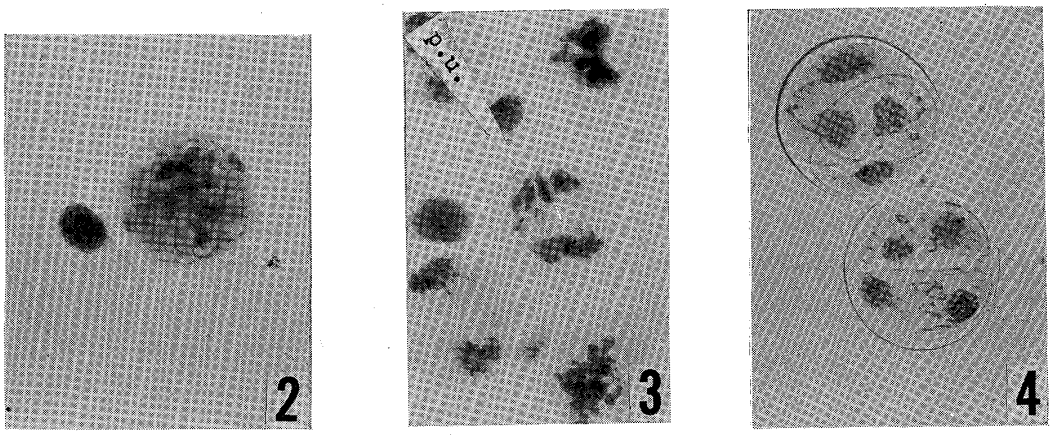


Fig. 2–4. Meiotic figures in microsporogenesis of triploid *Lilium tigrinum*. ×240. Fig. 2. Univalent at MI. Fig. 3. Precocious univalent (p. u.) at AI. Fig. 4. Conspicuously lagged chromosomes at TII.

At TI, there were found scarcely any lagged chromosomes, the derivatives of the univalents in the triploidy, and all the univalents, bivalents and trivalents were almost clearly distributed to poles of the spindle.

At TII, however, there was a conspicuous appearance of the lagged chromosomes (Fig. 4). While the univalents which have precocciously moved to the poles can be split normally in the second meiotic division,

and can be distributed there, those which are split later cannot be further split at MII. The latter lose the activity to move to the poles, and will be lagged on the equatorial plate at AII and TII. Thus, it looks as if the occurrence of precocious univalents reduced the frequency of the lagged chromosomes as assumed from the frequency of the univalent formation. In fact, however, the lagged chromosomes are considerably more frequent than expected above, and furtheremore, there were found at TII the lagged chromosomes with splitting structure. Thus comes out a question that the lagged chromosomes at TII would be resulted not only from the univalents of delayed splitting and the structural hybridity but also from the behavior of precocious univalents and normal bivalents and trivalents. The mechanism in this concern might be, for example, the balancing effect of the cell toward a harmonized state of the nucleus which has accepted an irregular set of chromosomes, or, the lack of the moving activity in the chromosomes as resulted from a physiological disorder in spore mother cells. The author rather likes the former proposition, though he does not know what really adequate is.

As described by Takenaka (1933) and Chandler, Porterfield and Stout (1937), the lagged chromosomes at AII are often cut off into fragments as the cytokinesis proceeds. Some may look chromosomes even at later stages of the pollen development, but most of them will be vacuolized, rolled up and eventually transformed into chromatic bodies. The fate of the lagged members can be followed to two ends (Takenaka, 1933; Chandler, Porterfield and Stout, 1937).

The chromatin granules (microcysts): The chromatic bodies which are not differentiated internally as nuclei.

The micronuclei: The chromatic bodies which are internally differentiated as nuclei.

During the process of the development of microspore cells into pollens, the chromatin granules are reduced in size and structure, while the micronuclei may develop to a considerable size.

The structural hybridity in the triploid *L. tigrinum* was well described and analyzed by Westfall (1940), who considered it to be an inversion heterozygosity. Since Sato (1937) and Stewart and Bamford (1943) also reported similar results, the structural hybridity might be rather widely distributed in this plant, but on the other hand, there were no such descriptions in the work of Takenaka (1933) and Chandler, Porterfield and Stout (1937). In the present materials of the study, there were clearly observed the formation of the chromosome bridges and the acentric fragments as caused by the crossing over in the inversion heterozygote. Among 506 pollen mother cells, the frequency of the cells in which the formation of anaphase bridges or of fragments were found was 11.4%.

For the above-mentioned reasons, the chromosomes in the spore cell nuclei will be distributed in an irregular manner. Such irregular distribution of chromosomes in the microspore cell nuclei has been confirmed by the observation of Chandler, Porterfield and Stout (1937). But almost all of the microspore cells separated just after the meiosis were morphologically normal, and they formed outside the exine having a characteristic relief pattern. The small cells, which are different from the normal tetrad cells, are often separated by an abnormal extra cytokinesis, and are also enveloped with a normally reliefed exine (Fig. 5), but they do not carry on further development. These small cells, the microcytes after Chandler, Porterfield and Stout (1937), are rarely formed without chromatic bodies, and thus it is thought that the formation of these microcytes may be attributed, at least in part, to the state of cytoplasmic functions in the spore cells, and also it may be suggested that there has already started the abnormal differentiation of cytoplasm within the sporic cells even before the separation of the tetrad cells.

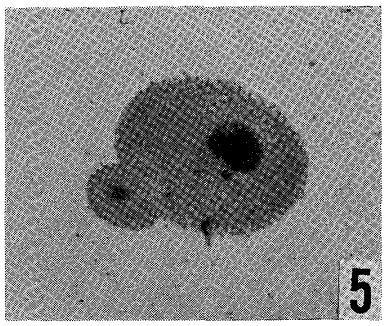


Fig. 5. Microcyte.

Various abnormalities may take place in the processes of the development of microspores to the male gametophyte, and finally there may be pollens with irregular outlook such as being too large or too small in size, abnormal in form, and irregular in thickness of the exine.

The present author could not come across abundant figures of the pollen mitosis, but could summarize in Table 1 the nuclear configurations in the pollen cells. It has interested to the author that the pollens with two vegetative nuclei are rather frequent, since there have been some evidences to show that the pollen nuclei are ill-differentiated in an unusual state of control in the cytoplasm. The frequency of completely abortive pollens increases to 39.6% in the flowers 2 days after the blossom.

Table 1. Nuclear configurations in pollen cells of *L. tigrinum*.

Nuclear configurations	%	
1 vegetative nucleus +1 reproductive nucleus	60.4[1)]	43.3[2)]
2 vegetative nuclei	15.2	19.2
1 vegetative nucleus	12.5	8.8
abortive or degenerative nucelus	9.7	27.6
other abnormal	2.2	2.1

1) Floral stage: abuot 1 week before the blossom. The pollen mitosis just has been completed.
2) Floral stage: just in the blossom.

The viability of the male gametophytes should be examined not only by staining method, but also through germination tests. As could be foreseen in other works, the germination rate in the present material was very low. Tested with various concentrations of sucrose and agar, and with various pHs, the highest rate was 4.5% of the total pollens on the medium which contained 10% of sucrose and 1% of agar at pH 5.8.

It has been pointed out that the addition of some kinds of substances to the sucrose-agar media could improve the germination rate of pollens (Ehlers, 1951; Hellmers and Machlis, 1956; Chandler, 1957; Sawada 1960; and etc.). The author also examined various kinds of growth regulators, vitamins, amino acids, and the extracts of germinating pollens, at various concentrations. So far, however, no remarkable effects could be found in them. Presumably the low germination rate of pollens in the triploid *L. tigrinum* may be due to the abnormal development itself of the cytoplasm, and the failure thus induced could not be saved by the application of such a simple means. There was not any notable difference in amino acid constituents of pollens between the triploid *L. tigrinum* and other self-fertile *Lilium* members as examined qualitatively by the paper partition chromatography. Perhaps, as the developmental stages of pollens of this species were not always uniform, an exact determination of the constituents could not be expected by such a means

2) *Macrosporogenesis and Female Gametophyte*

Westfall (1940) observed the macrosporogenesis and the formation of female gametophyte, but his interest was mainly in the question whether the chromosomal irregularities in male cells could also be observed in the process of female spore and gametophyte formation, and he concluded that there was something common in either processes. The object of the present author is mainly directed to examine normality and viability of the female gametophyte.

The ovaries were observed either by the ordinary paraffin method or the squash method. The major portion of the material was fixed with Nawashin's solution for 24 hr. and embedded as usual in paraffin. The sections of 25 microns in thickness were stained with Haidenhain's iron haematoxylin. The other portion was fixed with a mixture of absolute alcohol, glacical acetic acid and chloroform, 1:1:4, for 1/2–24 hr., and was macerated with 1N HCl at 60°C for 10 mins. Then the ovaries were taken out and further operated in a drop of 45% acetic acid under the binocular dissecting microscope, to isolate the female gametophyte. The stains was aceto-carmine or aceto-orcein.

The macrosporogenesis starts 3–4 days before flowering*. The behavior of the chromosomes in the first meiotic division, especially of of the univalents, is quite similar to that in the microsporogenesis. As far as the author observed, however, the formation of the anaphase bridge and the fragmentation in the macrosporogenesis seems to be less frequent than in the microsporogenesis.

The daughter nuclei gradually be separated from each other. The second meiotic division is much different from that process in the microsporogenesis. The lagged chromosomes, which were considerably many at TII in the microporogenesis, appeared not so conspicuous in the macrosporogenesis. The frequency of division figure in which the chromatin granules and/or the micronuclei were observed was only 21.1%. Westfall (1940) estimated the frequency as 20.0%. This may suggest that the male and female cells in sporogenesis differ somewhat in their physiological conditions. It may also be assumed that the macrospores are more complete in activity of cytoplasm and more effective in motive force for the chromosome movement. Thus, the chromosome members to be lagged in the macrosporogenesis can more easily moved to the poles than in the microsporogenesis. Or, it may be assumed that the balancing effect of the cell is not necessarily be exerted in the macrosporogenesis, especially of *Lilium*, for the reasons which will be considered later.

As the meiosis begins, the development of the female gametophyte in this genus also makes a start. The processes that follow are quite in accordance with those of the so-called "Fritillaria type", as has already been confirmed by Westfall (1940), who also established the irregular distribution of chromosomes in the macrospore nuclei.

One of the nuclei of the upper pair in the first 4-nucleated stage gradually moves downward, and the nuclear arrangement is altered to 1+3 (Fig. 11). In the next mitosis, the Bambaccioni phenomenon occurs on the charazal side (Figs. 12, 13), then the second 4-nucleated stage has a nuclear configuration of $2+2_{III}$. This process may occur 1 or 2 days before the flowering. As a result of the next synchronous mitosis, eight nuclei show a configuration of $4+4_{III}$. Thereafter, one of the nuclei of the upper is posited somewhat downward, one of the nuclei of the lower group moves upward, thus resulting in a nuclear configuration of $3+1\cdot1_{III}+3_{III}$ (Fig. 15). Then the cytokinesis is performed in both lower and upper groups of the nuclei.

In the middle part of the ovary, the developmental stages of the female gametophyte are almost synchronous, while the stages are less

* The age of flower was represented by the number of days before or after the flowering. The day of flowering was predicted by the appearance such as the length of the bud, which, however, may easily be influenced by the changes of the internal or external conditions. Thus the age used here may not be taken as absolute.

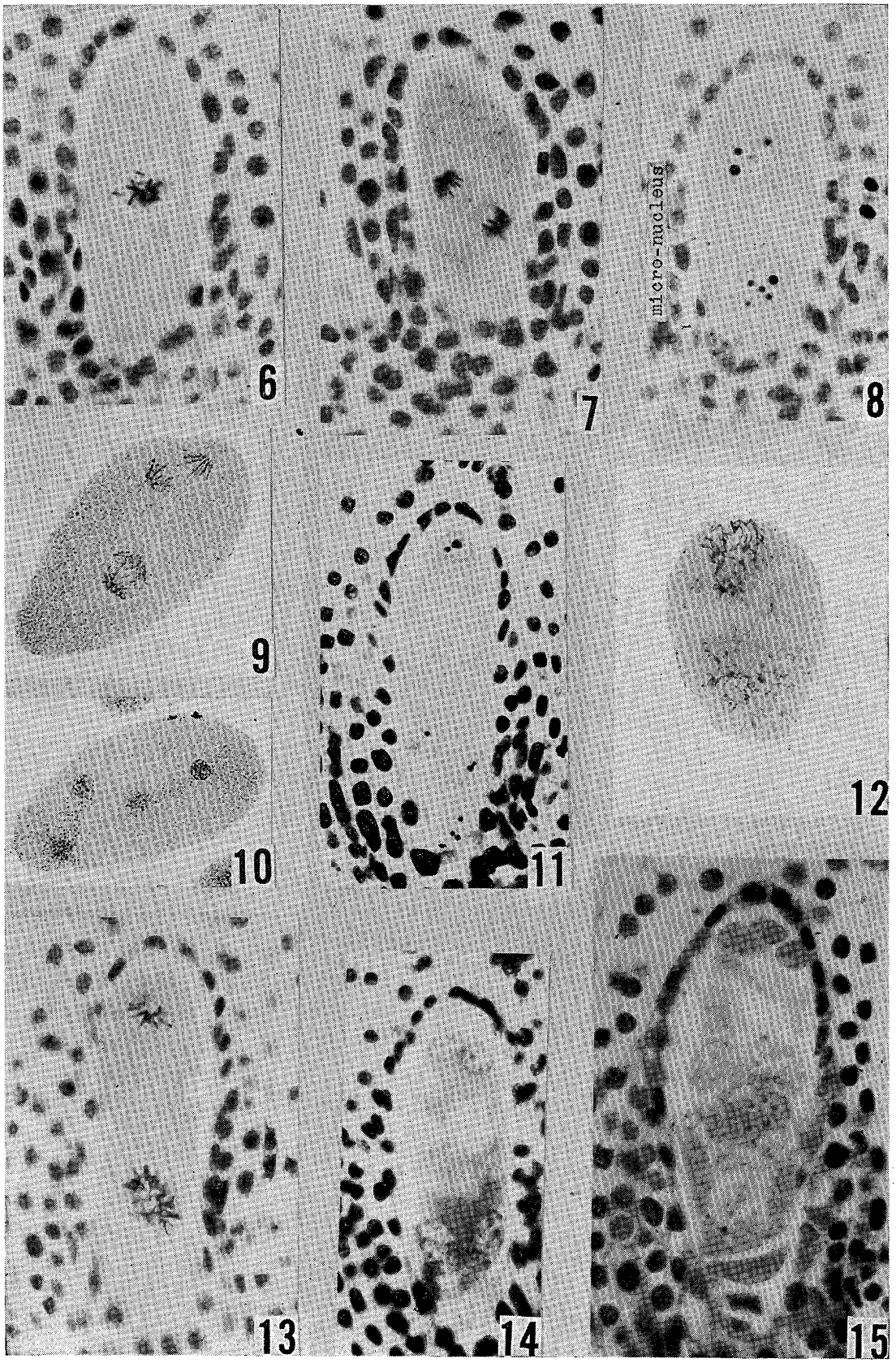


Fig. 6-15. Developmental stages of female gametophyte. ×240. Fig. 6. MI in macrosporogenesis. Fig. 7. AI in macrosporogenesis. Fig. 8. 1+1 stage of female gametophyte. Note the micronucleus. Fig. 9. AII of second meiotic division. Fig. 10. TII of second meiotic division. Fig. 11. 1+3 stage of female gametophyte. Fig. 12. Prophase of third synchronous nuclear division. Fig. 13. Bambaccioni phenomenon. Fig. 14. $1+3_{III}$ stage of female gametophyte. Fig. 15. Mature female gametophyte.

Table 2. The morphological normality of the female gametophyte in just blossomed flowers of the triploid *L. tigrinum*.

	Normal	Delayed	Abnormal
Number	448	96	65
%	73.5	15.8	10.7

advanced in the upper-most and lower-most parts. The stages in the middle part of the ovary in the flower which has just blossomed vary within a certain range. In Table 2 is shown the frequency of the morphological normality of the female gametophyte in just blossomed flowers, in which the dominant nuclear configuration is $3+1\cdot 1_{III}+3_{III}$. About more than 70% of the female gametophyte seem to complete the development normally, and the remaining 30% are in most cases in delayed stages and do not seem to carry out abnormal differentiations. An interesting fact to be noted is, that there occurs rather frequently the degeneration of the egg apparatus after the cell wall is established between its constituents. This suggests a possibility that the development of the female gametophyte is normal while the cell is in the coenocytic state, and that the separation with walls of the nuclei from each other may induce harmfull effects on the viability of the cell and the constituents of the egg apparatus, the effects being caused by the actions of unharmonized chromosome complemnts in each nucleus.

However, the above-mentioned 30% also induce some members which undergo unusual differentiations, especially in their nuclear arrangment, in manners that have never been observed in the normal development of the Fritillaria type female gametophyte. Their fate is still obscure, only being assumed to cease the unusual development and to degenerate.

3) *Pollen Tube Behavior in the Pistil*

The pollen tube behavior in some crosses and selfings of the triploid *L. tigrinum* has previously been reported (Niizeki and Iwamura, 1959). While the pollen tubes of *L. maculatum* and *L. Maximowiczii* can completely penetrate the styles of *L. tigrinum*, those of *L. tigrinum* do not pass on but stop to grow in the region 10–20 mm. below the stigmata. The present observations also agree in general with the former results. But, as the present material belongs to a clone different from the previous, the ability of the pollen tube growth in the selfing was a little different.

The styles were cut off at various intervals after the pollination, and were fixed with Carnoy's solution (3:1). They were macerated with 1N HCl at 60°C for 15–30 mins. Then the styles were placed on slides, dissected longitudinaly and carefully with fine needles, and were stained with cotton-blue in lacto-phenol. A few minutes after the

stain, the styles were gently squashed between two pieces of the slide glass.

a) In the normal pistils of the triploid *L. tigrinum.*

The pollen tubes appear 24 hr. after the pollination in the region 10 mm. below the stigma. Though the well-grown pollen tubes were only 30–40 in number at this time, many more pollens could germinate on the stigma to a further extent. The growth then stopped almost completely, and the tubes were 10–15 mm. 144 hr. after the pollination (Fig. 16, C). The tips of the tubes thus ceased growth are irregularly thickened or coiled. Since the style and ovary begin to wither about 1 week after the flowering, the pollen tubes in the triploid *L. tigrinum* will never be able to find a way into the ovarian cavity, and never be able to contribute to the fertilization processes, in the case of the selfing.

The pollen tubes of *L. maculatum* and of *L. Maximowiczii** will readily achieve a complete growth, and 72 hr. after the pollination, they will penetrate into the ovarian cavity of *L. tigrinum* (Fig. 16, A, B).

The bevavior of the pollen tubes of *L. Maximowiczii* in the ovaries of *L. tigrinum* were further investigated. The pollen tubes first take a path into the synergids where their tips are broken to liberate two sperm nuclei into the region between the upper polar nucleus and egg cell. It is believed that these moving sperm nuclei will not be enveloped by the cytoplasmic sheath, through the exact feature has not yet been analyzed. They are somewhat elongated spindle in shape, slightly twisted (Fig. 17).

The fertilized eggs begin to divide to form pro-embryos 2 weeks after the pollination (Fig. 18). The frequencies of the developing embryo and of the developing endosperm are shown in Table 3. If the pollen tubes of *L. Maximowiczii* have been able to fertilize all of the egg cells formed in an ovary of *L. tigrinum*, the frequency of the formation of physiologically functioning female gametophyte seems to be lower than that presumed from the view point of the morphological normality.

Table 3. Frequencies of the developing zygote and of the developing endosperm.

Developing zygote+Developing endosperm	32
Developing zygote+Undeveloping endosperm	1
Undeveloping zygote+Developing endosperm	16
Undeveloping zygote+Undeveloping endosperm	10
Total	59
% of normal seed developmeut	54.3

* The author is very grateful to the staff members of the Nikko Botanical garden, University of Tokyo, for their help in collecting the material.

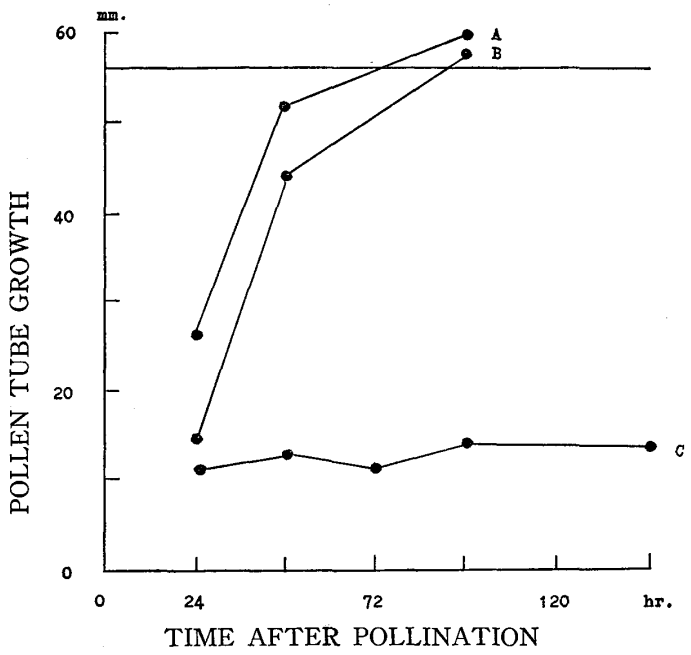
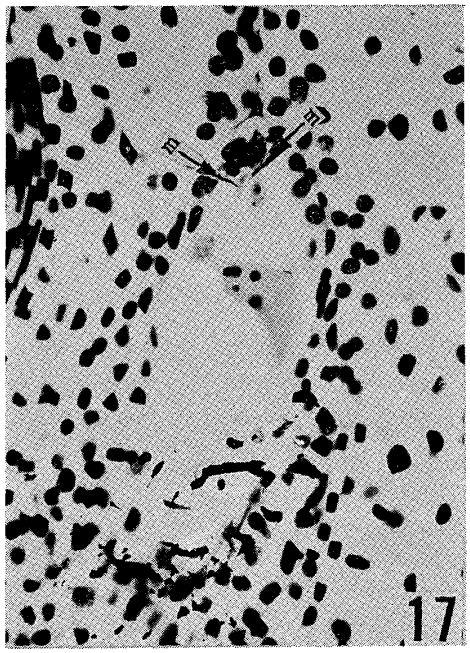


Fig. 16. Pollen tube behavior in the pistil of *Lilium tigrinum*.
A—*Lilium maculatum*: B—*L. Maximowiczii*: C—*L. tigrinum*.



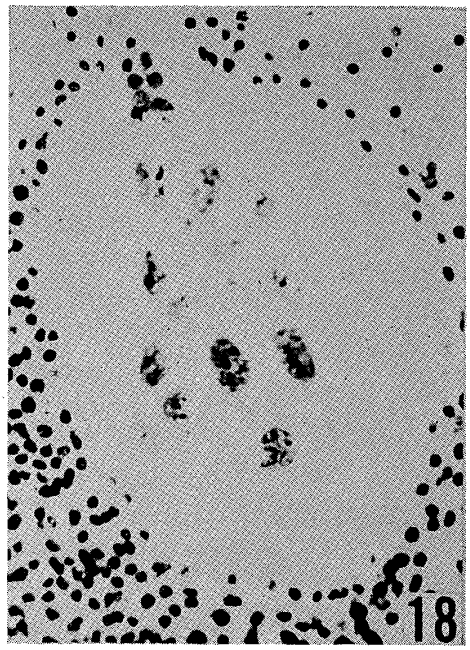


Fig. 17. Male nucleus in embryo sac. *m*, male nuclei. Fig. 18. Developing embryo and endosperm after the cross of *L. tigrinum*×*L. Maximowiczii*. Both, ×240.

In the cross experiments by the author of *L. tigrinum*×*L. Maximowiczii*, the frequency of the fruit set was 18.8%, while Shimizu (1953) could successfully get 70.1% in the crossing of the same combination. Difference of the clones or the environmental conditions might be responsible for these results, though there is no explanation yet.

Among the seeds obtained from mature fruits, the fertile seed in which the embryo formation has been completed showed a frequency of 21.8%.

b) Experiments on shortened styles.

As described before, the pollen tubes of *L. tigrinum* stopped growth after some elongation. In order to explain this phenomenon, the following assumptions may be proposed.

1) The cause of the suppression of growth may exist in the pollen itself. The nuclei in the pollen tubes have various types of the chromosome complements that might have been introduced as a product of meiosis in the triploid plants. Such unharmonized conditions in the nuclei may cause to alter the growth rate and may also limit the growth abilities of the pollen tubes.

2) The cause may exist in the style and may result in the so-called self-incompatibility, for in the style there may be some mechanisms which may interact with the pollen tubes in a certain manner that would suppress the growth of the tubes.

That the style may be shortened has already been shown experimentally by several authors (Yasuda, 1939; Buchholz, 1932; and etc.). Of course such experimental treatments alone could not reveal the real mechanism. Nevertheless, they are useful, for, if we accept the assumption 1) and abandon 2), the growth of pollen tubes should be retarded after a definite growth according to their nuclear condition, in the style shortened to any degree. If, on the other hand, we accept the assumption 2) but not 1), the pollen tube growth should be retarded in an earlier stage according to the degree of shortening of the style. Therefore, an experimental treatment to shorten the styles can at least be a means of choosing one or the other assumption.

As the first step of the experiment, a definite length of the styles and the stigmata were removed. Then the cut surfaces were covered with a thin film of sucrose-agar medium, and pollinated with a definite amount of the pollen grains. The tips of styles were covered with gelatine-capsules and vinyl bags. The pollen grains of both *L. tigrinum* and *L. Maximowiczii* were used in this experiment. The number of flowers for each treatment was ten. The behavior of the pollen tubes was observed 4 days after pollination, the styles being macerated in 1N HCl for 12–24 hr. at room temperature.

The results are schematically shown in Fig. 19. The behavior of the pollen tubes of *L. Maximowiczii* in the shortened style of *L. tigrinum* was quite similar to that in the control, and they easily can reach to the stylar base and penetrate into the ovaries. Though it was feared if the temperature within the bags would become so high that the pollen germination and the pollen tube growth might be influenced harmfully, the results indicated no such remarkable effects.

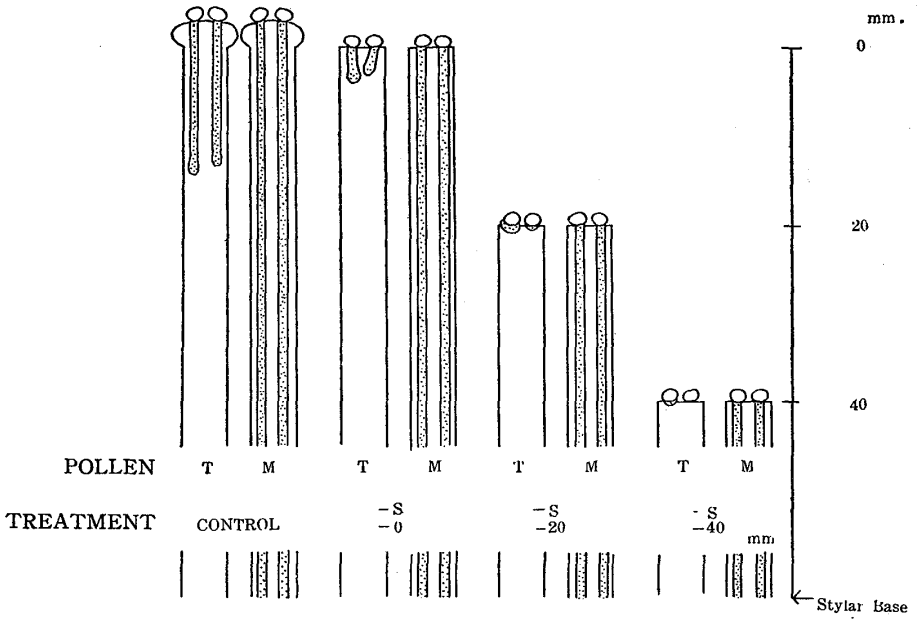


Fig. 19. Schema representing the results of style shortening experiment. −S: without the stigma. T: *L. tigrinum*, M: *L. Maximowizii*.

The pollen tubes of *L. tigrinum*, on the other hand, stop to grow after reached 10–15 mm. in the normal style with stigma. In the style from which the stigmatic part has been removed, their growth becomes worse and stops at a few mm. It is often observed that the orientation was reversed, i.e., the tips grew upward instead of downward. In the style of which upper 20 mm. were cut off, the pollen tubes were elongated only once or twice the diameter of a pollen, and more frequently upward. In the style which lost 40 mm. and the stigma, the germination rate was very poor.

These results may most reasonably be explained by taking the above assumption 2), that the suppressing mechanism lies in the style, as the grade of suppression paralleled the length of the lost part of it.

In this experiment, all of the shortened styles lacked stigmata. In the normal style, the suppressing mechanism develops when the pollen tubes have grown 10–15 mm. long, but on the other hand, the removal of the stigmatic parts (about 3 mm.) calls up an immediate suppresion. This suggests that a promoting agent lies in the stigmatic part, and that the removal of this agent results in the suppression in an earlier time in an upper region.

To confirm this supposion, the effect of the removal of only the stylar part was examined. First, the stigma was removed, and a definite length of the style was removed. Then the cut stigma was placed on the cut-surface of the shortened style with an attention not to turn the two parts around the axis, and a very small amount of 2% gelatin solution was applied to coat the slight gap between them.

Then, all the pistils were cut off at the base of the ovary. Each three styles were placed on a filter paper in a Petri dish. The pistils were kept oblique by pillowing on glass rod so as to avoid touching the stigmatic part with the paper. The ovarian parts were covered with cotton absorbent moist with distilled water (Fig. 20).

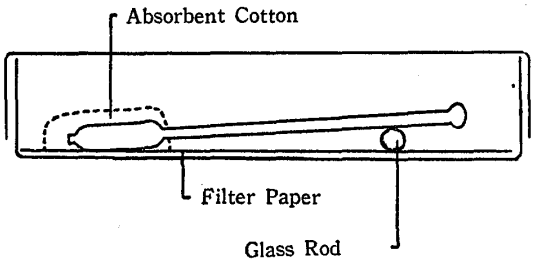


Fig. 20. Maintenance of treated styles.

Parallel with this operation, the removal of both stylar and stigmatic parts was also made. Only the pollen grains of *L. tigrinum* were used in this latter experiment. The experimental

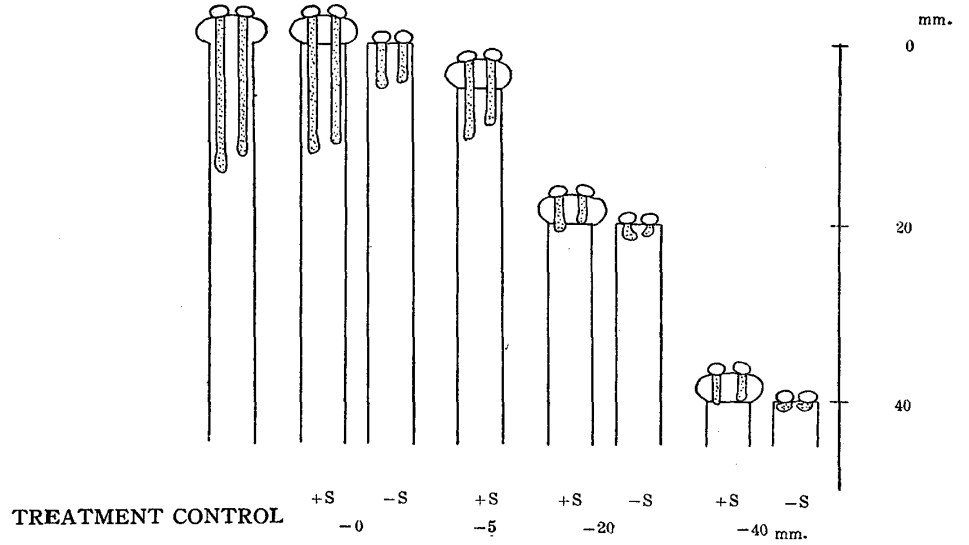


Fig. 21. Schema representing the results of style shortening experiment. +S: with the stigma.

and control dishes were kept at 30°C in the dark, and the observations were carried out 1 day after the pollination. Six pistils were examined for each treatment.

The results are schematically illustrated in Fig. 21.

In the pistils without stigmatic parts, the behavior of the pollen tubes was like that in the former experiments in general tendency, though the different experimental conditions seem to have improved somewhat the germination and growth. In the style from which only the stigmatic part was removed, the pollen tube growth was observed also after the elongation to about 3–4 mm. In the style which lost 20 or 40 mm. from top, the pollens could germinate and grow to some extent, but the orientation of the growth was again upward. The rate of the pollen germination and tube growth was slower in much shortened than in less shortened style.

In the control style, the pollen tubes could grow about 10–15 mm. long, and in the style in which the stigmatic part was cut but returned to the original plane without shortening the style, the pollen tubes could grow 11 mm. long, passing through the grafting plane. The cut plane does not seem to influence much the growth of pollen tubes, and it was generally concluded that the grafting of the stigmatic part gives better effects on the growth of the pollen tubes as compared with the case where the stigma was given up. In the style which lost 5 mm., pollen tubes could penetrate the stylar part about 6 mm. down the grafted plane. Even in the style without upper 20 mm., there were a few pollen tubes that passed the grafting plane and penetrated for a few mm. in the stylar part, but mostly the tubes stopped growth near the grafting plane. If 40 mm. of the style were removed, the pollen tubes could scarcely reach the grafting plane, and many of them stopped growth even before.

These results suggest that the growth promoting agent which to a certain extent may counteract the suppressing agent, may exist in the stigmatic part*. On the sugar-agar media, the pollen tubes of triploid *L. tigrinum* could grow only 1–2 mm. at most.

One of the proposed assumption that there be a physiological mechanism in the style of the triploid *L. tigrinum* to suppress or to limit the growth of the pollen tubes of the own species has also been confirmed from these results just described. Thus it is concluded that the selfsterility in the triploid *L. tigrinum* is partially due to the action of the so-called self-inocmpatibility system.

And, since the preliminary experiment of the same kind using the pollen grains of different clones also indicates the resemble result, the author surmises that the mechanism of the self-incompatibility may commonly distribute among the triploid species of *Lilium tigrinum*.

c) Delayed pollination and bud pollination.

In some plants which have self-incompatibility, it has often been reported that the suppressing mechanism in the pistil be changed in

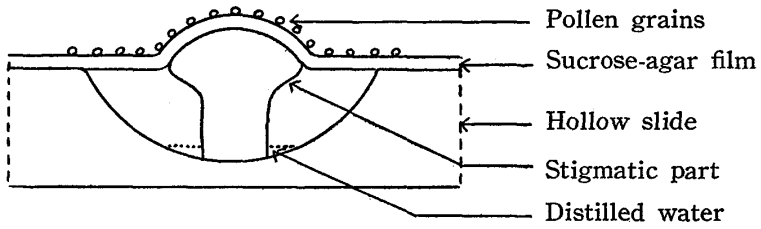


Fig. 22. Treatment of pollens with diffusives from the stigma.

* By the treatment of pollens as illustrated in Fig. 22, the rate of germination and also that of tube growth could be improved to some degree. This fact indicates that some promoting substances, which can diffuse into the agar, exist in the stigmatic part of *L. tigrinum*.

strength with the age of the flower. The bud pollination in *Nicotiana* (East, 1923), *Petunia* (Yasuda, 1929) and *Brassica* (Tatebe, 1937), and the delayed pollination in *Brassica* (Kakizaki, 1930) were reported as being effective respectively. The same situation may also be assumed in the triploid *L. tigrinum*. If it is really so, it will be expected that a self-sterile specimen of *L. tigrinum* can become self-fertile. Therefore, the pollination was examined in buds and older flowers.

The buds 2 days before the blossom and the flowers 2 days after the blossom were used for investigating bud pollination and delayed pollination respectively. The growth of pollen tubes was observed 1, 2, 4 and 6 days after the pollination.

Contrary to the expectation, in either case the pollen tubes stopped growth when they reached 10–15 mm. in length during 24–144 hr. after the pollination, which the result was the same either in the control or the experimental.

The behavior of the pollen tubes of *L. Hansonii* in the selfing much resembles that of the triploid *L. tigrinum*, and it was shown that the bud pollination in the former species could give some advantage to the pollen tube growth (Niizeki and Suzuki, 1960). As described just above, this does not hold good in the present material. Such a discord, which may be due to some difference in physiology of these plants, suggests that the mechanism of the self-sterility might have developed along the paths different with species.

d) Removal of bulbils.

Elwes (1880) stated "Mr. Hanson says that, to induce the plant to seed, all the bulblets must be removed". The present author carried out the following experiment to confirm this possibility.

The bulbils, when grew to the size recognizable with the naked eyes, were carefully removed with a fine needle or a pointed tweezers. All the flowers were allowed to blossom. The examinations were carried out on the crosses of the following combinations: treated × treated, treated × control, control × treated and control × control, in order to find out which would be affected the pollen tube growth or the pistillar part in the selfing.

Contrary to the expectation, all the results were negative, and the pollen tubes in all cases stopped growth when they were elongated to 10–15 mm., the maximal length even 6 days after the pollination. Thus it may be concluded that the removal of the bulbils, the organ of the vegetative reproduction, does not affect the behavior of the pollen tubes in the selfing of the triploid *L. tigrinum*.

e) adendum.

Some attempts to detect the presence of substances which inhibit the pollen tube growth in the style of the triploid *L. tigrinum* have been made, but so far clear result has not been obtained. An example

of the experiments is to compare the pollen tube growth of *L. tigrinum* and *L. Maximowiczii* on the sucrose-agar media containing the extracts of pistilar parts of *L. tigrinum*. Because, it is reasonably assumed that there may be something in the stylar extract which exhibits the promoting effect on the pollen tube growth of *L. Maximowiczii* and which exhibits the inhibiting effect on the pollen tube growth of *L. tigrinum*.

To prepare the extracts, the stylar part and the stigmatic part were homogenized respectively in distilled water (0.23 gr. f.w. of stigmatic parts in 1 cc of water; 1.60 gr. f.w. of stylar parts in 1.5 cc of water), then centrifuged. The supernatants were diluted to certain degrees, and mixed with equal volume of 20% sucrose-2% agar medium. The method for measurement of the pollen tube growth followed that proposed by Iwanami (1957). But in the case of *L. tigrinum*, since the pollen tube growth is not uniform, the growth is expressed as a mean of the length of pollen tubes appeared. The result does not accord with that expected from the assumption (Fig. 23). The extract of the stigmatic

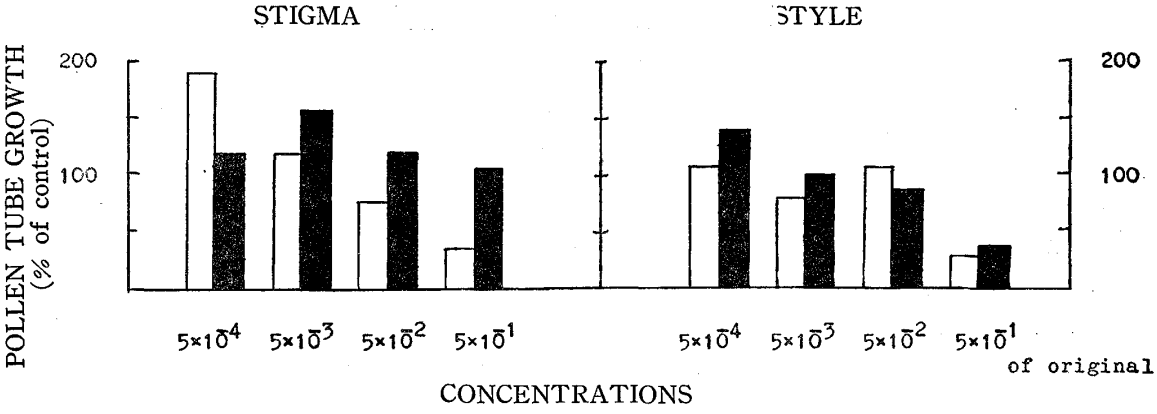


Fig. 23. Effects of some extracts upon the pollen tube growth of *L. tigrinum* and of *L. Maximowiczii*.

□ -- Pollen tube growth of *L. tigrinum*
■ -- Pollen tube growth of *L. Maximowiczii*

parts generally shows the promoting effect on the pollen tube growth of both species at suitable concentrations, while that of the stigmatic parts has not remarkable effect. According to the opinion of the present author, the structure of style in the genus *Lilium* may not be favorable for the study of this kind. The stylar part to which the pollen tubes come in contact is only one-cell layer, being the inner-most of stylar canal. And, if such inhibiting substances are secreted from this cell layer, the amount is thought to be too small in comparing with that secreted in the style, core of which is filled with the conductive tissue. Thus it seems reasonable to consider that the amount of such substances, if contained in the extract, is too small to be able to affect the pollen

tube growth on the media containing them. Then, the physiological back ground of the suppressing mechanism in the style of the triploid *L. tigrinum* is still unknown.

IV. Considerations

1) *Mechanism of Self-sterility in the triploid L. tigrinum*

The self-sterility is a phenomenon which can be resulted from various causes, and may take place at various stages of the life-cycle in plants. Following the proposition of Dobzhansky (1951), the primary causes of this phenomenon were divided into genic and chromosomal, but the present author has further revised this proposition and classified the causes as follows:

	Primary Cause		
Heritable	Genic Cytoplasmic Chromosomal	→	*Secondary Cause*
Non-Heritable	Environmental Nutritional		

Whichever the primary cause may be, some kinds of disharmony will be resulted. Various sorts of such disharmony may be called the secondary causes of sterility, and can be classified into chemical, physical and mechanical. From the physiological point of the view, the chemical disharmony may be based on either the complementary or the inhibitory actions (cf. Lewis, 1954). The physical disharmony may be caused by, for example, the osmotic pressure, and the mechanical may be caused by the incompatibility of the morphological or histological characters that occurs between the pistilar parts and the pollen or pollen tubes.

These causes provoke phenomenally the self-sterility at various stages of plant life. The time of occurrence of this sterility may be divided into pre-pollination and post-pollination stages, especially in regard of the male gametophyte. There is a reason to separate these stages, for, before and after the pollination, there is distinct difference in the growth type of the male gametophyte. The type before the pollination is rather like the type of cells just being differentiated from the meristematic condition and has begun to grow more or less three-dimensionally. The type changes after the pollination and becomes rather like the type of the germinating spores which show rather indefinite and filamentous growth, being more or less one-dimensionally.

Thus, the pre-pollination sterility means failure in development before the pollination, and includes the cases in which pollens or embryo-

sacs could not be fully mature. The post-pollination sterility, on the other hand, means the mis-development of the gametophyte, especially of the male, after the pollination, and includes the cases in which the growth of pollen tubes and the fertilization were not complete. This later sterility may be classified into two qualitatively different kinds. One is caused by the so-called stigmatic, stylar and ovarian inhibitions which occur in the period beginning with pollination and ending with the intrusion of the pollen tubes into ovular parts, and this is a result of the interaction between gametophyte and sporophyte. The second kind of sterility occurs after the tips of pollen tubes have reached the embryo-sacs. It takes place as the failure of cytogamy and karyogamy, a result of the interaction of different gametophytes. Niizeki (1959) has tentatively called these two kinds of sterility respectively the inter-sporogametophytic inhibition and the inter-gametogametophytic inhibition. The sterility that appears after the zygote has started development may called sporophytic.

The causes of self-sterility in the triploid *L. tigrinum* are thus manifold. The first is the triploidy which affects the developmental process of pollens and lowers the number of those which can germinate on the stigma. The second cause is the structural hybridity. It is generally said that the structural hybridity in diploid plants exerts a marked lethal effect during gametophytic development, and probably so it is in the triploid plant, *L. tigrinum*. For instance, the pollen abortion may be expected in this plant to occur with the deletion of the segments of chromosomes which were solitary in the microspore cells, without homologous partners. Both the first and second causes belong to the chromosomal sterility and they work in the pre-pollination stage.

It was further indicated that another mechanism of self-sterility also works in this plant. In the style of *L. tigrinum*, the growth-suppressing mechanism in the selfing shows a gradient increasing downward. Accordingly it works in the post-pollination stage. The background of this mechanism, so to say, the primary cause, is not yet known, though evident is that it does not involve the chromosomal which also may control in the other manner the growth of pollen tubes in the style. A more complete analytical study on selfed and crossed progenies and on physiological interactions will only make possible a more detailed discussion.

As mentioned above, the present author concludes that there occur two kinds of sterility, pre- and post-pollination, in the triploid *L. tigrinum*, operated by the primary causes qualitatively different from each other.

As to the limitation of the pollen tube growth in the selfing the data by Niizeki and Iwamura (1959) somewhat differ from those in the present study, and this difference may be due to a clonal difference of

the materials. In other words, the strength of the suppressing mechanism in the style may differ with clones. If a material with much weaker suppressing mechanism was chosen, the results of Sato (1937) might well be confirmed.

Though without direct relations to the self-sterility, certain other possibilities will be added. Comparing the viabilities of the zygotes at various stages, beginning with fertilization and ending, through the development of zygote, with the formation of mature seeds, it was found that mortality tends to decrease as the stage proceeds. This suggests that the sporophytic sterility also operates during the embryogenesis, and this may be caused by the primary cause of the chromosomal nature.

The author may add the importance of the effects of ovarian development, in considering the sterility. Lund (1956) observed that the increase in the hormonal level in the parts of the pistils is accompanied by the growing behavior of the pollen tubes, and suggested that these tubes have an ability to control the production of hormones in the pistils. And, in the crosses such as *L. Henryi* × *L. auratum* (Niizeki, 1959; Niizeki and Okazaki, unpub.) and *L. Hansonii* × *L. maculatum* (Niizeki and Suzuki, 1960), an ovary could often develop to form a fruit without normal seeds. In the first case the pollen tubes could reach embryo-sacs, and in the latter case the pollen tubes could reach the ovarian cavity. In the cross of *L. tigrinnm* × *L. Maximowiczii*, on the other hand, the fertilization seemingly occurred in high frequency in each ovary, but the rate of fruit-set was rather low. It is generally known that the parthenocarpic fruit set is rather common, but there has never been reported a case in which the seeds were mature without ovarian development. These facts suggest that the ovarian development must precede the seed development, and that a developing ovary may physiologically be a leading factor of the seed development. Not all of the well-grown pollen tubes induce the fruit development, and thus it may be assumed that there may be a specifically stimulating combination of the pollens as donor and the pistils as receptor. If the pollens are too poor in ability of activating the ovarian development, or if they have a certain key-action not fitted to induce the ovarian development, the sterility may be also take place in stages other than described above.

2) *Suggestions for higher Viability of Female Gametophyte in the triploid Plants*

In the triploid *L. tigrinum*, the development of the female gametophyte is performed with higher normality and viability than that of the male gametophyte. This tendency is more common in the polyploid or hybrid plants. Since the measure and expression differ with the

authors, an exact comparison of different data seems to be impossible, but some of them in the triploid are arranged in Table 4. It can be seen in the table that the formation of male gametophyte is always less efficient, and the rate of female gametophyte formation varies much within a considerable range as the material differs.

Table 4. Comparison of normality and viability between the male and female gametophyte in some examples of triploid plant.

	Triticum	*Citrullus*	*Iris japonica*	*Datura*	*Lilium tigrinum*
Male gametophyte					
Non-abortive	2	35	63.6	40-50	60.4
Normality		10.1			42.3
Viability*					4.5
General tendency	—	—	±	±	±
Female gametophyte					
Non-abortive					100
Normality			13.2	ca. 100***	73.5
Viability**	0.3-0.0	very rare			54.3
General tendency	—	—	±	+	+

All the values are expressed with %.

* Germiability of pollens.

** % of zygotic development.

*** Values including the ones in delayed stage.

To explain these different efficiencies, the author considers three factors which would play important roles in the gametophytic development, and assumes the inter-relations among them as schematically represented in Fig. 24.

In general, the state of dependence of the female gametophyte is thought to be a reason for the higher viability. Certainly a gametophyte can never carry on growing without exogenous nutritive supply. A sudden increase of the frequency of abortive pollens near the time of anther dehiscence is readily explained by this consideration. Though some of these nutritives may directly control the growth, most of them

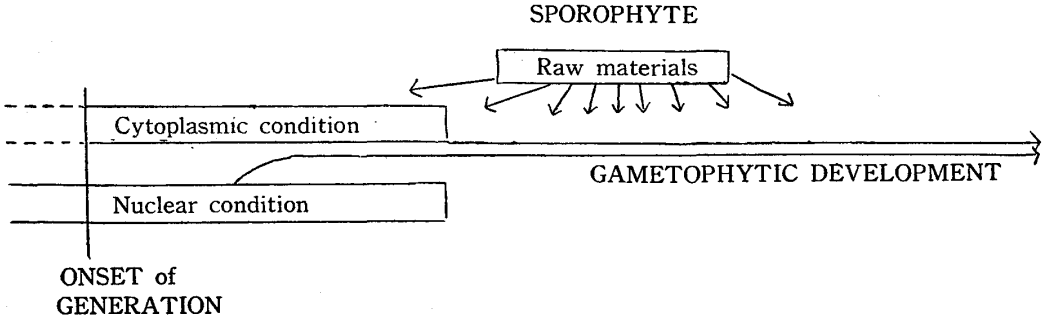


Fig. 24. Schema representing the interactions of three major factors needed for the gametophytic development. Explanation in the text.

must be transformed into the substances necessary for the development by the interaction of nuclei and cytoplasm in the gametophyte. Thus the author considers that one of the most important roles of the sporophyte is to supply raw materials for the gametophytic generation. It may be a matter of course that the gametophyte will degenerate or be delayed in growth if such supplies are inadequate, especially quantatively, to the need of nucleo-cytoplasmic interactions in the gametophytic cells.

For the onset of a generation, the most important factor is probably the cytoplasmic conditions in the cell. Throughout a series of generations, i.e., sporophytic → sporic → gametophytic → gametic → sporophytic and so on, the condition in nucleus, at least of gene complement, can be altered only quantitatively but not qualitatively, and the changes in successive generations should only be resulted from the alternations in their cytoplasm. To say otherwise, the cytoplasm at the onset of generation must be prepared as an end-product of the nucleo-cytoplasmic interactions during the preceding generation, and also must be prepared in such a way that the cytoplasm can start the exact processes characteristic to the next generation. If the cytoplasmic condition is ill-prepared, owing to a hybrid nature in the preceding generation, the following development cannot follow the way characteristic to the generation, and this may cause mis-localization or mis-division of nuclei or the abortion of cytoplasmic content. The importance of cytoplasm not only at the beginning but also during the gametophytic development has been emphasized by various authors. Considering the mechanism of the gametophyte formation and the double-fertilization, Gerassimova-Navashina (1957) pointed out that the nuclear repulsion is in some way connected with the organization of cellular elements, which is brought about by an interaction between the cytoplasm and the nuclei. But it seems to be more reasonable to say that the nuclear repulsion can be replaced by the cytoplasmic extension acting between the daughter nuclei just finished mitosis, and that the nuclear repulsion may be controlled by the state of the cytoplasm in which mitosis is performed. A similar situation was also recognized by Flint and Johansen (1958) that the striate-structure appearing in the cytoplasm has an important role in the nuclear differentiation in the female gametophyte. In his cytological studies on the hybrids of *Lilium*, Brock (1954) concluded that the errors in chromosome distribution in the female gametophyte may arise from the errors in cytoplasmic differentiation.

The cytoplasm can live without nucleus for a considerably long period of time, but the nuclei will not survive without cytoplasm. Furthermore, an enucleated cell may carry on internal differentiation and morphogenesis of its own specific type once after the genic actions

have been exerted onto the cytoplasm before the enucleation (Hämmerling, 1953). This fact suggests that the cytoplasm must be acted upon at least once by the nucleus at a certain stage of the cellular development. Then the nuclear condition may become a secondary factor. At the onset of a generation, the cytoplasm can select the genic actions needed for the following development of the cell characteristic to the generation, and the nuclear condition must be, at least once, in a perfect state. Unbalanced gene complement may also disturb development, because it forces cytoplasm a deficient or redundant selection and induces an abnormal state in the cytoplasm itself and also in further nucleo-cytoplasmic interactions which eventually will be cause a mis-development of the gametophyte.

The cell nuclei, however, seem belong either one of the two types. In one type the variation of the chromosome number has no remarkable effects on the survival of the nucleus, and in another survives only the nucleus with basic chromosome complements. For the sake of convenience, the former type of nucleus is called loosely conditioned, and the latter, strictly conditioned.

Another property of the nucleus must also be taken into account. There are accumulated many data showing that the nuclei which have accepted chromosomes in excess and become polyploid or heteroploid often discard some of them so as to keep a steady state of the whole cell. Perhaps this property may be exerted rather under the control of the cytoplasm, and it is suggested that such balancing-effect is an intrinsic and a more generalized nature of the cell or of the nucleus. Thus the lagged chromosomes observed frequently at AII of the microsporogenesis can be considered a product of the balancing phenomenon of the cell which has accepted an irregular set of the chromosomes, regarding not only the triploid *L. tigrinum* but also other triploid plants in general. Besides this elimination of chromosomes, there is often reported another mechanism of chromosome balancing called preferential segregation. Physiological nature of these phenomena are still unknown, but both can work so as to increase the viability of the cell, and may be accepted, at present, as the expression of the balancing ability of the cell or of the nucleus.

Even so, however, in the strictly conditioned nucleus of triploid nature the balancing effect may not be effective on the survival of the meiotic products, the tetrad cells, for such a balancing effect may be limited. On the other hand, a loosely conditioned nucleus, has more change to survive, exerting more effectively the balancing effect. Among the examples in Table 4, *Citrullus* and *Triticum* seem to be examples of strictly conditioned, and they show the lowest frequencies of survival in both of the male and female gametophyte. *Datura* seems to be an

example of the loosely conditioned type, and here the female gametophyte has the highest frequency of survival. Intermediate may be *Iris* in which the male gametophyte has a low and the female gametophyte also has a rather low frequency of survival.

In the triploid *L. tigrinum*, however, it is interesting that the lagged chromosomes appear conspicuously at AII of the microsporogenesis but appear not conspicuously at AII of the macrosporogenesis. This fact suggests that the failure of the balancing effect in the macrospore cell should induce a higher frequency of the disorder in the female gametophytic development. Contrary to the expectation, however, the frequency of normality and of viability of the female gametophyte in the triploid *L. tigrinum* is much higher than that of the male gametophyte, as clearly observed in the present study.

To explain this discrepancy, the author would like to make some assumptions, and to point out that the nuclear condition of the female gametophyte in *Lilium* is maintained in so favorable state that there is no need to evoke the balancing effect of the cell or of nucleus.

There are known three types of the female gametophyte formation, i.e., monosporic, bisporic and tetrasporic. The fundamental difference among these types is in the time to begin development, earlier or later. In other words, the development in the tetrasporic type has already started in the stage of megaspore mother cell, and the meiosis is included in the first step of the development, while in the monosporic type, the development starts after the meiosis is completed and the cell membrane is formed between the members of the tetrad. These are schematically compared in Fig. 25.

Accordingly, even if the sporophyte is in triploid condition, the initial cell of the gametophyte of tetrasporic type has a nucleus which

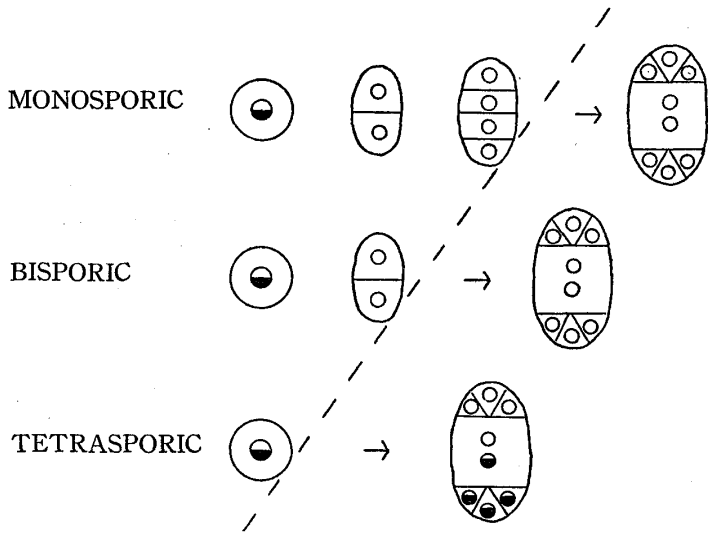


Fig. 25. Schema representing the difference of the time for the onset of the gametophytic generation among three types. Explanation in the text.

contains harmonized sets of chromosomes. The cytoplasm of the cell which has just started development can readily accept the actions and products of the harmonized sets of genes and can be directed to a normal development. Once well conditioned, such cytoplasm may control further development to some extent without being disturbed by unharmonized gene complements which may appear later. The type of the female gametophyte in the triploid *L. tigrinum* is, as described above, tetrasporic.

On the other hand, in the monosporic type, the nucleus of the cell which just started development has already received unharmonized sets of chromosomes as a result of meiosis, and the cytoplasm of the cell at the onset of the generation must accept inadequate products of unbalanced genic actions. Thus the initial cell cannot be directed normally to further development, and unbalanced interactions between the cytoplasm and the nuclei cause irregular differentations in cytoplasm and nucleus. The type of the male gametophyte formation in the triploid *L. tigrinum* is apparently comparable with the monosporic type of the female gametophyte formation, and the frequent production of pollens which are abortive or containing irregular nuclei can be explained in this manner.

As to the evolution of the self-incompatibility system, Pandy (1960) proposed a hypothesis that the higher is the degree of dependence of the gametophyte on sporophyte, the earlier appear the genic actions. This means that the genic action of the female gametophyte appears earlier than that of the male gametophyte, and this situation also seems to be favorable for the explanation mentioned above.

Even so, however, it must be noted that there is a variation as to the time of appearance of each genic action. Take, for example, the pollen grains. Form of the pollen grain and the relief pattern of the exine are generally determined by the genotype of the sporophytic cell or of the spore-mother cell, and the nature of the starch in the pollen grain is determined by the genotype of the gametophytic cell or of the spore cell. These are some extreme cases, but it must be remembered that not all of the genic actions appear simultaneously during the gametophytic development, especially at the onset of the generation.

Just before the completion of the development, the female gametophytic cell of the genus *Lilium* is a coenocyte, and contains all of the types of nuclei produced in a meiosis, theoretically, four types. These types are related complementarily with each other, and the genic actions of these nuclei can be compensated by each other. Thus the nuclei of the female gametophytic cell in the triploid *L. tigrinum* is considered to be in the so-called heterokaryotic condition at the level of chromosomes; they may exert harmonized actions in the cell as a whole throughout a

generation, even after the appearance of the nuclei which have accepted unharmonized sets of chromosomes, and thus they make smooth the internal functions of the cell during the generation. This is also a more privileged condition given to the tetrasporic type of the female gametophyte than is given to the monosporic type of the male gametophyte. Those often observed figures, in which the egg apparatus begins to degenerate after the completion of cytokinesis, may easily be explained in this way.

As considered above, the higher normality and viability of the female gametophyte in the triploid *L. tigrinum* can attributed not only to the higher degree of its nutritive dependence on the sporophyte, but also to the tetrasporic type of its formation. Nevertheless, the author's assumption concerning the development of generation, especially of the gametophyte, basing on the interactions of the cytoplasmic and nuclear conditions and raw materials, needs a further accumulation of data to be free of questions.

3) *Relations between the vegetative Reproduction and the Self-sterility in the triploid L. tigrinum.*

When a plant has a mechanism of the self-sterility in a wide sense, including the degeneration of floral structures, the species should be maintained by means of the vegetative reproduction. Accordingly, it is a matter of course that means of vegetative reproduction is much developed in the self-sterile plants in nature.

The relations between the vegetative reproduction and the self-sterility, i.e., degenerations in function of the sexual reproductive system, can be considered as follows.

a) Development of the means of vegetative reproduction may cause degeneration of the means of sexual reproduction. As has been often reported, some plants with well-developed organs of vegetative reproduction will recover, if such organs are disturbed, the means of sexual reproduction. In *Lycoris*, the excision of the bulb from the floral stem induces the fruit set, rarely the seed set (Tokugawa and Enomoto, 1930). In *Iris japonica*, suppression of the active development of stolons may cause production of fruits and rarely seeds (Yasui and Sawada, 1940). Using the technique of water culture, Sugawara (1940) succeeded in flowering of *Ipomoea*, and Tanabe (1956) also induced flowering in *Saxifraga cernua* by removing the bulbils just appeared. Explanation for these facts may be that the transfer of nutritive substances to the vegetative organs suppresses the development of sexual organs which will be formed later. Thus the self-sterility may be caused by the deviated distribution of nutritives in the plant body, and may be called nutritive sterility.

b) There is another explanation. Acquirement or the maintenance

of the vegetative reproductive system may act as a selective force for the survival of the plants which have acquired self-sterility. Also the degeneration of sexual functions may give a plant opportunity to develop the functions of vegetative reproduction. Apparently, this is not the alternative of the case explained above.

Lycoris radiata, distributed commonly in Japan, is known as triploid, and also exihibits the phenomenon of self-sterility. Nevertheless, Yasui (1958) reported a diploid form of this plant in which the mechanism of self-sterility as seen in the triploid form has already been set up, and concluded that the self-sterility in this species seems not to have originated in an irregular distribution of chromosomes in meiosis. As to the mechanism of the self-sterility in *Lycoris radiata*, Nakajima (1959) carried out some interesting experiments, and showed that the ovaries fail to develop into the fruits because of inhibiting actions of the substances produced in the bulbs. Though the origin and physiology of the self-sterility in triploid *L. tigrinum* are still unknown, the author would like to apply the above relation between diploid and triploid form of *Lycoris* to the like relations in *Lilium tigrinum*.

In the triploid *L. tigrinum*, as stated by Hanson (cited by Elwes 1880), it seems to be more reasonable to assume the explanation a), because the removal of the bulbils made the plant self-fertile. But even in such a case, the frequency of the seed formation is exceedingly small (cf. Sato, 1937). In the author's experiments in the present study, it has also been shown that the removal of the bulbils could not affect the growth of the pollen tubes in the selfings. Accordingly, it may be concluded that the mechanism of the self-sterility in the triploid *L. tigrinum* is in a considerably steady state, and that the development of the vegetative reproduction system did not result in the suppression of sexual organs. The author thus considers that the self-sterility and the vegetative reproduction in the triploid *L. tigrinum* are not related with direct physiological causality.

Presumably the self-sterile nature has been acquired in the diploid form of *L. tigrinum* which may be an ancestor of the triploid form of *L. tigrinum*, like in the case of *Lycoris*. But the means of vegetative reproduction such as the bulblet is a common character in the genus *Lilium*, and the self-sterile diploid *L. tigrinum* may have been able to reproduce with a slow rate. Among the plants thus reproduced, the triploid conditions may have been acquired through an unknown process. As regards the acquirement of the ability of the bulbil formation, there remain many to be decided, whether or not the ability was acquired with the triploidization, whether or not the ability was introduced with the triploidization, and whether or not the occurrence of the bulbil-forming plants by chance worked as a factor for the survival of the

plant, but, whichever the cause might be, the plant once acquired such a character may become distributed more dominantly than other forms, because of its durable nature, easiness of propagation and utility for mankind. The efficiency of the vegetative reproduction in the diploid self-sterile *L. tigrinum* seems to be much inferior to that of the diploid self-fertile and of the triploid self-sterile form, and thus it may be concluded that the former may encounter more chance of extirpation than the latter may.

As considered above, the author would propose a tentative hypothesis as to the the origination of the triploid *L. tigrinum*, though of course there remain some delicate points not explained so far.

V. Summary

The self-sterility in the triploid *L. tigrinum* has been investigated. The different steps in which the sterility mechanism acts are classified as follows.

1) The pre-pollination stage: The phenomenon seen in this step is the abortive or abnormal development of the male and female gametophytes. The triploidy causes irregular distribution of chromosomes in the spore cells, and the structural hybridity causes the deletion of chromosome fragments, which the results play important role in this step.

2) The post-pollination stage: The phenomenon in this step is the retardation of the pollen tube growth in the styles of the selfing. Some suppressing mechanism, distributed in the style increasingly downward, affects the pollen germination and the pollen tube growth.

The female gametophytes are more viable than the male. The tetrasporic condition in the formation of female gametophytes and their nutritive dependence on sporophytes may be the factors of better development.

Hypothetically the triploid *L. tigrinum* may have come through the stages of diploid self-fertility, diploid self-sterility, triploidization, and eventually triploid self-sterility.

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