Natural Science Report, Ochanomizu University, Vol. 11, No. 2, 1960
お茶の水女子大学 自然科学報告 第11巻 第2号

Self-sterility in *Lilium Hansonii*. I.

Pollen tube behaviors in some crosses.

Shigeya Niizeki and Hiroe Suzuki
(新関滋也)　　(鈴木宏枝)

Laboratory of Genetics, Faculty of Science,
Ochanomizu University, Tokyo

Introduction

Lilium Hansonii was described by several authors as a self-sterile plant. Generally, the phenomena of self-sterility are induced by the degeneration of functions of the reproductive organs or by the inhibitive actions of the pistilar parts to the pollen tube growth. And, though apparently there are many factors which exert some influences upon the various steps of these reproductive processes, the primary cause of the gametophytic self-sterility can be classified into the following two: chromosomal—being due to the unbalance in the chromosome complement, and genic—being due to the inhibitory or complementary actions of genes or heritable factors. Studying the meiotic figures of this plant, Haga (1943) found that *L. Hansonii* is normal diploid, and this fact suggests that the cause of sterility may be genic one.

The object of this study is to look for the primary cause of sterility. And in this paper, the preliminary observations on the chromosome morphology, viability of gametophytes, behaviors of pollen tubes will be reported.

Materials and Methods

Almost part of the materials of *L. Hansonii* was introduced from Sakata Seeds Co., and grown in our laboratory. The remaining part, used in the bud pollinations was obtained as cut flowers. *L. rubellum*, *L. maculatum* and *L. concolor* used as male parents were also get as cut flowers.

The schedule for observation of pollen tube behaviors in the styles accords with that of Niizeki (1959) except that the maceration of the styles is made by immersing them in 1N HCl for 24 hr. at room temperature. The somatic chromosomes in the root tip cells were stained with aceto-orcein or aceto-carmine after pretreatment with 0.002 M 8-hydroxyquinoline solution for 2–3 hr. at 25°C, or 0.2% colchicine solution for 0.5–1 hr. at 25°C, or their combination. Cold treatment for clearing

the heterochromatic regions of the chromosomes was also examined. But so far, the treatment at 0–5°C, for 24–72 hr. did not give good results. The ovaries fixed with acetic alcohol (3 : 1) were used for the examination of the pollen tubes in the ovaries and of the female gametophytes. The sections were made by the ordinary paraffin method, and stained with Haidenhain's iron haematoxylin. The fixation of ovaries, of course, was not suitable for cytological observation of fine structure.

Observations

Somatic chromosomes. The chromosome number of *L. Hansonii* was previously reported by several authors, and is to be $2n=24$. The meiosis could not be traced in the present study, and the chromosomes in the root tip cells and their idiogram are shown in Figs. 1 and 2.

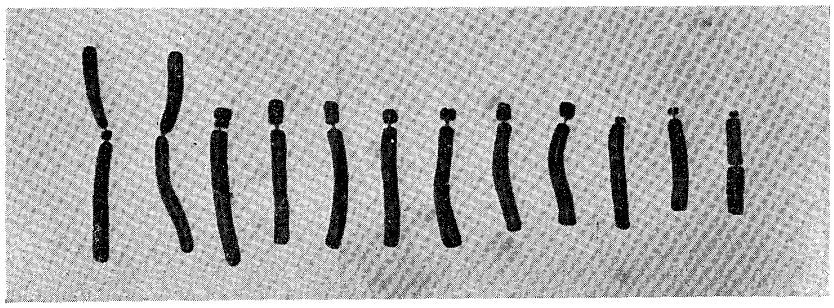


Fig. 1. The idiogram of half set (n) of somatic chromosomes of *Lilium Hansonii*. ×1200.

According to Stewart (1947), the occurrence of heteromorphic pairs of the chromosomes in the genus *Lilium* is frequent. The materials observed, however, are thought to have morphologically equall sets of the somatic chromosomes.

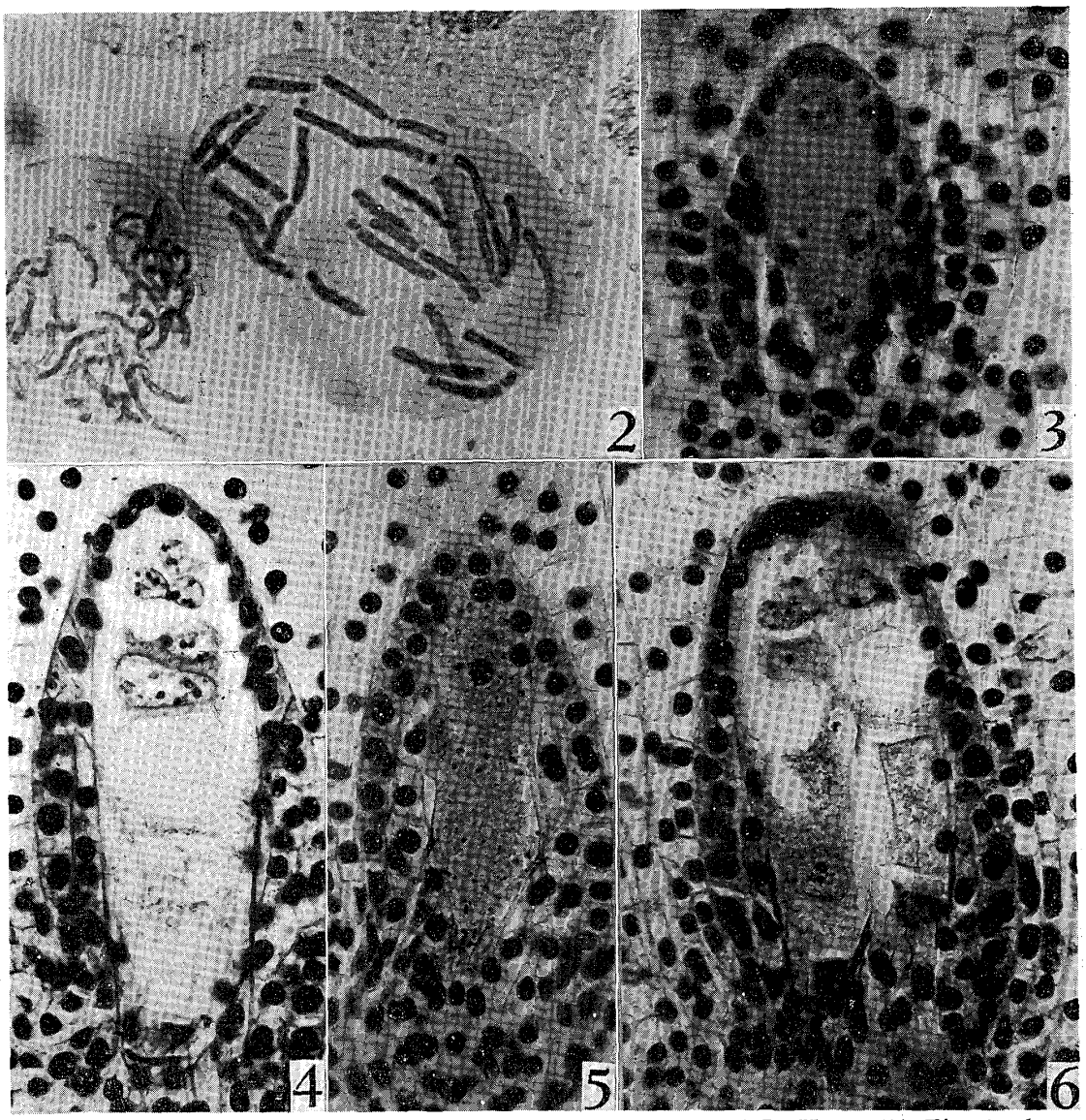
Viability of pollen. The stainability of the pollens with aceto-carmine is shown in the following :

Origin	Number of pollens		Total	% of stained
	stained	aborted		
Sakata	847	225	1072	79. 0
Cut flowers	916	170	1086	84. 3

The percentage of the stained pollens seems to be rather low, as that observed by Haga (1943) was 98.9%. But Brock (1954) reported that the percentage of the good pollen stained with cotton blue in lacto-phenol was 88.1%.

The staining of the matured pollens can only detect the existence

of the developmental failure of cytoplasmic contents during the maturation processes of pollen. Thus, the germination rate was measured. Optimal conditions for this measurement were previously selected, and these were: pH-5.6, sugar concentration-sucrose 7%, temperature-25°C. The measurements were carried out 9 hr. after sowing, and gave the values of 57.6-11.0%, mean 30%. The germination rates of other species tested parallelly were 65% for *L. maculatum*, 65% for *L. rubel-*



Figs. 2—6. The chromosomes and the embryo-sacs of *L. Hansonii*. Fig. 2, the somatic chromosomes in the root tip cell. Root was treated with oxyquinoline-colchicine solution for 3 hr. at 25°C. ×800. Fig. 3, the embryo-sac in the first four-nucleated stage. ×250. Fig. 4, the completely matured embryo-sac. The degeneration of the antipodal cell was very frequently observed. ×250. Fig. 5, the abnormal embryo-sac, in which the cytoplasm had developed to some extent while the nucleus had completely degenerated. ×250. Fig. 6, the double embryo-sac, of which formation seems to be abnormal as the number of nucleus counted in each is six. ×250.

lum, and 55% for *L. concolor* respectively.

Embryo-sac formation. Since the materials were fixed with Carnoy's solution, the figures were not suitable for fine cytological observation. But, as far as observed, the development of female gametophytes in *L. Hansonii* was normal. The earliest stage observed was the first four-nucleated stage (Fig. 3). Because the type of embryo-sac formation of the genus *Lilium* is thought to be tetrasporic, the abnormal behaviors of chromosomes, if exist, should be detected in this stage. As far as observed, however, the authors could not point out any figure as such.

In the ovaries of the flowers one day after opening, the almost embryo-sacs were in normal eight-nucleated stage of *Fritillaria* type, i. e. 416 (3, $1+1_{III}$, 3_{III})* (Fig. 4). In the uppest part of the ovary, however, the irregular behaviors of embryo-sac formation were frequently seen. They occurred in the regions between the base of the pistil and 1.5–2 mm. below, and were in earlier stage as first four-nucleated stage, while in the other regions almost all the embryo-sacs were uniformly in eight-nucleated stage. Measurements for the normality of embryo-sac formation are shown in the following:

Number of normal embryo-sac	Number of abnormal embryo-sac	Total	% of normality
38	39	77	49.4
200	0	200	100.0

These measurements were done in the regions in which the formation was uniform. Except an example shown upper in the above table, all the other individuals show 100% of the normality of embryo-sac formation. This is well comparable to that reported by Brock (1954). To explain the high percentage of the abnormal embryo-sac formation in the exceptional plant, it is possible to assume the existence of structural hybridicity of high degree as reported by Haga (1943). The nucleus to be contained in the embryo-sac had completely degenerated in the case of abnormal (Fig. 5). But the cytological study in detail of this plant remains for the future.

The double embryo-sac was observed in one case. The stage of development were the same to each other (Fig. 6).

Behaviors of pollen tubes of some species in the styles of L. Hansonii. Intra-specific pollinations were made both in the same individuals and between the different individuals. Inter-specific pollinations were made with *L. maculatum*, *L. concolor* and *L. rubellum*.

* This expression for the types of the embryo-sac formation was proposed by Maekawa (1950).

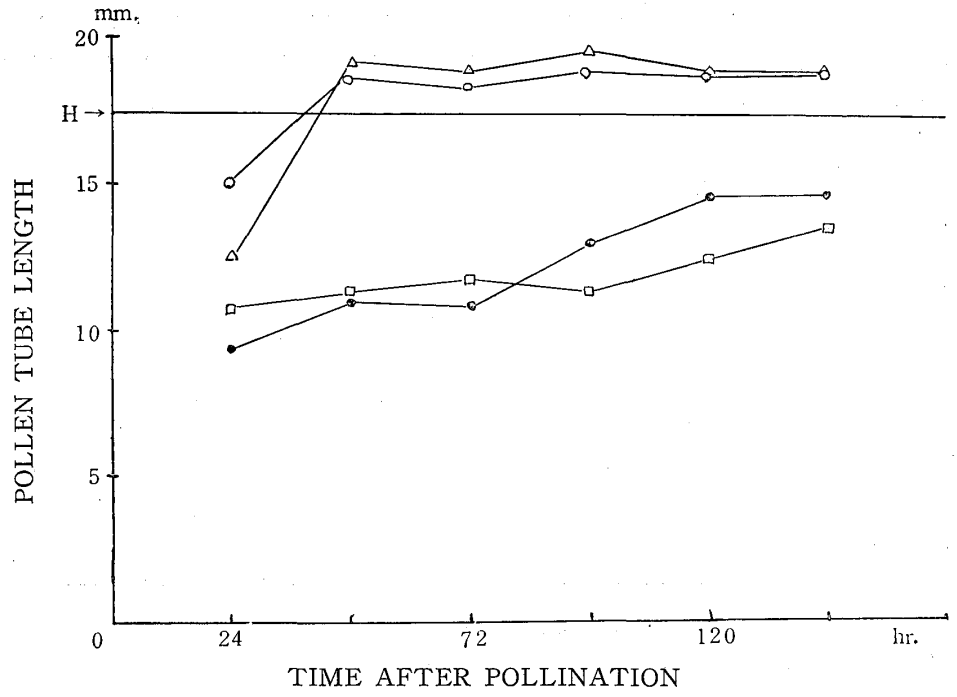


Fig. 7. The growth curves of pollen tubes in the styles of *L. Hansonii*: —△— *L. concolor*, —○— *L. maculatum*, —●— *L. rubellum*, —□— *L. Hansonii* (inter-individual pollination). H→indicates the position of the stylar base of *L. Hansonii*.

The results of inter-specific pollinations are summarized in Fig. 7. There can be pointed out two different tendencies as to the pollen tube behaviors. 1) the pollen tubes of *L. rubellum* and of *L. Hansonii* grow well in the 24 hr. period after pollination. But thereafter, their pollen tubes cannot pass through the stylar part of *L. Hansonii*. Those of *L. Hansonii* stopps their growth about 13.6 mm. below the stigma (Fig. 8). 2) the pollen tubes of *L. concolor* and of *L. maculatum* quickly take their growth, and pass through the stylar part. They can enter into the ovarian cavity, but thereafter, the growth is also retarded. The tips of the pollen tubes wander in the region 1.5–2 mm. below the base of style. As above mentioned, this is the site in which the irregular formation of embryo-sacs were observed. In only one case, approach of the tip of pollen tube to the micropylar end of the ovary was seen. Thus, the phenomena must be called as ovarian inhibition, and is not the intergameto-gametophytic inhibition.

Fig. 8. Region of style of *L. Hansonii*, in which the pollen tubes stopps their growth.

The comparison between the pollinations

of intra-individuals and inter-individuals does not reveal a remarkable difference as to the pollen tube behaviors, though there seems to be a scarce dominance of inter-individual pollinations (Fig. 9).

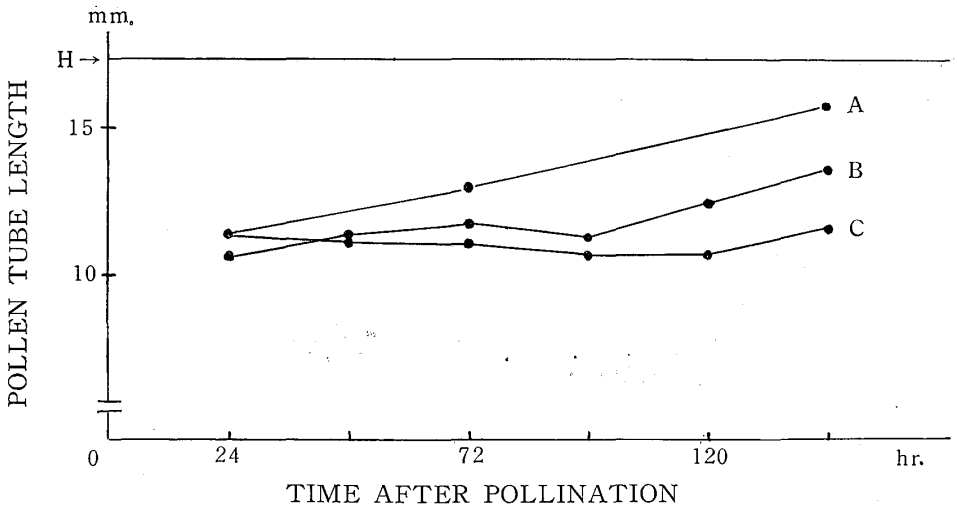


Fig. 9. The growth curves of the pollen tubes in the selfings of *L. Hansonii*. A—bud pollination. B—intra-individual pollination. C—inter-individual pollination. H→indicates the position of the stylar base of *L. Hansonii*.

The bud pollinations were also examined, following the suggestion made by Yasuda (1948). The bud pollinations gave some advantage to the pollen tube growth in the selfings (Fig. 9). However, the treatments were first done with the cut flowers, therefore it is unreasonable to make an exact comparison between this datum and that of the control plants grown in the field. The reexamination of this result in recent year, using the treated and the control plants under the same field conditions, showed the following results: in the styles of *L. Hansonii* of 1 or 2 days before flowering, the pollen tubes could penetrate through the stylar part, while in the styles of the control plants they stopped their growth in the stylar part. Thus, the tendency that the bud pollination gives more advantage to the growth of pollen tubes in the selfing has been certified.

Behaviors of pollen tubes of L. Hansonii in the styles of other species. *L. Henryi* and *L. concolor* were used as female plants. The stocked pollens were used in the former crosses, because the flowering time of each species were different. The germination rate of the pollens was decreased to 10–30%. The length of the styles of *L. Henryi* is about 2.8 times longer than that of *L. Hansonii* (*L. Henryi*–48.2 mm.: *L. Hansonii*–17.4 mm.). The behaviors of pollen tubes were very resemble to those in the styles of *L. Hansonii* (Fig. 10). In the crosses with *L. concolor*, the fresh pollens were used. As the length of the styles of *L. concolor* is considerably shorter than that of *L. Hansonii* (*L. con-*

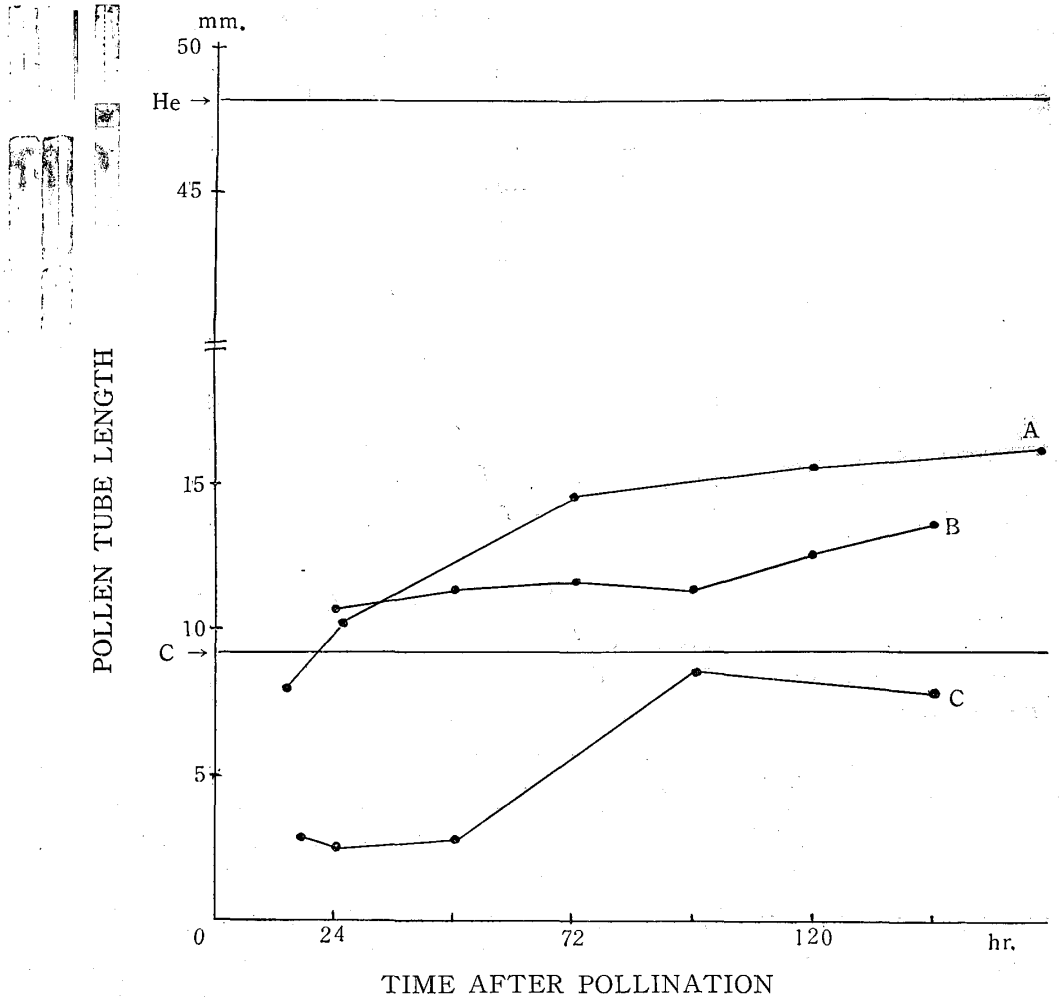


Fig. 10. The growth curves of pollen tubes of *L. Hansonii* in the styles of *L. Henryi* (A), of *L. Hansonii* (B) and of *L. concolor* (C). He→ indicates the position of the stylar base of *L. Henryi*, and C→ indicates that of *L. concolor*.

color–9.2 mm.), it may be posssible to assume that the pollen tubes of *L. Hansonii* which can grow about 13.6 mm. after the inter-individual selfings and about 14.6 mm. after the crosses with *L. Henryi*, should be able to reach to the ovarian part of *L. concolor*. Contrary to the expection, as shown in Fig. 10, almost all of the pollen tubes stopped their growth in the region about 7–9 mm. below the stigma.

Fruit development. In the flowers pollinated with the pollens of *L. Hansonii*, *L. rubellum* and *L. maculatum*, the styles fell off by 10th day after pollination, and ovaries also fell off by 15th day after pollination,

In the flowers pollinated with the pollens of *L. concolor*, however, the thickening growth of the ovaries was distinctly observed in the 10 days-period after pollination. Out of 5 ovaries pollinated, 2 ovaries developed well and matured early in Octover. A sample of these fruits are shown in Fig. 11. But these are thought to be produced

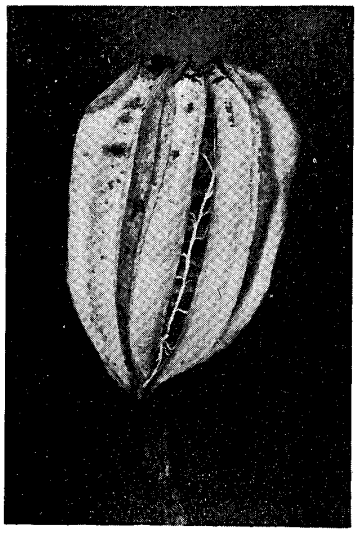


Fig. 11. Capsules obtained by the cross of *L. Hansonii*× *L. concolor*. ×1.5

by the parthenocarpic development, since the cytological observation did not reveal any sign of fertilization. The seeds obtained were all abortive, and composed only with seed coat.

Considerations

The phenomena of gametophytic self-sterility may be occurred in various steps during the development of gametophyte, and these will be considered in more details in the following paper. As above mentioned, the primary cause of these phenomena can be roughly divided into chromosomal or genic one.

The meiotic figures in *L. Hansonii* were previously studied by Haga (1943). According to his data, all except one among several dozens of the plant showed normal processes of meiosis, and almost all of their pollens were fully matured. Though the pollens were well matured, all of these plants were perfectly self-sterile. The remaining one was confirmed to be a reciprocal translocation heterozygote, and also the bridge formation was observed. This one, having a structural hybridicity in high degree, produced the abortive pollens in extreme high percentage, as about 99.8%. Comparing the normal and the exceptional hybrid plant, it may be said that the structural hybridicity, which can result the deficiency or duplication of chromosomal segments in single genome of the gametophyte, have an important role in the disturbance to the development of the gametophyte, and results effectively the "pollen abortion". But, though the structural hybridicity may be an effective cause of self-sterility, it is a rather exceptional occurrence, and the phenomenon of self-sterility in *L. Hansonii* has more wide occurrence, i. e. occurrence on the species-rank. Thus the primary cause should be accepted as genic or physiological phenomena.

There has been known the production of some hybrids using *L. Hansonii* as male parents. According to the list of Woodcock and Stearn (1950), such examples are as follows: *L. martagon*×*L. Hansonii* (*L.* "Backhouse hybrids", *L. dalhansonii*, *L. Marhan*), *L. medeoloides*×*L. Hansonii*. These possibilites for hybridization also make a reason to believe that the cause of self-sterility in *L. Hansonii* is not due to the incomplete development of gametophyte. In the observation carried out here, the morphological normality of embryo-sac formation and

the germiability of pollens were established. The pollen tubes can emerge on the stigma, and grow downward in the style. But the growth is soonafter ceased, and the retardation of pollen tube growth in the style seems to be a direct cause of the failure of fertilization.

Apparently, the pollens of many species of *Lilium* contain in themselves the nutritional storages for the germination and the pollen tube growth. But the possible growth brought about by these storages under the limited environmental conditions as on the sucrose-agar medium, is rather short. And the maintenance of the further growth, which can be observed in the styles of suitable combinations, may depend upon the further supply of essential substances other than sugars, from the stylar part.

The pollen tubes of *L. Hansonii* can travel about the same length in the styles of *L. Henryi* and in those of *L. Hansonii*. This fact first makes us a following assumption that both of the styles of *L. Hansonii* and of *L. Henryi* have very resemble conditions to response to the nutritional requirements of the pollen tube growth of *L. Hansonii*, but the conditions set up are quite insufficient. Accordingly, in the style of both species, the pollen tubes of *L. Hansonii* are able to grow only to such an extent as limited by the qualitative or quantative nutritional storages in themselves. This assumption that the styles of *L, Hansonii* are in incomplete conditions for the complementary interactions between the styles and the pollens is further supported by the statement of Backhouse (1959). The statement is that whereas *L. martagon* crossed easiy with *L. Hansonii* it had never suceed to make the reverse crosses. This suggests that the pollens of *L. Hansonii* can carry out the fertilization but the pistils does not accept the growth of pollen tube growth in some combinations.

If the status of the pistils of *L. Hansonii* above mentioned means that they are in degenerative process, the opinion of Shimizu (1957) concerning to the development of the mechanism of self-sterility in *L. Hansonii* is interesting. His opinion is as follows. In the native land, Utsuryo-Island, *L. Hansonii* has a nature of fertile. After the importation long years ago of these fertile form of the plants into Japan, they were mostly accustomed to be propagated artificially by the vegetative means. This condition might differentiate a mechanism of self-sterility in *L. Hansonii*, i. e. the customary usage of the vegetative reproduction makes the system of sexual reproduction to degenerate. This explanation is very interesting one, and will be discussed in more details in relation to the self-sterility of *Lilium tigrinum* in the following paper.

The pollen tubes of *L. maculatum* can pass through the stylar part of *L. Hansonii*, and penetrate into the ovarian part. But these pollen

tubes cannot pass the stigmatic tissue of *L. Henryi* (Niizeki, 1959). This fact bears a discrepancy in the assumption above mentioned that the styles of *L. Hansonii* and *L. Henryi* have a resemble nutritional supply. One of the explanations is that, though the necessary factors for the pollen tube growth are sufficient, other factors which actively inhibit the penetration of pollen tubes through the stigmatic tissue may contribute. Another possibility is over-sufficient complementary action of the stigmatic tissue to the pollen tube growth. And the exact explanation may be a problem for the further research.

Bud pollination gave an advantage of the pollen tube growth to some extent. The resemble results were previously obtained by Yasua (1948) in *Petunia*. Yasuda explained his results by assuming the existence of "individual substance "or" special substance", which presents an inhibiting action on the pollen tube growth in the selfing. Accordingly, in the case of *L. Hansonii*, it is also possible to assume the same situation with that of Yasuda. And also, the fact that the pollen tubes of *L. Hansonii* stopped their growth in the styles of *L. concolor* after shorter growth than that in the styles of *L. Hansonii* may be more easily explained by the inhibiting action hypothesis. Tokugawa (1914) also reported similar retardation of the pollen tube growth in the crosses of *L. speciosum*×*L. Hansonii*. And contrary to the data in the selfings of *L. Hansonii* of the present authors, he observed the penetrance of the pollen tubes into the ovarian part, using the cut flowers.

As above considered, the authors tend to take a complementary action hypothesis for explaining the mechanism of cross- and self-sterility in *L. Hansonii*, but the problems are too complicated to be solved simply and the conclusive evidence is not yet obtained.

Summary

The preliminary observations on the self-sterility in *Lilium Hansonii* has been made with special reference to the pollen tube behaviors.

1) The number of somatic chromosomes is $2n=24$, and the idiogram is shown in Fig. 1.

2) The morphological normality of embryo-sac seems to be 100%.

3) The modes of pollen tube behaviors of some *Lilium* members in the pistils of *L. Hansonii* can be classified into two types: 1) stopps in the stylar part (*L. Hansonii, L. rubellum*), 2) stopps in the ovarian part (*L. maculatum, L. concolor*).

4) The pollen tubes of *L. Hansonii* stopps their growth in the stylar part of *L. Henryi* and of *L. concolor*.

4) The bud pollination gives some advantage to the pollen tube growth in the selfing.

Acknowledgement

The authors wish to express their sincere appreciation to Prof. F. Maekawa, University of Tokyo, for his helpful guidance during the course of study.

References

Backhouse, W. O. 1959. Brief history of the Backhouse lily hybrids. R. H. S. Lily Year Book, 22: 30-31.

Brock, R. D. 1954. Fertility in *Lilium* hybrids. Heredity 8(3): 409-420.

Haga, T. 1943. A reciprocal translocation in *Lilium Hansonii* Leicht. Cytologia 13(1): 19-25.

Maekawa, F. 1950. A new system, showing embryo-sac formation types. (in Japanese) Miscel. Rep. of Res. Inst. for Natural Resources, No. 17-18: 145-149.

Niizeki, S. 1959. Cross experiments in *Lilium*. I. Pollen tube behaviors in the crosses of *Lilium Henryi* with some species of *Lilium*. Nat. Sci. Rep. of Ochanomizu Univ., 10(2): 61-67.

Shimizu, M. 1957. *Lilium Hansonii* and its culture. (in Japanese). Agric. and Hortic. (Tokyo) 32(9): 1346-1350.

Stewart, R. N. 1947. The morphology of the somatic chromosomes of *Lilium*. Amer. Jour. Bot., 34(1): 9-26.

Tokugawa, Y. 1914. Zur Physiologie des Pollens. Jour. Coll. Sci., Tokyo Imp. Univ., **35** Art. 8: 1-53.

Woodcock, H. B. C. and W. T. Stearn, 1950. Lilies of the world. London.

Yasuda, S. 1948. Physiology of reproduction in higher plants. (in Japanese). Tokyo.

(*Received Sept. 1, 1960*)