EXPERIMENTAL STUDIES ON THE STERILITY OF SOME *LILIUM* SPECIES

By

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(with 14 text- and 23 plate-figures)

Contents

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Introduction

About ninety five wild species of *Lilium* have been discovered in the world. All of them are confined to waste areas of the northern hemisphere and nearly half of them are indigenous to Asia.

Many beautiful lilies, such as the easter lily, Japanese lily, auratum lily, rubellum lily, *L. concolor*, speciosum lily, dahurian lily etc. are indigenous especially to our country.

Bulbs of these valuable species are cultivated in this country and are exported to foreign countries in large quantities, in addition a considerable number of bulbs are also consumed in Japan.

In spite of the above, scientific work in the field of lily-breeding is limited to a very small scale in Japan and only a few workers have endeavoured to breed them since Meiji era as described below.

TOKUGAWA (50) observed the pollen-tube growth in the stylar cavity of the cross-pollinated easter lily and Thunberg lily. SHIMIZU (39) conducted experiments on the inter-specific crosses of many lilies and reported the possibilities of raising seeds. NAKAJIMA (24) crossed the formosa lily with pollens of many other lily species: *L. longiflorum* var. *albo-marginatum*, the Bermuda lily, Thunberg lily, auratum platyphyllum lily, Henry lily, regal lily, auratum lily, *L. cernuum*, madonna lily, Japanese lily, and successfully obtained hybrid seeds from all crosses. However, all of the F_1 plants had flowers similar to the mother plant. He also reported the abnormal seed formation in the two inter-specific crosses; speciosum lily (♀)×auratum lily (♂) and speciosum lily (♀)× Japanese lily (♂). According to him, while seeds from these crosses did not germinate by normal management, their embryos grew steadily when they were embryo-cultured. MYODO (22) reported that the seedling varieties of the easter lily contained 7.6% abnormal pollen grains and he allso observed the pollen-tube

development and growth of the same varieties on both the artificial germinating bed and the stylar cavity when self- and cross-pollinated. NIIZEKI and SUZUKI (27) made surveys on the pollen-tube growth of *L. concolor*, *L. maculatum*, *L. rubellum* and *L. Hansonii* in the style of *L. Hansonii*.

According to them, the pollen-tubes of *L. concolor* and *L. maculatum* could traverse the style in 48 hours after pollination, but the tubes of the latter two species could not. Another survey by the same authors were made on the pollen-tube growth of *L. Hansonii* in the stylar canals of *L. Henryi* and *L. concolor*. The pollen-tubes could not pass through the style in both cases.

Scientific work on lily breeding have also been carried out in foreign lands, especially in U.S.A. OLIVER (28) attempted to raise easter lily bulbs from seeds about sixty years ago, when the majority of bulbs of that lily demanded in U.S.A. were imported from Japan and when the cultivation of them in his country had been damaged seriously by virus diseases. STOUT (45, 46, 47) studied the self-incompatibilities found in many species of this genus. PFEIFFER (30, 31) reported that the longevity of pollens exceeded ten months when stored in proper conditions of low temperature and suitable moisture. BRIERLEY, EMSWELLER and MILLER (6) tested the seed production in self- and interclonal crosses between varieties of the easter lily and found that self-incompatibility was prevalent in most varieties of that lily. In the later years EMSWELLER and STUART (15) found a possibility of overcoming this incompatibility by using a growth-promoting substance. EMSWELLER and LUMSDEN (14) and EMSWELLER (11) reported that they had raised tetraploid easter lilies, and further cytological investigations were carried out by the latter author. Recently EMSWELLER, UHRING and STUART (16) reported that the use of naphthalene acetamide alone or in combination with potassium gibberellate greatly increased seed set in selfing of the 'Georgia' cultivar. of the easter lily. They also reported that Naphthalene acetamide used at the time of pollination delayed senescence of the embryosacs of the some cultivar.

In spite of the extensive basic research on lily breeding as mentioned above, numerous points remain to be clarified especially on the behaviors of pollen-tubes in the inter-specific pollinations and on the occurrence of apomixis.

The present paper deals with surveys on (1) the pollen-tube growth when inter- and intra-specifically pollinated and also when forced pollinations on the cut surface of the style were carried out, (2) the development of the embryosacs in relation to the time when the pollen-tube arrives at the ovule and (3) the development of the normal and the apomictic embryos in the seeds.

List of scientific names and their common and Japanese names of lilies which will appear in this paper

Scientific names	Common names	Japanese names
L. amabile PALIBIN	Korean lily	Koma-yuri
L. auratum LINDL.	Auratum lily	Yama-yuri
L. auratum var. *platyphyllum* BAKER	Auratum platyphyllum lily	Tametomo-yuri Saku-yuri
L. callosum S. & Z.	Slimstem lily	Suge-yuri
L. candidum LINN.	Madonna lily	
L. cernuum KOM.	Nodding lily	Matsuba-yuri
L. concolor SALISB.	Concolor lily	Hime-yuri
L. concolor var. *coridion* BAKER	Yellow concolor lily	Kihime-yuri
L. concolor var. *mutsuanum* MAK.		Mutsuhime-yuri
L. concolor var. *pulchellum* REGEL		Chosenhime-yuri
L. concolor var. *pulchellum* f. *partheneion* WILS.	Red concolor lily	Akahime-yuri
*L.×Crow's hybrids	Grow's hybrid lily	
L. dauricum KER-GAWL.	Dahurian lily	Ezosukashi-yuri
L. dauricum 'Kogane'		Kogane-yuri
L. dauricum 'Kongojo'		Kongojo
L. formosanum WALLACE	Formosa lily	Takasago-yuri
L. Hansonii LEICHTL.	Hanson lily	Takeshima-yuri
L. Henryi BAKER	Henry lily	Kikanoko-yuri
L.×hollandicum BERGMANS	Umbell lily	
L. japonicum THUNB.	Japanese lily	Sasa-yuri
L. lancifolium var. *flaviflorum* MAK.	Yellow tiger lily	Ogononi-yuri
L. lankongense FRANCH.	Lankong lily	
L. Leichtlinii var. *Maximowiczii* BAKER	Maximowicz lily	Akahirato-yuri
L. Leichtlinii 'Akatsuki'		Akatsuki-yuri
L. Leichtlinii 'Motomura'		Motomura-yuri
L. leucanthum var. *centifolium* STEARN	Centifolium lily	
L. longiflorum THUNB.	Easter lily	Teppo-yuri
L. longiflorum var. *albomarginatum* T. MOORE		Chotaro-yuri
L. longiflorum 'Croft'	Croft lily	
L. longiflorum var. *eximium* BAKER	Bermuda lily	
L. longiflorum 'Floridii'	Florida lily	

Scientific names	Common names	Japanese names
L. maculatum THUNB.	Thunberg lily	Iwato-yuri
L. maculatum atrosanguineum hort.		Uedabeni-sukashi
L. maculatum 'Benisukashi'		Beni-sukashi
L. maculatum 'Chigusa'		Chigusa-yuri
L. maculatum 'Kisukashi'		Ki-sukashi
L. maculatum 'Kusuda'		Kusuda-yuri
L. maculatum 'Oku-kinbusen'		Oku-kinbusen
L. maculatum 'Taisho-beni'		Taishobeni-sukashi
L. martagon LINN.	Martagon lily	
L. medeoloides A. GRAY	Wheel lily	Kuruma-yuri
L. nepalense D. DON	Nepal lily	
L. nobilissimum MAK.	Noble lily	Tamoto-yuri
L. philadelphicum LINN.	Wood lily	
L. pumilum DC.	Coral lily	Itoha-yuri
L. pumilum 'Golden Gleam' hort.	Golden Gleam	Ki-itoha
L. regale WILS.	Regal lily	
L. rubellum BAKER	Rubellum lily	Otome-yuri
L. Sargentiae WILS.	Sargent lily	
L. speciosum THUNB.	Speciosum lily	Kanoko-yuri
L. speciosum f. *album-novum* hort.		Mineno-yuki
L. speciosum f. *rubrum* hort.	Speciosum rubrum	Aka-kanoko
L. speciosum var. *tametomo* S. & Z.	White speciosum	Shiro-kanoko
L. superbum LINN.	American turkscap lily	
L. tigrinum KER-GAWL.	Tiger lily	Oni-yuri
L. tigrinum 'Kakuta'		Kakuta-yuri
***L.*×*Cameo* HYBRIDS	Cameo hybrid lily	
***L.×'Wase-Chigusa'		Wase-chigusa
*****L.*×*Aurelian* HYBRIDS	Aurelian hybrid lily	

* parents are said to be *L. sulphurgale* and *L. imperiale* 'G. C. Greelman'.
** hybrid between *L. auratum* and *L. japonicum*.
*** hybrid between *L. maculatum* 'Chigusa' and *L. dauricum*.
**** hybrid between *L. regale*, *L. Sargentiae* and *L. Henryi*.

Chapter I. Pollen-grains, pollen-tube development and growth

1. Abortive pollen grains contained in the anther

POST (32) reported on the abortive pollen grains contained in anthers of six varieties of the easter lily grown in a greenhouse. He specified empty and deformed grains as the abortive ones while all others which swelled on the germinating beds were considered as good ones. According to him var. *Harrissii* contained the most abortive pollens, 44.82%, and var. *Croft* the least, 2.5%. Other varieties examined were var. *Creole*, var. *Erabu*, var. *White Queen*, and var. *Giganteum* which contained 10～4% abortive pollen grains. He also showed that the percentages of abortive grains were neither correlated with the frequency of the abnormalities of meiotic division of pollen mother cells nor the appearance of virus diseases in mother plants. MYODO (22) reported that the percentage of abortive pollen grains contained in the seedling var. of the easter lily was 3.8% and all other grains swelled well when they were sown on the germinating beds prepared by the secretion of stigma of the same lily instead of the artificial ones, but that 17.4% of all swollen grains did not develop tubes at five hours after sowing on the secretion at 28～30°C. These pollen grains were considered to be unable to germinate by a certain physiological disturbance, and it was conjectured that if the test were carried out on artificial beds, which are not be quite in an optimal condition this percentage for pollen grains failing to germinate may increase slightly.

It seems clear that the number of the abortive grains contained in anthers may be changed by the condition under which the mother plants have been cultivated, so the pollen grains in the following report were taken from the plants which had been cultivated in the same field of floriculture at the Hokkaido University, Sapporo, with some exceptions being marked in the Table 1.

Material and Method

Pollen grains were removed by needle tip from dehiscent anthers and attached to the germinating bed consisting of a drop of water containing 0.5 Mol. sugar. In each observation at five hours after sowing, the number of swollen pollen grains and abortive grains in one microscopic field of vision were calculated. The calculations were made for six colonies of pollen grains from six different anthers of each kind.

Fifty four species and varieties of *Lilium* were used as materials for this observation, forty nine of which were cultivated in the field while four kinds were cultivated in the greenhouse and in the remaining Noble lily, its pollens

were supplied from Kyushu.

Results

The results of observations are summarized in Table 1. The percentages of abortive pollen grains contained in anthers of all examined species are distributed over a wide range, the smallest value is 0% for *L. martagon* and the largest is 57.95% for *'Cameo Hybrids'*. The number of kinds which contain more than twenty per cent are ten, and those which contain less than ten per cent are thirty four and the remaining ten contain 10～20 per cent abortive grains.

TABLE 1. Abortive pollen grains contained in anthers of fifty four kinds of lilies

Species and varieties	Abortive Grains (per cent)	Group
L. amabile	22.45±2.13	N D
L. auratum	8.95±2.11	N S
L. auratum var. *platyphyllum*	9.00±1.83	N S
**L. aurelian* 'Sunburst Strain'	2.35±0.20	H D
L. callosum	1.20±0.17	N S
L. 'Cameo Hybrids'	57.95±3.21	H S
L. concolor var. *coridion*	3.12±0.31	V D
L. concolor var. *mutsuanum*	1.53±0.07	N D
L. concolor var. *pulchellum*	3.25±0.50	N S
L. concolor var. *pulchellum* f. *partheneion*	23.99±1.74	V D
L. concolor var. *pulchellum* f. *partheneion* (a new form)	4.16±0.36	H S
L. Crow's Hybrids	2.85±0.41	H S
L. dauricum	5.51±1.17	N S
L. dauricum 'Kogane'	11.34±0.98	H D
L. dauricum 'Kongojo'	20.80±1.55	V D
L. Hansonii	34.80±2.76	V D
L. Henryi	4.78±0.82	N S
L. hollandicum 'Tangerin'	5.83±0.35	H D
L. japonicum	1.40±0.47	N S
L. lancifolium var. *flaviflorum*	10.66±0.83	V D
**L. lankongense*	20.33±1.08	N S
L. Leichtlinii var. *Maximowiczii*	1.85±0.56	N S
L. Leichtlinii 'Akatsuki'	22.46±1.63	V D

Species and varieties	Abortive Grains (per cent)	Group
L. Leichtlinii 'Akatsuki' (a new form)	14.41±1.88	H S
L. Leichtlinii 'Motomura'	2.75±0.56	H D
L. leucanthum var. *centifolium*	1.77±0.11	N S
L. longiflorum (a green stemmed form)	1.18±0.42	N S
L. L. longiflorum var. *albo-marginatum*	11.00±1.15	V D
L. longiflorum 'Floridii'	3.61±0.22	V D
L. maculatum	2.41±0.59	N S
L. maculatum 'Benisukashi'	10.50±1.26	H D
L. maculatum 'Chigusa'	18.84±1.21	H D
L. maculatum 'Chigusa' (a new form)	3.68±0.32	H D
L. maculatum 'Kisukashi'	26.08±2.58	H D
L. maculatum 'Kusuda'	8.39±0.62	H D
L. maculatum 'Oku-kinbusen'	7.52±0.35	H D
L. maculatum 'Taisho-*beni*'	3.52±0.28	H D
L. martagon	0.00±0.00	N S
L. medeoloides	12.04±1.18	N S
L. nepalense	1.04±0.11	N S
****L. nobilissimum*	14.22±1.35	N S
***L. philadelphicum*	9.52±1.03	N S
L. pumilum	2.15±0.44	N S
L. pumilum 'Golden Gleam'	21.42±1.89	V D
L. rubellum	0.99±0.15	N S
L. Sargentiae	1.47±0.13	N S
L. speciosum f. *album-novum*	4.68±0.31	N D
L. speciosum f. *rubrum*	1.67±0.42	N D
L. speciosum var. *tametomo*	12.54±1.79	V D
L. speciosum var. *tametomo* (a new form)	3.39±0.28	H S
***L. sperbum*	20.37±2.28	N S
L. tigrinum	15.71±1.51	V D
L. tigrinum 'Kakuta'	4.13±1.32	V D

N D : natural forms and hitherto propagated asexually.
N S : natural forms and hitherto propagated by seeds.
V D : seedling variants and hitherto propagated asexually.
H D : hybrid origins and hitherto propagated asexually.
H S : hybrid origins and hitherto propagated by seeds.
*: Pollens were taken from plants in the greenhouse.
**: Pollens were sent from Kyushu.

A general view on Table 1 shows that (1) the kinds of natural origin and hitherto propagated by seeds (NS) have abortive pollens in very low levels of percentage while the kinds known as seedling variants and hiterto propagated asexually (VD) have in high levels, the other kinds of ND, HD and HS group are intermediate, (2) anthers grown on the plants which have been cultivated in the greenhouse seem to contain a higher percentage of abortive pollens even though they belong to NS group as seen *L. lankongense* and *L. superbum*, (3) newly raised forms contain a definitely lower number of abortive pollens than the older ones in spite of their resemblance in characteristics as in the cases of Akatsuki-yuri, Chigusa-yuri and their new forms.

2. Pollen-tube development and growth in relation to temperature and sugar concentration of the germinating beds

A few reports can be referred to with regards to the influences of enviromental conditions on the pollen-tube development and growth of lilies.

TOKUGAWA (50) observed a more rapid growth of pollen-tube of *L. maculatum* in the stylar cavity of the same species at 80°F than at 60°F. Shisa showed in his publication (42) that the optimum pH for pollen-tube development in *Lilium* species was 5.5 when the germinating bed consisted of gelatine and sugar. SHINOTO (41) reported in his publication that the optimum pH to develop their tubes was 4.7 for pollens of the easter lily, formosa lily and tiger lily when agar and sugar were used for the germinating bed. MYODO (22) attempted to find the optimum temperature and sugar concentration for the pollen-tube development in the easter lily and reported that the optimum temperature was about 30°C on the agar-sugar germinating bed, and that the optimum sugar concentration was about 8 per cent when water solution was used for the bed. He observed also the pollen-tube growth in the stylar cavity and reported that the optimum temperature for the growth was the same as that for tube development on the artificial germinating bed. And while they could grow in a wide range of 11°C～35°C, they could not grow or burst in a few hours at 40°C. He suggested that a considerable number of pollen-tubes could be damaged when paper bags were used to isolate the emasculated and pollinated lilies from contamination by undesired pollens, because the temperature in the bag was apt to rise to 40°C in the flowering season of the easter lily even at the latitude of Sapporo (North 43.4).

Material and Method

Eight species and varieties of Lilium, which flowered in different seasons

between the middle of June and the beginning of August in Sapporo, were used for materials in the germinating tests under various temperatures as well as various sugar concentration. The germinating beds used were drops of water solution of sugar in VANTIEGHEM's cells, and on one drop 20～120 pollen grains were sown by a needle tip. These were placed in the incubators of respective temperature and at five hours after sowing, the number of germinating pollen grains was estimated. The pH of the germinating beds were between 5.6～5.8. The percentages shown in Table 2 were obtained from good pollens only and the abortive ones were omitted from these data.

Results

The results obtained in this experiment are summarized in Table 2.

TABKE 2. Germination of the pollens of eight species under different temperatures and sugar concentrations: percentages at five hours after sowing

(A) *L. concolor* var. *pulchellum* f. *partheneion*

Sugar (Mol.)	Temperature (C)				
	40	30	25	15	10
1.17	0.81	0	0	0	0
0.58	9.84	16.66	0	0	0
0.29	15.69	19.30	19.73	0	0

(B) *L. japonicum*

Sugar (Mol.)	Temperature (C)					
	40	30	25	20	15	10
1.00	0	7.80	0	0	0	0
0.58	3.30	0	0	0	0	0
0.29	4.16	8.25	13.04	0	0	0
0.25	0	0	47.09	28.04	0	0

(C) *L. maculatum* 'Kisukashi'

Sugar (Mol.)	Temperature (C)				
	40	30	25	15	10
1.17	0	0	0	0	0
1.00	0	3.25	0	0	0
0.58	2.98	14.40	0.25	0	0
0.29	6.47	9.93	26.14	0.95	0

(D) *L. auratum*

Sugar (Mol.)	Temperature (C)				
	30	25	20	15	10
1.00	34.73	0	0	0	0
0.25	13.63	37.71	35.50	0	0

(E) *L. medeoloides*

Sugar (Mol.)	Temperature (C)				
	30	25	20	15	10
1.00	25.00	34.18	0	0	0
0.25	33.67	32.55	8.00	0	0

(F) *L. pumilum*

Sugar (Mol.)	Temperature (C)				
	30	25	20	15	10
1.00	56.25	51.38	5.74	0	0
0.25	42.85	42.85	31.57	5.00	0

(G) *L. dauricum*

Sugar (Mol.)	Temperature (C)				
	30	25	20	15	10
1.00	0	63.63	0	0	0
0.25	35.21	66.66	11.11	2.00	0

(H) *L. concolor* var. *pulchellum*

Sugar (Mol.)	Temperature (C)				
	30	25	20	15	10
1.00	58.13	5.26	0	0	0
0.25	25.00	43.47	15.15	10.00	0

These results indicate that the optimum temperature for pollen-tube development is found between 25°C and 30°C for all species tested. The flowering season of lilies is widely spread between the middle of June and the beginning of August by the species but the optimum temperatures of pollen-tube develop-

ment in respective species are generally the same. The lowest temperature where the tubes can develop within five hours, is 15°C for the early flowering species and varieties; *L. maculatum* 'Kisukashi', *L. medeoloides*, *L. pumilum*, *L. dauricum*, *L. concolor* var. *pulchellum*. For the other three lilies 20°C is the lower limit of temperature for tube development and they flower in the field after the middle of July at Sapporo.

The optimum temperature for the pollen-tube development, as seen in the tables, changes by the sugar concentration of the beds viz. the optimum temperature tends to rise slightly in the higher sugar concentrations.

3. Pollen-tube growth in the style

MYODO (22) estimated the rapidity of pollen-tube growth in the stylar cavity of the easter lily when cross- and self-pollinated. His report showed that when cross-pollinated, the pollen-tube grew as long as 48.0 mm in the first 24 hours and then 72.3 mm in the next 18 hours but when selfed it was 30.0 mm in the first 24 hours and 44.3 mm in the next 18 hours under the optimum temperature, 28°C. As seen in his report, the pollen-tubes when self-pollinated, grew very slowly or stopped their growth after they reached the lower part of the style. He further showed that pollen-tubes developed well on beds of secretion from own and other stigmas, but they could only grow as long as 1.63 mm~1.70 mm in five hours after sowing. If the emergence of tubes from pollen grains occurred at 3 hours after they were sown, the rapidity of tube-growth was about 0.8 mm per hour, which was a very smaller value than in the style. The pollen-tube, therefore, may possiblly accept a certain nutritive substance from the wall of the stylar cavity or an inviting substance from the ovary.

The writer wishes here to report some results of experiments on the best age of styles for the pollen-tube growth and on the pollen-tube behaviors in self- and cross-pollination and also in the inter-specific pollination.

i) Pollen-tube growth when pollinated in the styles of different ages

In breeding operations of the lily, only a little attention is paid generally to the time of pollintion, which is carried out on the day of flowering of the mother plant. The lily has a strikingly great numer of ovules along the central axis of the long ovary, and they mature at slightly different times according to their positions on the axis. When the tubes are abundant some of them can find their partners in the ovary. But in cases when the number of pollens placed on the stigma is very small or the number of abortive embryosacs and pollens are very large, it would be important to pollinate at the best time for the seeds to grow well.

Material and Method

In this experiment two species were used: easter lily and speciosum lily. Because of the fairly strong self-sterility in both of the lilies they were pollinated with other clones of respective species.

In the first experiment (A) of the easter lily, flowers with styles of proper ages were cut and coss-pollinated and then placed in an incubator at 23°C. They were previously emasculated and their stem bases were kept in water during this experiment.

In the next two experiments of speciosum lily (B, C), flowers were cut one day before flowering and kept in a room with their stem bases in water, and the emasculation was carried out on the same day. The flowers were placed in an incubator at 25°C., immediately after pollination.

In all three experiments, the length of pollen-tubes was estimated by the longest one in each sample, the method used is as follows: a style previously pollinated at 48 hours prior to that time was cut off at the top of the ovary. This was split open along the longitudinally median plane and cotton blue solution was deposited on the cut surface so as to penetrate the stylar cavity. The colored pollen-tubes on the wall of the cavity were traced by microscope and the tip of the longest tube was determined and the distance between this point and the surface of the stigma was estimated.

Results

The results obtained are summarized in Table 3, 4 and 5.

From the results here mentioned it seems clear that the appearance of the secretion on the stigma is a fairly correct indication of maturing of the style. The easter lily secretes frequently even on the day of flowering when cultivated in the field, but in general the secretion is seen on the following (2nd) day. The speciosum tily is usually slow in secreting which occurs at two or three days after flowering.

The best time of pollination, from the standpoint of the tube-growth, is considered to be 1～2 days after the flowers have opened for the easter lily and 2～3 days after for the speciosum lily. Though the pollen-tubes can grow by pollination conducted on the day before or after the best day, their growth gradually slows. If the secretion is absent on the expected optimum day, the tubes grow very slowly.

(A) *L. longiflorum*

TABLE 3. Pollen-tube growth of the easter lily in styles of different ages

(at 23°C, inter-clonal cross)

ages of style	stigmatic secretion	when pollinated	length of style (mm)	length of tubes (mm) after 48 h.	velocity of tube-growth per h. (mm)*
1 day before flowering	absent	Aug. 15	90	45	1.00
1st day of flowering	absent	July 30	85	82	1.82
1st day of flowering	present	Aug. 16	87	87+x	1.93+x
3rd day of flowering	present	Aug. 16	87	66	1.46

(B) *L. speciosum* f. *rubrum*

TABLE 4. Pollen-tube growth of the speciosum lily in styles of different ages

(at 25°C, style: rubrum, pollen: tametomo)

ages of style	stigmatic secretion	when pollinated	length of style (mm)	length of tube (mm) after 48 h.	velocity of tube-growth per h. (mm)*
1 day before flowering	absent	Sept. 3	65	33	0.73
"	"	"	65	35	0.77
"	present	"	66	42	0.93
"	"	"	66	48	1.06
1st day of flowering	absent	"	67	40	0.88
"	"	"	67	45	1.00
"	present	"	68	55	1.22
"	"	"	68	48	1.06
3rd day of flowering	"	Sept. 7	70	70+x	1.55+x
"	"	"	70	70+x	1.55+x
5th day of flowering	"	Sept. 15	67	38	0.84
"	"	"	67	50	1.11
7th day of flowering	"	Sept. 17	70	19	0.42

(C) *L. speciosum* var. *tametomo*

TABLE 5. Pollen-tube growth of the speciosum lily in styles of different ages

(at 25°C, style: tametomo, pollen: rubrum)

ages of style	stigmatic secretion	when pollinated	length of style (mm)	length of tube (mm) after 48 h.	velocity of tube-growth per h. (mm)*
1 day before flowering	absent	Sept. 2	59	43	0.95
"	"	"	59	42	0.93
"	"	"	64	49	1.08
"	"	"	64	43	0.93
1st day of flowering	"	Aug. 26	63	58	1.28
"	"	"	63	55	1.22
2nd day of flowering	present	Sept. 5	60	60+x	1.33+x
"	"	"	60	60+x	1.33+x
3rd day of flowering	"	Sept. 6	61	61+x	1.35+x
"	"	"	61	61+x	1.35+x
4th day of flowering	"	Sept. 7	61	61+x	1.35+x
"	"	"	61	61+x	1.35+x
5th day of flowering	"	Sept. 8	63	63+x	1.40+x
"	"	"	63	63+x	1.40+x
"	absent **	"	55	52	1.15
"	" **	"	55	55	1.22
7th day of flowering	present	Sept. 15	65	25	0.55
"	"	"	65	33	0.73

*: the emergence of tubes was assumed to be at three hours after sowing.
**: secretion was absent, the cause of which was unknown.

ii) Pollen-tube growth in cross- and self-pollination

Several reports have mentioned the strong characters of high self-sterility in some species of *Lilium* (6, 45, 46, 47). MYODO (22) reported that pollen-tubes of the easter lily travelled down normally as far as about a half to two-thirds of the whole length of the style when self-pollinated, but they stopped or elongated very slowly thereafter.

Some other species of *Lilium*, however, are self-fertile (49, 50), and TOKUGAWA (50) reported the pollen-tubes of the auratum lily grew much more slowly when cross-pollinated than when selfed.

YASUDA (53) sugested, in his studies on self-sterility in Petunia, that a growth-

inhibiting substance for pollen-tubes may be secreted from ovules in the ovary. EAST and PARK (10) reported in their studies on self-sterility on Nicotiana that in the compatible cross the style possibly secreted a certain substance which was favourable for the growth of pollen-tubes, in the incompatible cross, however, this substance was absent in the style and the pollen-tubes could not elongate as easily as in the former.

It seems interesting to determine the reason why the pollen-tube can travel and reach the embryosacs better, in the compatible crosses than in the incompatible ones. Is there a growth-promoting substance for pollen-tube in the compatible or a growth-inhibiting substance in the incompatible? Possibly the causes may be different for different plants.

Here the writer wishes to supply some data on the pollen-tuhe behaviors both in compatible and incompatible crosses using four species of *Lilium*.

Material and Method

One of the four kinds for this experiment was the formosa lily which was self- and inter-clonal cross-fertile, the other three were self-sterile. Flowers with styles to be pollinated were cut one day before they opened and placed in water. Pollination was carried out on the stigmas between the first and third day of flowering, and the flowers were held at 25°C throughout the experiment.

Result

The results obtained are summarized in Table 6, 7, 8 and 9.

(A) *L. concolor* var. *pulchellum* f. *partheneion*

TABLE 6. Pollen-tube growth of the 'Akahime-yuri' after self- and cross-pollination

age of style	when pollinated	combination	length of style (mm)	length of tube (mm) after		velocity of tube-growth per h. (mm)*
				7 h.	24 h.	
1st day of flowering	July 21	cross (interclonal)	10	1.5		
"	"	"	"		7.5	0.35
"	"	"	"		7.5	0.35
"	"	"	"		10.0+x	0.47+x
"	July 27	self	11		6.0	0.28
"	"	"	"		7.0	0.33

From the results mentioned in Table 6, 7, 8 and 9 it was shown that in species with high self-sterility the pollen-tubes from self-pollinated grains travelled

(B) *L. speciosum* f. *rubrum*

TABLE 7. Pollen-tube growth of the speciosum rubrum lily after self- and cross-pollination

age of style	when pollinated	combination	length of style (mm)	length of tube (mm) after			velocity of tube-growth per h. (mm)*
				24 h.	48 h.	72 h.	
4th day of flowering	Aug. 26	cross (interclonal)	66	42			2.00
"	"	"	66		66+x		1.46+x
"	Aug. 29	self	69		33.5		0.74
"	"	"	69			37	

(C) *L. speciosum* var. *tametomo*

TABLE 8. Pollen-tube growth of the speciosum tametomo lily after self- and cross-pollination

age of style	when pollinated	combination	length of style (mm)	length of tube (mm) after			velocity of tube-growth per h. (mm)*
				24 h.	48 h.	72h.	
1st day of flowering	Aug. 26	cross (interclonal)	65	21			1.00
"	"	"	63		58		1.28
"	"	"	63		55		1.22
"	"	"	58			58+x	1.28+x
"	"	"	58			58+x	1.28+x
"	Aug. 30	self	65		28		0.62
"	"	"	65		30		0.66
"	"	"	66			37	0.53
"	"	"	66			39	0.56

(D) *L. formosanum*

TABLE 9. Pollen-tube growth of the formosa lily after self- and cross-pollination

age of style	when pollinated	combination	length of style (mm)	length of tube (mm) after		velocity of tube-growth per h. (mm)*
				24 h.	48 h.	
1st day of flowering	Aug. 31	self	84	57.0		2.71
"	Aug. 29	"	90		90+x	
"	Aug. 27	cross (interclonal)	84	58.5		2.78
"	"	"	79		79+x	

down for about a half to two-thirds of the whole length of the style, by the time when the tubes from the cross-pollinated grains had passed through the styles and reached the top of the ovary. This observation was the same in the easter lily as reported previously by the writer (22).

The tips of pollen-tubes from self-pollinated grains showed no signs of swelling or twisting when they had stopped.

In the formosa lily, which is a self-fertile species, the velocity of pollen-tube growth was more or less the same in both cases of self- and cross-pollinations.

As reasons for slowing the pollen-tube growth in the incompatible cross, SHISA (42) introduced four factors: a) the absence of growth-promoting substances, b) the presence of growth-inhibiting substances, c) an excess of nutritive substances in the conducting tissue and d) the deficiency of nutritive substances.

In species of *Lilium* the reason for the slowing of the pollen-tube growth in selfing was considered to be a) as SHISA indicated, for the three following reasons (1) the tips of tubes showed no abnormality in their shape (2) there could be observed no indication for rise and fall of growth-inhibiting substances when pollinated on styles of diferent ages, for example, pollen-tubes of the speciosum tametomo lily in selfing grew about 30 mm long in 48 hours after pollination in both styles of flowering day and three days after flowering. Pollen-tubes in cross-pollinations, on the other hand, grew about 48 mm and 70+x mm respectively in styles of flowering day and three days after flowering, (3) the nutritional conditions in the style could no be a factor in this case for the conditions were quite the same in both cases of self- and cross-pollinations.

iii Pollen-tube growth in cut styles

SHIMIZU (39) reported that seeds could be obtained in some lilies even when pollinated on the cut suaface of the style, but in the incompatible cosses this pollination on the cut surface was also unsuccessful.

In the previous experiments above described, it is clear that the pllen-tubes could grow fairly well for the first half way of the style even in the incompatible crosses after which they stopped or continued to but very slowly.

The writer wishes to introduce a growth-promoting substance for the pollen-tube, which was probably secreted from ovules in the compatible crosses. Here, the writer attempted to present another experiment by cutting or shortening the style, and to observe the pollen-tube growth in the remainder of the style.

Material and Method

Styles of two kinds of lilies viz. *L. longiflorum* and *L. speciosum* were used as materials. On the day of flower-opening the styles were cut transversely removing more than half of the style, and immediately after that the pollen

grains were smeared on the cut surface so that the pollen grains could creep into the stylar cavity, and coated by lanolin as shown in text-fig. 1.

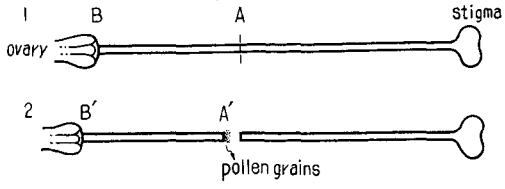

Fig. 1. Schematic diagram of the operation of the style for the easter lily.

In the case of the speciosum lily pollen grains were smeared on the longitudinally split surface at the top of the stump and this portion was covered by a thin wire, as shown in text-fig. 2.

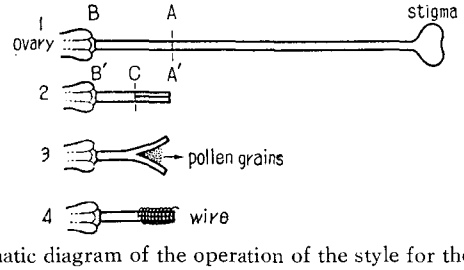

Fig. 2. Schematic diagram of the operation of the style for the speciosum lily.

These operations were carried out using cut flowers which were then placed in an incubator at 25°C, with their stems dipped in water.

The pollen-tube length was estimated at 24 hours after smearing the pollen grains for the easter lily and at 48 hours for the speciosum lily.

(A) *L. longiflorum*

TABLE 10. Pollen-tube growth of the easter lily and the 'Kakuta-yuri when pollinated on the cut surface of shortened styles of the easter lily

age of style	when pollinated	combination	length of A′~B′ (mm)	length of tube (mm) after 24 h.	velocity of tube-growth per h. (mm)*
1st day of flowering	July 30	cross (interclonal)	13.0	13+x	0.61+x
"	"	"	15.0	15+x	0.71+x
"	July 29	"	15.0	15+x	0.71+x
"	"	self	15.0	15+x	0.71+x
"	July 30	"	15.0	15+x	0.71+x
"	"	pollens of 'Kakuta'	16.0	12	0.57

(B) *L. speciosum* f. *rubrum*

TABLE 11. Pollen-tube growth of the speciosum rubrum lily when pollinated on the cut surface of the shortened styled

age of style	when pollinated	combination	length of C~B′ (mm)	length of tube (mm)	velocity of tube-growth per h. (mm)*
1st day of flowering	Sept. 3	cross ** (interclonal)	15.0	15+x	0.33+x
〃	〃	〃	15.0	15+x	0.33+x
〃	〃	〃	20.0	20+x	0.44+x
〃	〃	〃	20.0	20	0.44
〃	〃	self	20.0	20+x	0.44+x
〃	〃	〃	20.0	15	0.33
〃	〃	〃	20.0	20+x	0.44+x
〃	〃	〃	20.0	20+x	0.44+x

*: the emergence of the tubes was assumed to be at three hours after sowing.
**: Pollens: speciosum tametomo lily.

Results

Results obtained are shown in Table 10 and 11 for respective species.

The style was cut at A, pollen grains were smeared at A′. B and B′ are the tops of the ovary.

From these two experiments above mentioned, it is clear that pollen-tubes can grow steadily in the basal portion of the style. Accordingly there can not be any growth-inhibiting substances for pollen-tubes to grow. The reason for stopping and slowing of the growth of pollen-tubes in the basal portion of the style when pollinated on the stigma in the incompatible cross, is therefore considered to be a deficiency of growth-promoting substances from the ovary. Pollen-tubes of *Lilium* can grow to a certain length on various media which do not supply any nutritive or growth-promoting substances as the writer reported previously (22). This limit of length to which the pollen-tube can grow without any growth-promoting substances probably coincides with about half of the length of the style.

The pollen-tube growth of 'kakuta-yuri' in the basal part of the style of the easter lily was only 12 mm long after 24 hours at 25°C, and in this interspecific combination it was noted that the tips of tubes swelled abnormally.

iv) Growth-promoting action for pollen-tubes in the compatible cross-pollination

It was conjectured in the previous experiments that in compatible crosses

the pollen-tube growth was promoted by a certain substance from the ovary.

In the incompatible crosses this substance was considered to be non-functional.

Further tests will be described here, which suggest also the presence of the growth-promoting substance in the style, using the formosa lily which is a self- and cross (interclonal)-fertile species.

Material and Method

The material in this experiment was one of the clones of an early flowering strain of the formosa lily, and pollens of the other clone were used. Pollen grains were sown in two ways: in the first experiment they were sown on the cavity wall of opened pieces of the style. In the second experiment they were sown on the transversely cut surface of the style so as the pollen-tubes could grow into the cavity of the closed style. Both of these two experiments were carried out under a temperature of 25°C, and after 9 (in the first experiment and 15 (in the second) hours the length of pollen-tubes were estimated.

In the first experiment the style was cut into four pieces, each of which was almost of the same length (a, b, c and d in text-fig. 3, A). Each of the four pieces was split longitudinally along the median plane and the stylar cavity was exposed. Pollen grains were sown on the exposed cavity wall at the central point of each piece (text-fig, 3, B). In the 'a' piece, however, they were sown in the cut surface of the stigma as shown in text-fig. 3, C.

Each of the pollinated pieces was placed in a cell of high humidity and the length of pollen-tube which grew along the wall or cut surface was estimated at 9 hours after pollination.

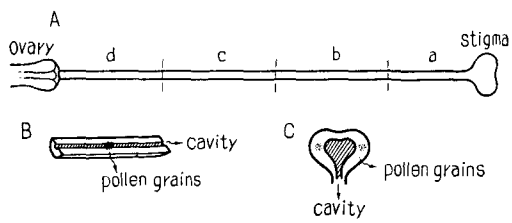

Fig. 3. Schematic illustration showing the cut pieces of styles and the points where pollinated.

In the second experiment the style was cut transversely into two pieces, which were almost of the same length. Pollen grains were sown on three cut surfaces (B, B′ and C in text-fig. 4), and on the stigma (A in text-fig. 4).

The two pieces which had been pollinated were placed in cells of high humidity at25°C and the pollen-tube length along the cavity wall was estimated

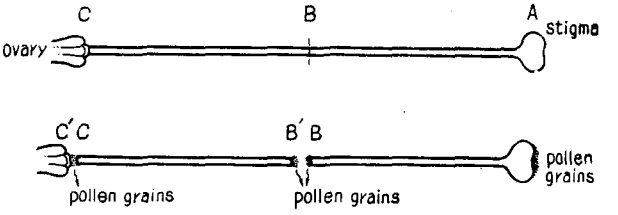

Fig. 4. Schematic illustration showing the whole style and operation in the second experiment.

at 15 hours after the pollination.

Results

In the first experiment, the pollen-tubes from grains sown on the cut surface of the stigma in 'a' piece, grew in random directions as shown in text-fig. 5, but a larger number of tubes were pointed towards the inside of the stigma than in other directions. On the other hand, the pollen-tubes which were sown on the cavity wall grew in two directions viz. the stigma and the ovary (text-fig. 6).

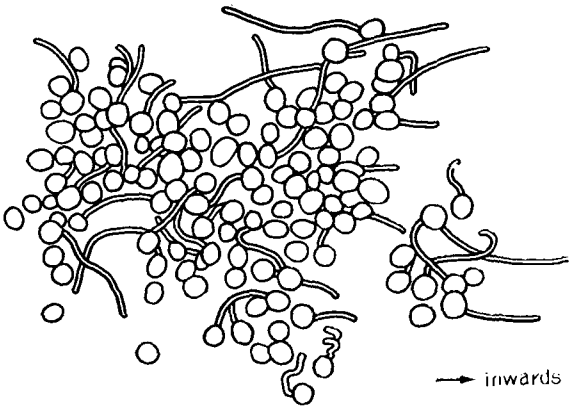

Fig. 5. Pollen-tubes grown from grains sown on the cut surface of the stigma. More tubes point towards the inwards than towards other directions. after 9 h. × ca. 45.

In pieces 'a' and 'b' the pollen-tubes did not grow as long as in pieces 'c' and 'd', where tubes grew to 2.20~2.80 mm in 9 hours for both the stigma and the ovary. The reason why there was no polarity in the pollen-tube growth, could not be understood, but it was clearly seen in the data that pollen-tubes grew more in the basal portion of the style which had been split in the longitudinally median plane than in the top, and it would suggest that there

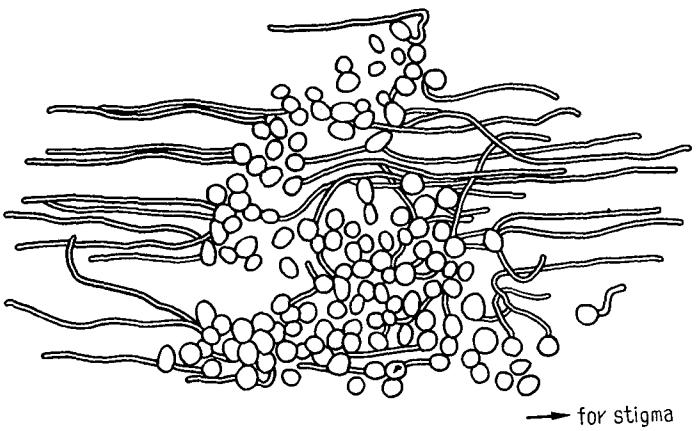

Fig. 6. Pollen-tubes grown from grains sown on the stylar wall of 'c' piece of the first experiment. after 9 h. × ca. 45.

was a higher content of growth-promoting substance distributed in the basal portion of the style than in the upper.

In the second experiment, pollen grains were sown on the stigma and on the transversely cut surfaces. 15 hours after sowing, each piece of the style was split longitudinally, then the tubes were stained with cotton blue solution and the length of tubes were estimated.

Pollen grains sown on the stigma (at A in text-fig. 4) and on the transversely cut surface of B′ developed tubes towards the ovary, while pollen grains sown on the cut surfaces of B and C developed tubes towards the stigma.

TABLE 12. Pollen-tube growth of the formosa lily on the cut and exposed surface of the four pieces of style. after 9 h.

age of style	when pollinated	piece of style	length of tubes (mm)	
			for the stigma	for the ovary
1st day of flowering	Aug. 30	a	1.21, at random	
"	"	b	1.70	1.00
"	"	c	2.60	2.20
"	"	d	2.70	2.80

From the results shown in Table 12 and 13 it was clearly seen that pollen-tubes grew very well towards the ovary along the cavity wall of the styles which had not been split longitudinally as compared with pollen-tubes towards

TABLE 13. Pollen-tube growth of the formosa lily in the closed stylar cavity which was cut transversely in two. after 15 hours.

age of style	when pollinated	point where pollens were given	length of tubes (mm)	
			for the stigma	for the ovary
1st day of flowering	Aug. 30	A		33.33
"	"	B	6.16	
"	"	B′		37.83
"	"	C	6.00	

the stigma, and also that the length of tubes from B′ (the basal portion of the style) was slightly longer than from A (the stigma).

From the two experiments above described a growth-promoting substance is suggested to be secreted from the ovary and this substance is distributed in the style for a certain period, and the concentration of that substance in the style is higher in the basal part than in the top. Accordigly, the pollen-tubes grew more rapidly in the basal portion. The polarity in the growth of pollen-tubes clearly seen in the second experiment is probably caused by the difference in the concentration of the growth-promoting substance distributed in the style as above stated. If the style is cut into small pieces or split longitudinally as in the first experiment, no decided polarity could be recognized in the pollen-tube growth.

v) Pollen-tube growth when inter-specifically cross-pollinated

Many efforts have been made by a number of lily breeders since the end of the last century to raise new hybrid lilies by inter-specific crosses (18, 34) and a small number of combinations were proved to be successful, from which some well known hybrids have been bred. However, most of the other crosses between different species of *Lilium* have been proved unsuccessful. On one hand, in some kinds, regal lily and formosa lily for instance, apomixis has been reported to be prevalent when they were used as seed parents and crossed with other species.

The writer observed in the next three experiments the pollen-tube development and growth when the pollens were sown on the stigma and on the stigmatic secretion of the other species.

In the first experiment pollens were sown on the secretion of the stigma of other species, in the second the inter-specific pollinations were tried on the stigma and in the last experiment pollens were sown on the cut surface of the short-cut styles of the other species.

(A) Pollen-tube growth on the secretion of stigma of other species

Material and Method

A drop of the stigmatic secretion was placed on a glass slide directly from the mature stigma, and pollen grains were sown on the drop. The tube development and growth progressed in the Vantieghem's cells under a temperature of 25°C with exceptions of the noble lily (22°C) and the tiger lily (25°~30°C).

Results

The results obtained are shown in the Table 14. Here we can see that the pollen-tube can grow fairy well on the stigmatic secretion of the other species and this fact will suggest that the stigmatic secretion is quite excellent as a germinating medium for pollens of any other species as well as of the same species of *Lilium*.

TABLE 14. Pollen-tube growth of some lilies on the stigmatic secretion of the other species

when pollens were given	temperature (°C)	parents of		length of tube (mm) after		
		stigmatic secretion	pollens	2 h.	4 h.	20 h.
July 28	25~30	easter lily,	tiger lily	0.1	1.2	2.0
July 31	25	"	Henryi lily			0.6
"	"	"	'Motomura'			0.4
"	"	"	'Benisukashi'			0.6
"	"	"	'Kisukashi'			0.8
"	"	"	japonicum			1.0
"	"	"	Hanson lily			0.4
"	"	"	'Chosen-hime'			0.8
"	"	"	amabile			1.0
"	"	"	'Ueda-beni'			0.5
"	"	"	speciosum			0.8
July 28	"	'Kakuta-yuri'	easter lily	0.2	1.8	6.5
July 31	"	"	Henryi lily			0.6
"	"	"	'Akatsuki'			1.1
July 4	22	dahurian lily	noble lily		1.0	

(B) Pollen-tube growth in the style of the other species

The previous experiment has shown us that the pollen-tubes could develop and grow very well on the stigmatic secretion of the other species, but it was not known whether they could grow in the stylar cavity and whether they

travel down or not. In some plants other than lily the pollen-tube can travel down along the conducting tissue of style of an incompatible as well as of compatible plant (1, 21, 25, 38, 43, 48, 49). In many other plants, however, they can not grow or travel down in the incompatible style, and some of them are arrested decidedly and show abnormal shapes of the tube tips or they burst (7, 17, 19, 29).

NIIZEKI (26) observed the behaviors of pollen-tubes in crosses of *L. Henryi* with four different species viz. *LL. maculatum*, *auratum*, *speciosum* and *longiflorum*. In one cross with *macratum* he noticed a stigmatic inhibition but in two crosses with *auratum* and *speciosum* the pollen-tubes passed through the style. NIIZEKI and SUZUKI (27) showed that the pollen-tubes of *L. concolor* and *L. maculatum* could pass through the style of *L. Hansonii*, while *L. rubellum* could not. The writer wishes here to report on the pollen-tube growth of some lilies in styles of the other species in 24～72 hours after the pollination under temperatures of 22°～30°C.

Material and Method

Flowers with styles on which the pollen grains were to be placed had been cut one day before the pollination, and put in water. Pollen grains were placed on the stigmas at the time they were recognized to be mature.

Results

The results are shown in Table 15.

Table 15 will show us that in some combinations of these inter-specific crosses the pollen-tubes are arrested decidedly in the upper part of the style and they can grow but for a very short distance as the pollen-tubes of the formosa lily, regal lily, Maximowicz lily, speciosum album-novum, Chosenhime and Henryi lily in the style of the easter lily. In other combinations, on one hand, pollen-tubes can grow very well and arrive at the ovarian part within 72 hours after pollination as seen in the cases of the pollen-tubes of the Henryi lily in the style of Maximowicz lily, wheel lily and Chigusa in the Hanson lily, wheel lily in the Akahime-yuri, Chigusa-yuri and Akahime-yuri in the wheel lily, speciosum rubrum lily in Henryi lily, the auratum lily in Henryi lily, Henryi lily in the speciosum rubrum lily, the auratum lily in the speciosum rubrum lily, Maximowicz lily in Thunberg lily and Thunberg lily in the tiger lily. In the last group of combinations the pollen-tubes can grow more than two-thirds of the whole length of style but they can not arrive at the top of the ovary within 72 hours after pollination as in the cases of the Akahime-yuri in the style of Maximowicz lily, Henryi lily in the auratum lily, Maximowicz lily and speciosum tametomo in the Henryi lily, Maximowicz lily in the Thunberg lily.

TABLE 15. *Pollen-tube growth of lilies in some inter-specific crosses*

female parent (style)	length of style (mm)	when pollinated	pollen parent	tempera-ture (C)	length of tube (mm) after				
					20 h.	24 h.	30 h.	48 h.	72 h.
formosa lily	87～ 91	1st day of flowering	*Motomura*	25			30	44.3	48.0
easter	95～109	〃	formosa	25	9.0	14.0			
〃	91～108	〃	regale	25		3.9	3.9		
〃	102	〃	Maximowicz	25				8.1	
〃	103	〃	speciosum album-novum	25				15.5	
〃	106	2nd day	Thunberg	25		16.0		30.7	47.0
〃	102	1st day	Chosen-hime	30					5.0
〃	102	〃	Kakuta	25		12.5		22.5	62.5
〃	102～109	〃	Henryi	25		2.0			
Thunberg	55～ 56	〃	easter	25		11.0		17.0	
〃	65	〃	Sargent	25				8.2	
tiger	59.5	〃	Henryi	23				7.7	
〃	51	2nd day	Sargent	25				8 2	
easter	105	1st day	Red Thunberg	25		8.0			
speciosum tametomo	65	〃	Henryi	25		24.0		26.0	
Maximowicz	39	2nd day	Henryi	25				39+x	
Hanson	16	1st day	wheel lily	25		16+x			
〃	18	〃	Chigusa	25				18+x	

Akahime	10	2nd day	wheel lily	25		9.0		10+x	
wheel lily	19	1st day	Chigusa	25				19+x	
"	19	"	Akahime	25					19+x
Henryi	61	"	speciosum rubrum	25					61+x
"	60	"	auratum	23～25		13.3		36.7	60+x
speciosum rubrum	67	"	Henryi	25				67+x	
"	70	"	auratum	25		24.5		57.4	70+x
tiger	54	2nd day	Thunberg	25		34.5		54+x	
"	55	"	Maximowizx	25					55+x
formosa	91	1st day	easter	25～27	39.5			88.0	
Maximowicz	45	"	Akahime	25		21.2		33.3	
Okukinbusen	61	"	wheel lily	25				32.0	
auratum	80～103	"	speciosum rubrum	25		38.5		52.0	
"	81～101	"	Henryi	25		32.0		68.7	
Henryi	59	2nd day	Maximowicz	25				46.5	
"	55	1st day	speciosum tametomo	25				40.0	
speciosum rubrum	63	"	Maximowicz	25				30.5	
Thunberg	56	"	Maximowicz	25		31.7			56+x

We would say that the first step to raise the hybrid seeds is a smooth growth of the pollen-tubes in the style and therefore we can expect greater possibilities to obtain hybrid seeds in the second and third groups of conbinations than in the first group.

(C) Pollen-tube growth in the basal part of the styles of the other species

Material and Method

The style was cut off in the mid-way of the style and pollinated on the cut surface of the basal stump. Pollens were smeared at 'A' in the text-fig. 7 so that the pollen grains could penetrate into the stylar cavity, which was then covered with lanoline paste.

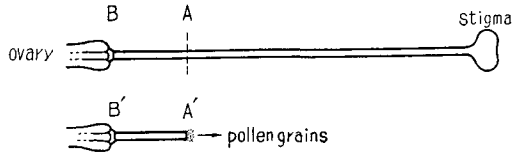

Fig. 7. Schematic illustration showing the operation of the style.

Results

The results are shown in Table 16. Pollen-tubes grow well for some distance, as seen in the table, even in the basal part of the style and their behaviors are very similar to that of self-pollinated ones in the self-incompatible species.

TABLE 16. Pollen-tube growth of the Maximowicz lily and 'Kakuta yuri' in the shortened style of the easter lily

when pollinated	temperature (°C)	parent of		length of A′~B′ (mm)	length of tube (mm) after	
		style	pollens		24 h.	48 h.
July 29	25	easter lily	Maximowicz	15		15+x
July 30	25	"	Kakuta	12	10.0	

vi) Distribution of pollen-tubes in the style

The length of pollen-tubes hitherto described has been shown by the longest one out of the mass of the tubes. Accordingly there were also many tubes which were shorter than the longest one. The writer attempted here to survey the distribution of them in the stylar cavity.

Material and Method

Now, it was demanded at first to know the number of the whole pollen-

tubes. Therefore a controlled pollination was carried out so as 120～150 pollen grains were given on the stigma. This number of pollens was kept fairly strict by the preparatory training. The abortive pollens were assumed to be at most twenty per cent of the entire number for the easter lily, so about 100 pollen grains were expected to develop their tubes which grew into the stylar cavity.

As the materials for this experiment, seedling varieties of the easter lily were used. Inter clonal cross-pollinations between them were tried under the temperature of 22°～24°C, and at 72 hours after the pollination, the styles were cut into pieces as shown in the text-fig. 8, then the number of pollen-tubes which were on the way or had passed through in each of the three pieces (a, b, and c) was counted. Each piece had the same length of 10 mm. but was at different distance from the stigma as shown in the figure.

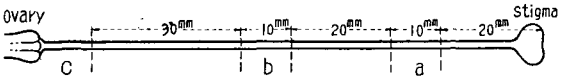

Fig. 8. Schematic illustration showing the operation of the style.

Results

The results obtained are summarized in Table 17 and in text-fig. 9.

TABLE 17. Distribution of the pollen-tubes at 72 hours after pollination in three parts of the style which were in different distances from the stigma

age of style	part	distance from the stigma (mm)	number of pollen-tubes observed
1st day of flowering	a	25	11
"	b	55	4
"	c	95	1

Out of the expected 100 pollen-tubes from the stigma, 89 did not reach the top of the piece 'a' and 11 were travelling in 'a' and had passed through it, in the piece 'b' four tubes were observed and in the 'c' only one tube was observed which had penetrated into the top of the ovary. From the results it would be clear that a very small number of pollen-tubes out of all pollinated grains could travel down the stylar cavity smoothly and the remaining majority of them grew far slower than the former.

In the summer of 1953, the writer tried controlled pollinations on the Croft lily with about 200 pollen grains of a variety of the easter lily. These

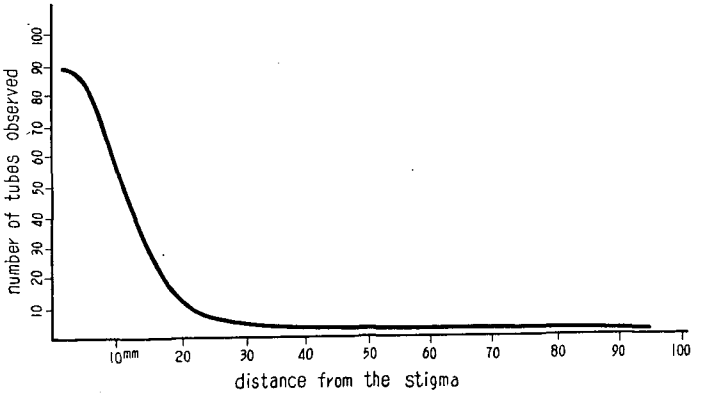

Fig. 9. The distribution of pollen-tubes in the style at 72 hours after the controlled pollination with about 150 grains.

pollinations were conducted on the stigmas of four different ages of (a) one day before flowering, (b) the first day, (c) the second day and (d) the fourth day of flowering. From these pollination trials the writer obtained the results mentioned in Table 18.

TABLE 18. Pollination tests on Croft lily with controlled number of 200 pollen grains of the other clon of the easter lily

age of style	secretion	when pollinated	appearance of ovary after two weeks	number of good seeds per capsule
1 day before flowering	absent	July 28	green fading away	0
"	present	"	healthy	10
1st day of flowering	"	July 29	"	3
"	"	"	green fading	0
2nd day of flowering	"	July 30	"	0
"	"	"	"	0
"	"	"	"	0
4th day of flowering	poor	Aug. 1	decaying	0

Table 17 and 18 will permit us to suppose that the pollen-tubes which grew smoothly could be of use for the fertilization while the majority of others could not.

Chapter II. Development of Embryosacs

Until recent years, the process of embryosac-development in *Lilium* was considered to be the simplest one, commonly called a lily type. In this type, the archesporecell which was in the subepidermal layer of the nucellus, became an embryosac mother cell, then divided three times establishing a complete embryosac which contained eight nuclei.

BAMBACIONI reported, however, a different process of macrosporogenesis in 1930 using *Fritillaria persica*, in which the nucleus of the embryosac mother cell divided actually four times and grew to a complete eight nucleate embryosac. After the first two meiotic divisions four nuclei were observed, then three of them united into one which divided successively into two, on the other hand, the remaining one out of four also divided into two simultaneously. As the result of these divisions the embryosac included again four nuclei, but the two at the charazal part were much larger than the micropylar two. These four nuclei divided themselves once more and lastly an eight nucleate (four large and four small nuclei) embryosac was established.

This curious phenomenon in the macrosporogenesis has been reported to be widely found in other Liliaceae as mentioned below;

BAMBACIONI, V. (1930)	in	*Fritillaria persica*
BAMBACIONI, V. and GIMBINI, A. (1930)	in	*Tulipa Gesneriana*
BAMBACIONI, V. and MEZZETTI, V. (1932)	in	*Lilium bulbiferum*, *L. candidum* and *Tulipa praecox*
COOPER, D. C. (1935)	in	*L. Henryi*
SANTOS, J. K. (1937)	in	*Lilium philippinense*
MYODO, H. (1953)	in	*Lilium longiflorum* var.

The writer's attempt in the present paper is to survey the rapidity of the development of the embryosac in connection with the pollen-tube growth after the pollination as well as with the fertilization.

1. Embryosac grown in the ovary of the easter lily

Embryosacs of the easter lily were in very different stages of development according to the positions they attached to on the central axis of the ovary as reported by the writer in 1953 (23). Embryosacs which grew in the middle and slightly upper part of the ovary developed earliest and those which grew in the basal part, developed very late.

Two data from his observations in 1950 are shown in the next two tables

19 and 20, in the former table the ovary examined was taken from the green stemmed varieties of the easter lily three days before flowering. The ovary was cut transversely into five pieces of the same length as shown in the text-fig. 10. The extreme parts of the top and base of the ovary, where embryosacs did not grow, were omitted for about 3 mm and 5 mm respectively. The observations on the diverse stages of the embryosac-development in each piece of the ovary are shown in Table 19.

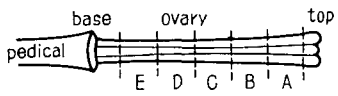

Fig. 10.

Schematic illustration showing the ovary cut into five pieces. The extreme parts (top and base) were omitted.

In the second observation, stages of embryosac-development in the middle part of the ovary were examined in several ages of the ovary, the results being shown in Table 20. From the Table 20 it will be understood that by the second day of flowering, about 75% of all embryosacs were established, but the remaining 25% were not yet complete.

TABLE 19. Number of the embrosacs underway in each developmental stage by the different positions in the ovary (three days before flowering)

stage		position of the piece				
		A*	B	C	D	E
2nd division (homoeotypic)	prophase					
	metaphase					1 (2.94)
	anaphase					
	telophase					
	***resting stage after the 2nd division	3 (10.34)**				4 (11.76)
	***three nuclei fusing					11 (32.35)
3rd division	prophase					
	***metaphase	1 (3.44)				3 (8.82)
	anaphase					1 (2.94)
	telophase					2 (5.88)
	resting stage after the 3rd division	25 (86.20)	30 (100.0)	43 (100.0)	37 (100.0)	12 (35.29)
	total number of observed embryosacs	29	30	43	37	34

* A, B, C, D and E are the same marks as in text-fig. 10.
** The number in the brackets shows the respective % of the total number.
*** see Plate figs. 15, 16, 17.

TABLE 20. Embryosac-development in the middle part of the ovaries of different ages

age of ovary		10days before fl.	7 days before	5 days before	3 days before	1 day before	the day of fl.	2 days after fl.
length of ovary		6.0 cm	8.0 cm	11.0 cm	12.0 cm	13.5 cm	14.5 cm	15.5 cm
1st division (hetero typic)	first synapsis	1 *(8.3)						
	open spireme	8 (66.6)	21 (14.6)					
	2nd synapsis	1 (8.3)	8 (5.5)					
	diakinesis	1 (8.3)	29 (20.2)					
	metaphase	1 (8.3)	16 (11.1)					
	anaphase		1 (0.6)					
	telophase		8 (5.5)					
	interkinesis		41 (28.6)					
2nd division (homoeo typic)	prophase		3 (2.0)	1 (0.3)				
	metaphase		5 (3.4)	4 (1.5)				
	anaphase		2 (1.3)	3 (1.1)				
	telophase		4 (2.7)	27 (10.4)				
	resting stage		5 (3.4)	19 (7.3)	3 (2.6)			
	3 nuclei fusing			2 (0.7)	1 (0.8)			
3rd division	prophase			11 (4.2)	7 (6.1)	1 (3.0)	1 (0.6)	
	metaphase			3 (1.1)	2 (1.7)			
	anaphase			5 (1.9)	2 (1.7)			
	telophase			169 (65.5)	89 (78.0)	6 (18.1)	10 (6.3)	
	resting stage			12 (4.6)	10 (8.7)	4 (12.1)		
4th division	prophase			2 (0.7)		5 (15.1)	7 (4.4)	
	metaphase					3 (9.0)	4 (2.5)	
	anaphase					1 (3.0)	1 (0.6)	
	telophase					13 (39.3)	35 (22.1)	7 (24.1)
	resting stage						100 (63.2)	22 (75.8)
	**cell formation							
total number of observed embryosacs		12	143	258	114	33	158	29

* the number in the brackets shows the respective % of the total number.

** see Plate fig. 18.

The difference of times of the embryosac-development by their positions in the ovary (Table 19), is considered to be a principal reason for the production of the variously deformed capsules when pollinated only once and by a small amount of pollens.

In the summer of 1952, the writer attempted to get deformed capsules of the easter lily. He cross-pollinated once on each of the stigmas of different ages. The styles were cut off above the ovary at 72 hours after the pollination. Plate-fig. 1 show the capsules produced where A and B are capsules from the pollinations on the day of flowering, D and C are from the pollinations on the second and fourth day of flowering respectively, and E is an openly pollinated one.

From this experiment it would be supposed that the ovules in the ovary secreted a certain substance which attracted pollen-tubes during a limited period, but stopped the secretion thereafter. The pollen-tubes, accordingly, passed by the too old ovules and reached the active ones.

2. Embryosacs grown in the ovary of the speciosum tametomo lily

In this variety of the speciosum lily, observations were carried out on the embryosacs in the ovaries of three different ages viz. two days before flowering, 1st day and the third day of flowering. Embryosacs here observed were attached in the middle part of the ovary. The results are shown in Table 21.

TABLE 21. Development of the embryosacs of the speciosum tametomo lily in the ovaries of three different ages

age of ovary	number of observed embryosacs	number of established ones	number of immature ones	number of abnormal ones
2 days before flowering	40	18 (45.0)*	12 (30.0)	10 (25.0)
1st day of flowering	32	21 (65.6)	5 (15.6)	6 (18.8)
3rd day of flowering	33	24 (72.7)	2 (6.1)	7 (21.2)

* the number in the brackets shows the respective percentages of observed embryosacs.

Table 21 shows us that in the speciosum tametomo lily, the percentage is about 65 for the established embryosacs on the first day of flowering which is nearly the same situation with the easter lily. The stigma of the speciosum lily begins to secrete about 48 hours after the flowers open and the styles can

properly accept pollens for a period of three days after the secretion has appeared as reported in chapter 1.

About twenty per cent of all observed embryosacs are abnormal ones, the qualities of abnormality here observed are (1) 5～6 nuclei are seen in the resting stage after the fourth division, (2) the established embryosac with 4～6 cells, (3) the eight nuclei are of the same size in the resting stage after the fourth division, (4) the established embryosac includes 9～10 nuclei and (5) the established embryosacs without synergids.

When we cross-pollinate on the speciosum tametomo lily with pollens of the type speciosum lily, we always gain capsules of abnormal shape with a small amount of seeds ,neverthless, in the reverse pollination a plump one with many good seeds can result if sufficient pollens are given (Plate-fig. 1, 2 and 3). This would be caused by the high level of percentage for the abnormal embryosacs contained in the ovary of the speciosum tametomo lily.

3. Embryosacs grown in the ovaries of the 'Benisukashi-yuri'

This form of the Tunberg lily always bears fruits with a very scanty number of seeds. For the observations the ovaries of the fourth day of flowering were used. The observed embryosacs had grown in the middle part of the ovary, the results being shown in Table 22.

TABLE 22. Stages of the embryosac-development in ovaries of the 'Benisukashi-yuri' on the fourth day of flowering

age of ovary	number of observed embryosacs	number of established embryosacs	number of immature embryosacs	number of abnormal enbryosacs
4th day of flowering	58	5 (8.6)	45 (77.6)	8 (13.8)

* the number in the brackets shows the percentage.

It was a striking fact that 77.6% of all observed embryosacs were immature by the fourth day of flowering and 13.8% were more or less abnomal, the characters of the abnormality were as follows: (1) only 5～6 nuclei were present in the resting stage after the fourth division (2) the established embryosacs constituted of 5, 6, or 9 cells (3) the 8 nuclei in the established embryosacs were of the same size (4) only 3 nuclei were observed in the resting stages after the second division and (5) the nuclei were entirely absent in the vicinity of the micropyle.

In the early summer of 1953, the writer tried two series of crosses viz.

'Benisukashi-yuri' (♀) × Dahurian lily (♂) and 'Chigusa-yuri' (♀) × Dahurian lily (♂). The fruits contained various number of seeds as shown in Table 23.

TABLE 23. Number of good seeds in each capsule in two crosses between the two cultivated varieties of the Thunberg lily and the wild dahurian lily

capsule	combinations	
	Benisukashi × dahurian lily	Chigusa × dahurian lily
1	49	101
2	26	80
3	24	77
4	19	74
5	13	72
6	11	56
7	4	52
8	2	52
total number of seeds	148	564
number of seed per one capsule	18.5	70.5

In the eight capsules of Benisukashi-yuri, 49 was the highest number of seeds grown in one capsule and the lowest one was only 2. In the capsules of Chigusa-yuri, on the other hand, the highest and lowest number were 101 and 52 respectively.

The pollen-tube growth of the dahurian lily in the style of these two forms of the Thunberg lily was also observed and it was seen that the pollen-tubes could grow but very slowly in styles of Benisukashi-yuri as compared with the

TABLE 24. Pollen-tube growth of the dahurianlily in styles of two forms of the Thunberg lily at 22°C

age of style	when pollinated	combination		length of style (mm)	length of tube (mm) after	
		(♀)	(♂)		24 h.	48. h.
1st day of flowering	July 12	Benisukashi	dahurian lily	52.5	8.5	
〃	〃	〃	〃	50.0		26.5
〃	July 17	Chigusa	〃	53.5	23.5	
〃	July 18	〃	〃	50.0		50+x

cases of Chigusa-yuri, the results being shown in Table 24.

This delay of pollen-tube growth in the styles of Benisukashi-yuri was considered to be caused by the delayed development of embryosacs of this lily, accordingly by very poor secretion of the growth-promoting substances for pollen-tubes from the ovule.

The scanty number of good seeds in the capsules of this lily was probably caused by two reasons (1) the delay of the pollen-tube growth and (2) the prevalence of abnormal embryosacs in the ovary.

Chapter III. Fertilization and Seed Formation

1. Fertilization and normal seed formation

For the observations here conducted four species of *Lilinm* were used; the easter lily, Benisukashi-yuri and the formosa lily.

In these four species of *Lilium* the pollen-tubes under favourable conditions can reach the top of the ovary in 48～72 hours after pollination as observed in chapter I, 3. Accordingly it would be expected that some tubes out of all pollinated are able to arrive at the ovules situated in the middle of the ovary within 72～96 hours after pollination.

WENIGER (51) reported in his paper that the male nucleus was in contact with the egg nucleus about 60～120 hours after pollination, using the wood lily and the easter lily.

In the present chapter the writer's observation will extend further on the fertilization of polar- and egg-nuclei and the cell membrane-formation of the endosperm nuclei in four species cited above which have been grown in our experimental farm. The results of these observations are shown in Table 25.

After the pollen-tube intruded into the embryosac, each of the two male nuclei stood near by the egg and two polar nuclei for the conciderably long

TABLE 25. Number of days after pollination when the first division of the primary endosperm- and fertilized egg-nuclei, and the beginning of cell membrane-formation of the nuclear endosperm were seen in four species of *Lilium*

species	division of primary endosperm nucleus	division of fertilized egg	beginning of cell formation
easter lily	10～12	13～14	
speciosum lily	8～ 9	12～13	20～23
Benisukashi	8～ 9	12～13	
formosa lily	8～12	12～15	30～35

time of 4～8 days. The fertilization of the polar nuclei preceded that of the egg nucleus, spireme were formed in each of the second male nucleus and two polar nuclei, and then the nuclear membranes disappeared at the point of contact of the nuclei and the segmentation of spiremes occurred on the spindle, and at last the division was accomplished by a longitudinal split of the chromosomes. Both of the fertilized polar- and egg-nuclei divided themselves successively in a short time after the fertilization. The fertilization and successive division progressed in a similar manner to that of the three nuclei which fused and divided previously to the third division in the development of the embryosac.

After the first division of the primary endosperm nucleus, further division succeeded, and when the third or fourth division of the endosperm nucleus finished, the fertilization of the egg nucleus was observed.

The first male nucleus primarily and the egg nucleus next developed their spiremes respectively, and then they fused together. The fertilized egg stayed for only a short time in the condition of a vacuolated cell, then divided by a transverse membrane and formed a two-celled proembryo.

The development of the embryo in *Lilium* was considered to be irregular in its process, but it was ascertained in the present observation on four species of *Lilium* that the basal cell (micropylar one) of the two-celled proembryo supplied the suspensor later on, and the top one supplied the cotyledon, hypocotyl and radicule (Plate fig. 4, 5, 6 and 7).

The suspensor cell divided itself twice by a longitudinal membrane and formed, in the early period of development, a column of four cells the length of which was about 4～5 times of the thickness of the column.

The cotyledon, hypocotyl and radicule were differenciated in the very late period from the cylindrical mass of tissue which had originated in the top of the young embryo.

The cell membrane began to from around the individual endosperm nuclei after the daughter nuclei were distributed uniformly along the inside surface of the embryosac, and it was observed during 20～35 days after the pollination for the respective species.

2. Abnormal seed formation in some inter-specific crosses

Though the occurrence of abnormal seed in *Lilium* was reported by some investigators (24, 40), no attempts were made on the elucidation of the mechanism.

NAKAJIMA (24) reported that the obtained abnormal seeds from the speciosum rubrum lily which had been pollinated with pollens of the auratum lily and

though they could not germinate by a common management of culture, the embryos could easily germinate if they were embryo-cultured. The most striking character of the abnormal seeds resulted from this cross was the juicy endosperm in contrast with the hard one found in the normal seed.

Seeds from the same crosses as in NAKAJIMA's report, however, were often reported also by the other lily breeders to be gained and grown before and after his report by the common management of culture.

These facts would show us that some clones of the speciosum rubrum lily produce normal or nearly normal seeds when crossed with the auratum lily that are able to germinate under common management.

The writer has obtained abnormal seeds of the same qualities by some other inter-specific crosses in *Lilium*, which will be summarized in Table 26.

TABLE 26. Examples of the abnormal seeds produced by inter-specific crosses other than speciosum and auratum

parents		qualities of endosperm	seed germination percentage by usual culture
(♀)	(♂)		
Akatsuki	maculatum 'waseki'	jelly	0
Akatsuki	amabile	jelly	0
Maximowicz	concolor pulchellum	juicy	0
Maximowicz	amabile	jelly	57
lancifolium flaviflorum	dauricum 'Kogane'	jelly	66

The writer wishes here to present a detailed description on the process of the abnormal seed development by the cross on speciosum rubrum lily with auratum pollens.

i) Pollen-tube growth in the cross on the speciosum rubrum lily with auratum pollens

The pollen-tube growth of the auratum lily in the style of the speciosum rubrum lily was observed and compared with that of the speciosum tametomo pollens in the style of the speciosum rubrum lily. The results are summarized in Table 27, which is shown by a mean value of six samples of each combination. The experiment was carried out in an incubator at 22°C constant, and the styles used were grown on the cut flowers.

The results shown in Table 27 indicate that the pollen-tube of the auratum lily grows normally down the style of the speciosum rubrum lily without any disturbance and can reach the top of the ovary within 72 hours after the pollination on the stigma at 22°C which is almost the same rapidity with the

TABLE 27. Pollen-tube growth of the auratum and speciosum tametomo lily in the style of the speciosum rubrum lily

combination	lenght of tube (mm) after		
	24 h.	48 h.	72 h.
speciosum rubrum (♀)×auratum (♂)	24.5	57.4	70+x
speciosum rubrum (♀)×speciosum tametomo (♂)	21.3	44.2	66+x

case of the speciosum tametomo's pollens in the style of the rubrum.

ii) Fertilization and the development of the embryosac and endosperm after two crosses; speciosum rubmum (♀)×auratum (♂) and speciosum rubrum (♀)×speciosum tametomo (♂)

The pollen-tube growth of the auratum lily in the styles of the speciosum rubrum was positively normal as reported above, while the fertilization and the development of both the embryo and the endosperm after the arrival of the pollen-tube at the embryosac were delayed gradually as compared with the case of the interclonal cross (rubrum and tametomo) as mentioned in Table 28.

TABLE 28. Numbers of days after pollination when the first division of the fertilized egg- and polar nuclei and the beginning of the membrane-formation of endosperm nuclei were observed in two crosses; speciosum rubrum (♀)×auratum (♂) and speciosum rubrum (♀)×speciosum tametomo (♂)

pollinations: Aug. 15, 1952, outdoors

combination	division of fertilized endosperm nuclei	division of fertilized egg nuclei	beginning of membrane formation of endosperm nuclei
rubrum (♀)×auratum (♂)	11~12	14~16	28~30
rubrum (♀)×tametomo (♂)	8~ 9	12~13	20~23

The division of the endosperm nuclei in the cross of the rubrum × auratum were very inactive, the cellular membrane formed later on was very thin and the resulting cells were very poor in their contents as compared with the case of the interclonal cross (Plate fig. 10 and 11). The cells of the endosperm, accordingly, were very large and thin membraned, each nucleus of which was sparsely distributed as shown in the Plate fig. 8 and 9.

The development of the embryos from both crosses progressed almost in the same way for about forty days after pollination, but after that time the growth of the embryo from the cross with auratum was strikingly delayed as

compared with that from the cross with tametomo as seen in Table 29 and text-fig. 11.

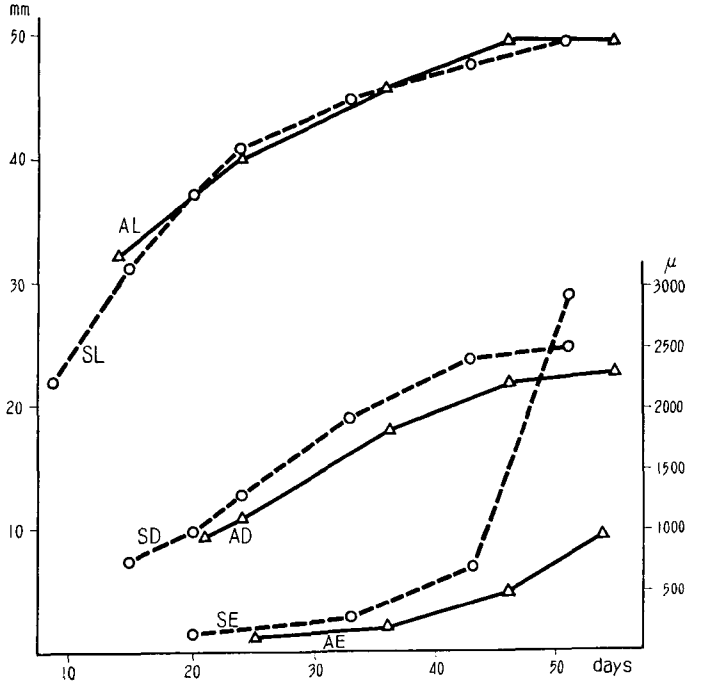

Fig. 11. Diagramatic illustration of the growth of the ovary and the embryo in two crosses; speciosum rubrum×auratum and speciosum rubrum× speciosum tametomo. This figure was deduced from Table 29.

AL : length of ovary in rubrum×auratum
SL : " rubrum×tametomo (cm)
AD: diameter of ovary in rubrum×auratum
SD: " rubrum×tametomo (mm)
AE: length of embryo in rubrum×auratum
SE: " rubrum×tametomo (μ)

The antipodal cells were active for a long time in both cases, but they became inactive and their cell membranes were thick at an earlier date in the former cross (rubrum × auratum) as compared with the latter as shown in the Plate fig. 12 and 13.

From the observations above described it will be proper to consider that the common difficulty in the germination of abnormal seed is caused by the defficiency of nutritive substances in the endosperm which are demanded for the initiation of root growth of the embryo. Endosperm cells which are very poor in the cytoplasm contents and thin membraned, are probably incapable

TABLE 29. Growth of the ovary and the embryo in two crosses of speciosum rubrum (♀)×auratum (♂) and speciosum rubrum (♀)×speciosum tametomo (♂)

	combination	days after pollination											
		9	14	15	20	21	24	33	36	43	46	51	56
length of ovary (cm)	rub.×aur.		3.2				4.0		4.6		5.0		5.0
	rub.×tamet.	2.2		3.1	3.7		4.1	4.5		4.8		5.0	
diameter of ovary (mm)	rub.×aur.					9.5	11.0		18.0		22.0		23.0
	rub.×tamet.			7.5	10.0		13.0	19.0		24.0		25.0	
length of embryo (μ)	rub.×aur.						125		230		520		950
	rub.×tamet.				150			300		775		2920	

of taking the nutritive substances through the ovary walls and these substances steadily accumulate there, which is possibly the main reason for the frequent developments of the adventitious roots from the ovary wall of the speciosum rubrum lily when crossed with the auratum lily as shown in the Plate fig. 14 and also when crossed with the Parkman's lily as reported by Mr. C. A. BEST in 1953 (5).

iii) Apomictic seed formation

Many references to the apomictic seed formations in *Lilium* have been given in papers on lily breeding, especially the regal lily has been reported to set its seeds well when pollinated with almost any other kinds of lilies, but the F_1 and F_2 plants were much the same with the seed parent. The seeds, therefore, have been considered to be spomictic.

RAPPLEYE (35) carried out controlled pollination on the regal lily with pollens of twenty one other kinds of lily and obtained, however, none of seeds with the exception of *L. leucanthum* var. *chloraster* which is a very closely related species to *L. regale*.

Recently, EMSWELLER (12) cross-pollinated on the regar lily with a series of pollens of other lilies and he also could not obtain any seeds. From these tests, EMSWELLER conjectured that the alleged apomixis in *Lilium* was less prevalent than commonly suspected.

There have been two reports on the morphological observation of the occurrence of the apomixis in *Lilium*, one of which was made by COOPER (9). His observation was, however, on the synergid embryo neighbouring the normal one grown from the fertilized egg. The other one was made by RAPPLEYE (36). He tried a series of coth inter- and intra-specific pollinations using the

regal lily as a female parent. He concluded from his observations that all of the embryos of various types; normal, abnormal and intermediate ones which grew in the embryosacs of the regal lily from controlled pollinations with the easter lily, Maximowicz lily, and *L. leucanthum* var. *chloraster* were not apomictic, they developed only when the pollen-tubes were not precluded to penetrate into the ovaries. Thus both of COOPER and RAPPLEYE seem to eliminate diploid gametes and sporophytic budding as the origin of the apomictic seeds.

The writer has been attempting to gain a datum on the developmental morphology of the apomictic embryo and seed. He cross-pollinated the formosa lily with pollens of many other kinds of lily for seven years since 1947, and managed to obtain a small number of seeds only when he used the pollens of the 'Motomura-yuri' (a form of *L. Leichtlinii* var. *Maximowiczii*). Ten flowers were pollinated each year with pollens of the 'Motomura-yuri' and in 1947 and 1950 he obtained no seeds. In 1951, however, he obtained the greatest number of 98 seeds from ten capsules as shown in Table 30.

TABEL 30. Number of apomictic seeds in each year grown in ten capsules of the formosa lily when they had been cross-pollinated with the 'Motomura-yuri'

year of pollination	1947	1948	1949	1950	1951	1952	1953
number of apomictic seeds	0	20	18	0	98	10	5

It was unknown by what reasons the apomictic seeds did grow in some years and not in the others. In parallel with these cross- pollination the writer made observations on the pollen-tube growth in the style after the pollination. The pollen-tubes of the 'Motomura-yuri' grew well for the first half or two-thirds way of the style, but they stopped there and the tips of them swelled abnormally about 72 hours after the pollination, while the pollen-tubes in self-pollination reached the top of the ovary in 48 hours after. Table 31 shows the results.

The majority of the embryosacs of the formosa lily which had been pollinated with the 'Motomura-yuri' stopped their growth soon after a slight growth. The outer seed coat, however, grew normally and formed a wide and thin wing which surrounded a tiny and blasted embryosac in the center as shown in text-fig. 12.

A small number of embryosacs, on the other hand, grew fairly well in their length and width but not in depth. Because they were devoid of their

TABLE 31. Pollen-tube growth of the Motomura-yuri and the formosa lily in the styles of the latter lily

age of styly	pollen parent	when pollinated	temperature (°C)	length of style (mm)	length of tube (mm) after 24 h.	48 h.	72 h.	96 h.
1st day	Motomura	Aug. 26	25	88	30			
of	"	"	25	91		44		
flowering	"	"	25	91		48		
	"	"	25	91		42		
	"	"	25	91		43.5		
	"	Aug. 29	25	81		44		
	"	"	25	77			48*	
	"	Aug. 28	20~32 (out doors)	87				50*
	"	"	"	87				58*
	self	Aug. 31	25	84	59			
	"	"	25	84	55			
	"	Aug. 29	25	90		90+x		
	"	"	25	90		90+x		

* tips of the pollen-tubes were abnormal in shape.

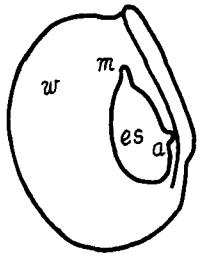

Fig. 12. Blasted seed.
a : antipodal portion
es : blasted embryosac without embryo

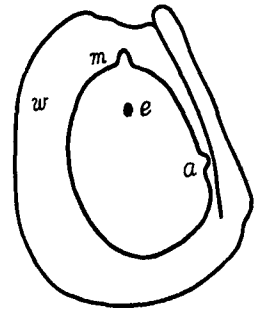

Fig. 13. Apomictic seed.
m : micropyle
e : apomictic embryo
w : seed coat

endosperm and embryo, the inner space of the embrosac was pressed by both sides. A group of cells of the nucellar tissue began to divide themselves and formed an apomictic embryo as shown in text-fig. 13. This fact will suggest that the alleged apomictic seed is a sporophytic budding in its origin. The process of the development of the apomictic embryo from the nucellar tissue

was not a constant one and the number of cells that took part in the formation of the embryo was not constant.. The embryo suspensor could be distinguished morphologically in some samples but not in the others (Plate figs. 19, 20, 21, 22).

The location of cells that began to divide themselves to fom an apomictic embryo was not constant, but in most of the examples it was in the vicinity of the micropyle. The frequency of their location in the nucellar tissue was surveyed and the results were summarized in Table 32.

TABLE 32. Frequencies of the occurrence of the apomictic embryo in four parts of the nucellus

A, B, C and D should be referred to the text-fig. 14

part of nucellus	number of developed embryos	percentage
A	34	87.17
B	5	12.82
C	0	0
D	0	0
Total	39	

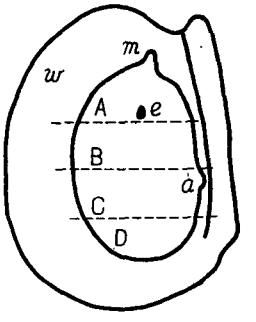

Fig. 14.
Four parts of the nucellus showing the occurrence of the apomictic embryos in the table 31.

'A' was the nearest portion to the micropyle of the four and about 87 per cent of all observed apomictic embryos initiated in this area. The other 12 per cent were in 'B' area which was located next to 'A'. In the antipodal parts of 'C' and 'D' none of them was observed.

Chapter IV. Discussion

One of the fundamental problems in plant breeding is to clarify the process of the development of seeds including the formation of pollens and embryosacs, pollen-tube growth, fertilization and seed formation; the present study on lilies is in connection with these problems.

Abortive pollen grains are generally contained in higher percentages in hybrid species and species which have been propagated asexually for a long time than in the wild species and species which are commonly propagated by seeds.

EMSWELLER and BRIERLEY (13) reported that many irregularities in metaphase pairing were caused in two varieties of *L. longiflorum* which were in meiosis, by an exposure to abnormally high temperature (45～46°C) during

a short time (thirty minutes). These irregularities would probably be one of the occurrence of the abortive pollen grains. For the observation of chapter I most of the pollen grains were selected from flowers which were grown in the field under the natural temperatures (20～30°C).

The observation of POST (32) was carried out using forced lilies in the green house which were in a different condition from that of the present experiment. *L. longiflorum* var. *Harrissii* in POST's report contained abortive pollen grains at a strikingly high percentage of 44.82, while *L.longiflorum* var. *albo-marginatum* in the present observation contained only 11.0 per cent which was the highest value in three varieties of *L. longiflorum* examined by the writer.

Lily species and varieties which contain many abortive pollen grains may also contain many abnormal embryosacs. For instance, *L. speciosum* var. *tametomo* contained 12.54 per cent abortive pollen grains and about 20 per cent abnormal embryosacs, and the 'Benisukashi-yuri' contained 10.50 per cent abortive pollen grains and 13.8 per cent abnormal embryosacs.

The contamination by abnormal pollen grains and embryosacs may be one of the causes of the occurrence of non-fertilization and the abortion of the seeds.

In the majority of cases of the incompatible cross, the pollen-tubes can grow well as long as about a half of the style, but they stop there. Why do they stop their growth? The writer would like to suggest a growth-promoting substance for the pollen-tubes in the compatible cross, which substance is non-functional in the incompatible crosses, the reasons of which should be as follows: 1) in most cases of the incompatible cross, the pollen-tube tips do not show any signs of abnormality, 2) in compatible crosses, the pollen-tube can grow better in the base than in the top of the style, which will be the result of a higher content of the growth-promoting substance in the base than in the upper part of the style, 3) the pollen-tubes pass by the old ovules in the top and travel down towards the young and active ones which are secreting that substance. The ovules secrete the growth-promoting substance during a certain period and they, probably, stop the secretion when they are too old, 4) the growth-inhibiting substance for pollen-tubes as suggested by YASUDA in PETUNIA (53) cannot be expected in general cases of *Lilium*, for there are no signs of rise and fall in the secretion of that substance, 5) the pollen-tubes can grow well in the basal part of the style even in the incompatible crosses when the style is cut short and pollinated on the cut surface.

Pollen-tubes forced to travel in the conductive tissue of the ovary by shortening the style, cannot intrude into the ovules which do not secrete the inviting substance for the present pollen-tubes.

There will be a great possibility for overcoming the cross- as well as self-incompatibilities in *Lilium* if an artificial and external administrator of certain substance which will promote the growth of the pollen-tube as reported by EMSWELLER and STUART (15) and EMSWELLAR et al. (16) can be made.

The optimum temperature for the pollen-tube growth was observed between 25°~30°C for all lilies in this experiment, in the lower temperatures they grew but very slowly or stopped their growth without any remarkable damage, while in the higher temperatures they could scarcely grow and when higher than 40°C they were completely damaged in a short time (22). It would be better therefore, to use a soda straw, as reported by EMSWELLER (12), instead of a paper bag for preventing the stigma from contamination with undesired pollen grains, for the temperature within the bag is apt to rise strikingly on a fine midday, while the soda-straw will keep the stigma cool,

About eighty per cent of all the embryosacs in the middle part of the ovary, where they develop earliest, establish themselves by the third day of flowering in many species of *Lilium*. In species which secrete abundantly on the stigma such as the easter lily, the timely pollination will be carried out on the day when the secretion begins to appear (generally the 1st day of flowering) because the tubes can grow best and most of the embryosacs will be established by the third or fourth day of flowering when the pollen-tubes can reach the ovules.

The general difficulty in the germination of the abnormal seeds in *Lilium* is considered to be caused by the deficiency of nutritive substances in the endosperm rather than the presence of the inhibiting substance for the embryo to grow. This deficiency of the nutritive substances may be caused by two facts; 1) the poor constitution of the endosserm cells which cannot absorb enough nutritive substances from the ovary wall, 2) the curious development of the roots originating from the ovary wall.

Many suggestions and questions have been reported about the apomixis in *Lilium* and it has been demanded to supply a morphological datum on the development of the apomictic seeds. COOPER (9) noticed the occasional occurrence of the twin-embryo in seeds of some species of *Lilium* and reported that one of the twins resulted from the normally fertilized egg and became diploid, but the other grew from one of the synergids and was haploid. The alleged apomictic seed in horticulture is a different one from COOPER's report, and the question here is whether the single embryo held in the seed is apomictic or not. EMSWELLER (12) and RAPPLEYE (35) seemed to have strong questions on the occurrence of apomixis in *Lilium* because of their negative data of pollination tests on the regal lily with pollens of the other species. The writer's

observation showed that apomictic embryos often originated in the nucellar tissue of the formosa lily when cross pollinated with the 'Motomura-yuri'. The positions where the apomictic embryos developed were always in the vicinity of the micropyle, so the initiation of cell division of the nucellar tissue was possibly caused by the so-called pollen-hormone.

Chapter V. Summary

A series of the problems in connection with the fertilization in *Lilium* was studied, the results of which are summarized as follows:

(1) The abortive pollen grains contained in the anthers are between 0 and 57.95 per cent according to the varieties of lily. Generally speaking, the species of hybrid origins and those which have been commonly propagated asexually by bulblets or scales for a long time, contain abortive pollen grains at high levels as compared with species of wild origins and those which are commonly propagated by seeds.

(2) The optimum temperatures for the pollen-tube development in all lily species tested here are between 25°~30°C. The lowest temperature, however, is a little lower in the early flowering species than in the late flowering ones. The optimum temperature for each species is inclined to turn higher when the sugar concentration of the germinating bed beccomes high.

(3) The age of the style is an important factor for the pollen-tube to grow well. The best age of the style for the tube-growth is one day old (the 1st day of flowering) for the easter lily and two to four days old (2nd to fourth day) for the varieties of the speciosum lily. The pollen-tubes can grow at a rapidity of about 2.0 mm and 1.5~1.6 mm per hour respectively for the easter lily and the speciosum lily under the temperature at 25°C. They can, accordingly, reach the top of the ovary within 48 hours after the pollination.

(4) The pollen-tubes in the incompatible crosses can travel as far as a half to two-thirds of the whole length of the style, but there they stop, the tips of the pollen-tubes being normal with a very few exceptions. The tubes can grow well even in the basal part of the style, if the style is cut short and pollinated on the cut surface.

(5) The pollen-tubes grow better in the basal part of the style than in the upper part in the case of the compatible cross.

(6) Pollen-tubes from pollen grains which have been given on an older stigma, pass by the old ovules in the top of the ovary and reach the active ones in the basal part The capsule produced in such a case is a deformed one, in which the good seeds grow only at the base of the ovary.

(7) The writer suggested the presence of a growth-promoting substance

for the pollen-tube in the compatible crosses, the substance would be non-functional in the incompatible crosses by reason of the results mentioned in (4), (5) and (6) of the present summary.

(8) The rapidity of the embryosac-development is considerably different according to the positions where the ovules grow. They develop earliest in the middle, next in the upper and latest in the basal part of the ovary.

(9) The abnormal embryosacs contained in the ovary were observed using two species, the percentages were about 20 and 14 for the speciosum tametomo lily and the 'Benisukashi-yuri' respectively. The qualities of the abnormality were also described.

(10) By the third day of flowering eighty to ninety per cent of all embryosacs grown in the middle part of the ovary are established in both of the easter lily and the speciosum tametomo lily, but very few (8.6%) are established by the same day in the 'Benisukashi-yuri'.

(11) The process of the abnormal seed-formation in the cross of *L. speciosum rubrum* × *L. auratum* was described. From the observation, the alleged difficulty of germination of the abnormal seeds was considered to be caused by a deficiency of the nutritive substances in the endosperm rather than by the presence of a certain growth-inhibiting substance for the embryo. Because the endosperm cells of the abnormal seeds were very poor in their contents, they could, probably, not absorb the nutritive substances through the ovary wall. The initiation of the roots out of the ovary wall observed by the writer and Mr. C. A. BEST would suggest the accumulation of that substances left over in the wall.

(12) The process of the apomictic seed formation was observed in the formosa lily when pollinated with pollens of the 'Motomura-yuri'. A group of nucellar cells began to divide themselves about 30 days after cross-pollinated and they formed apomictic embryos which were found only in the vicinity of the micropyle. The apomictic embryo, therefore, was a sporophytic budding in its origin and was considered as a product by the influence of a sort of pollen hormone of the 'Motomura-yuri'.

The writer wishes to express his heartfelt thanks to the late Prof. Dr. TOKUJIRO MAEKAWA for the kind and proper suggestions for the present study in its early stage.

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Explanation of Plate-figures

PLATE I.

Fig. 1. The deformed capsules of the easter lily pollinated at different ages of the style. $\times \frac{3}{10}$

A and B were pollinated on the first day, C on the 2nd and D on the 4th day of flowering. E was from open pollination.

Fig. 2. A plump capsule of the speciosum rubrum lily pollinated with the speciosum tametomo lily. $\times \frac{1}{2}$

Fig. 3. A capsule with poor contents of the speciosum tametomo lily crossed with the speciosum rubrum. $\times \frac{1}{2}$

Fig. 4. Embryosac of the 'Benisukashi-yuri' 10 days after the pollination with the dahurian lily. Fertilized egg is in prophase of the first division, four endosperm nuclei are present, two of which appear in this figure. $\times$ 154

Fig. 5. A 18-celled embryo of the 'Benisukashi-yuri' 15 days after pollination. $\times$ 82

Fig. 6. About 30-celled embryo of the 'Benisukashi-yuri' 18 days after pollination. $\times$ 40

Fig. 7. The normal embryo of the formosa lily 45 days after pollination, in the longitudinal section perpendicular to the flat surface of the seed. $\times$ 40

Fig. 8. Embryo of the speciosum rubrum lily crossed with the auratum lily, 46 days after pollination. $\times$ 40

Fig. 9. Embryo and endosperm of the same cross with fig. 8, 54 days after pollination. Seet the thin membrane and poor contents of the endosperm cells. $\times$ 40

Fig. 10 and 11. Embryo and endosperm of the speciosum rubrum lily when crossed with the speciosum tametomo lily. 43 (for fig. 10) and 51 (for fig. 11) days after pollination. $\times$ 40

Fig. 12. Antipodal part of the speciosum rubrum lily 25 days after the pollination with the auratum lily. The membrane is noticeably thickened. $\times$ 154

Fig. 13. The active antipodal part of the speciosum rubrum lily 20 days after the pollination with the speciosum tametomo lily. $\times$154

Fig. 14. Adventitious roots from the capsule of the speciosum lily crossed with the auratum lily. $\times \frac{3}{1}$

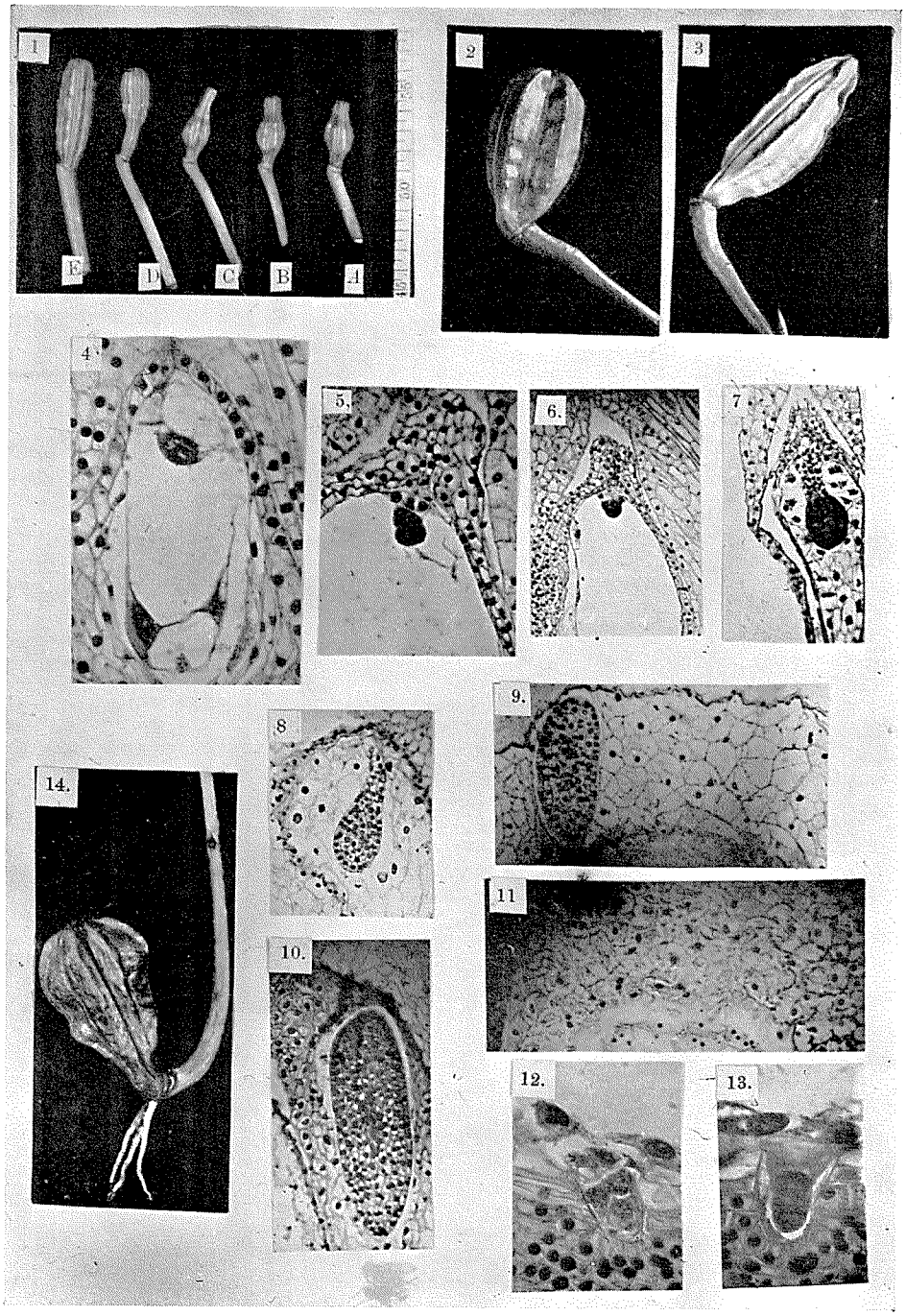
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PLATE II.

Fig. 15. Resting stage after the second division of the embryosac of the easter lily. × 262

Fig. 16. The fusing stage of the three nuclei whice is preceding to the third division during the embryosac development. × 427 (easter lily)

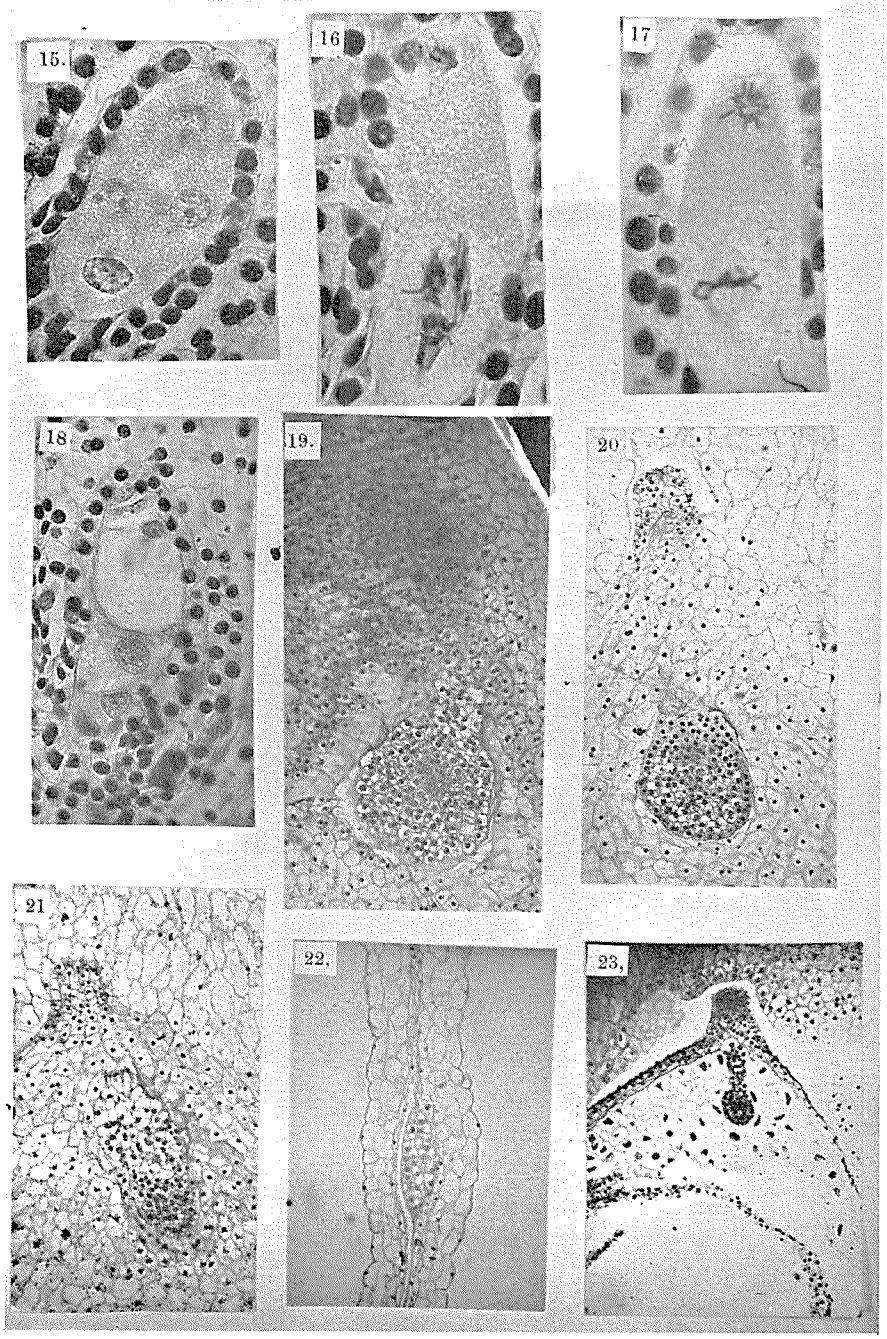
Fig. 17. Metaphase of the third division of the embryosac. × 307 (easter lily)

Fig. 18. An established embryosac of the easter lily. × 127

Fig. 19, 20 and 21. Apomictic embryo of the formosa lily as seen in the longitudinal section parallel to the flat surface of the seed. 56 days after pollination. Note the embryo surrounded by the nucellar tissue. × 43

Fig. 22. Apomictic embryo of the same lily as seen in the longitudinal section perpendicular to the flat surface of the seed. 65 days after pollination. × 43

Fig. 23. The normal embryo of the formosa lily as seen in the longitudinal section parallel to the flat surface of the seed. 45 days after pollination. Note the embryo surrounded by the endosperm tissue. × 43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