

## Cross experiments in *Lilium* I.

### Pollen tube behaviors in the crosses of *Lilium Henryi* with some species of *Lilium*.

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#### Introduction

A phenomenon, cross-sterility is thought to play an important role in various fields of biology. And, while the physiology on the processes of fertilization in the vascular plants has long been neglected mainly for the technical difficulties, the phenomenon of sterility, especially of self-incompatibility, has been rather well analysed both physiologically and genetically (cf. Lewis, 1954), as it is an unfavorable barrier for plant breedings. But, there can not be found a uniform model to explain the mechanisms among the results obtained by various authors.

This series of studies deals with the mechanisms of inter-specific cross-sterility in the genus *Lilium*. Because, in general, the plants belonging to the genus *Lilium* have large flowers and large gametophytes, they are good materials for studying the phenomenon of sterility, both cytologically and physiologically. In order to classify the various grades of cross-sterility, as a first step, the pollen tube behaviors in some crosses, using *Lilium Henryi* as a female plant, were observed, and these results will be described in this paper.

#### Materials and Methods

The female plant, *L. Henryi* BAKER, having long been cultivated in our laboratory garden, were used. The other species of *Lilium* taken as male plants were as follows:

*L. auratum* LINDL.

*L. longiflorum* THUNB.

*L. speciosum* THUNB.

*L. maculatum* THUNB.\*

The details of other crosses, *L. Henryi* × *L. tigrinum* and *L. Henryi* ×

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\* As the basis of classification and nomenclature of *Lilium* members, the author adopted those of Woodcock and Stearn (1950).

*L. Hansonii* will be reported elsewhere.

As a rule, fresh pollen grains were used, but those of *L. maculatum* were stored for about one month in a desiccator with  $\text{CaCl}_2$  at about  $0^\circ\text{C}$ , since the flowering time was earlier than that of *L. Henryi*. Female plants were emasculated one day before flowering, and pollinated on the day just flowers open. Behaviors of pollen tubes in the style were determined by the following schedule: styles cut off with suitable intervals after pollination, were immersed in 1N HCl at room temperature for 20 minutes or more, and thereafter cut open longitudinally with fine needles carefully, stained with cotton-blue in lacto-phenol, and squashed between the slide-glasses. The concentration of sucrose solution suitable for the artificial germination of *Lilium* pollen grains were previously decided for some species as between 5 to 10 per cent, then 7 per cent were used for sucrose-agar media throughout this work.

### Results and Considerations

*L. Henryi*  $\times$  *L. maculatum* The method used for the pollen storage was not suitable, and the rate of germination on the artificial media was very reduced to 30 per cent. The pollen tube growth at 48 hours after sowing reached in length to five to twenty-six times of the longer diameter of the pollen grains. 20 flowers were crossed, but 19 flowers did not show a sign of ovarian development. The remaining one would seem to start the ovarian development, but soon after, stopped its growth and dropped at 25th day after pollination.

The pollen grains on the stigma began their germination, but the tubes thus formed could not pass through the stigmatic tissue. The

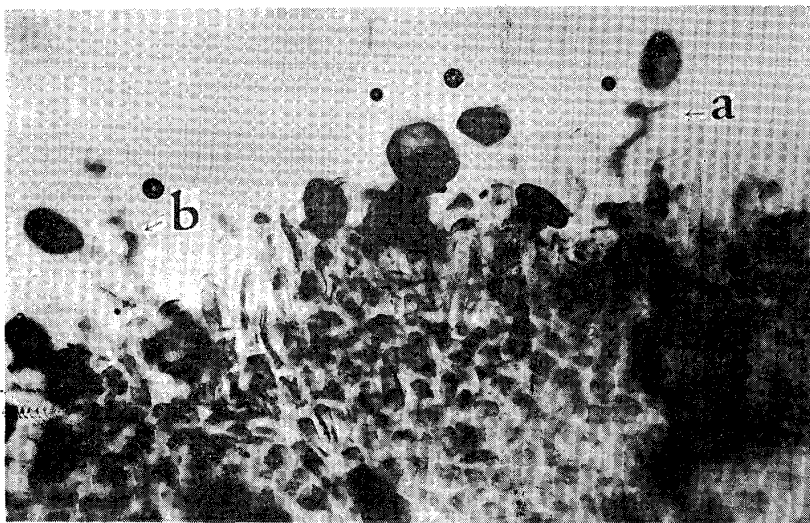


Fig. 1. Pollen tube behaviors of *L. maculatum* 'Chigusa' on the stigma of *L. Henryi*. a shows a coiling tube, b shows a tube which seemingly lost the normal growth-orientation.

growth of tubes were very retarded, and appeared to lose the growth-orientation in the stigmatic tissue. The coiling of tubes, as reported by Stout (1931) in the self-incompatible plant, *Brassica pekinensis*, was also very frequently observed. 'Kisukashi' was used at first, then 'Chigusa' was also examined. Both of them showed the similar tendency as to the pollen tube behaviors.

*L. Henryi* × *L. auratum* About 100 per cent of the pollen grains could germinate on the artificial media. During 45 or 50 hours after sowing, the pollen tubes reached to the length of forty-five to sixty times of the longer diameter of the pollen grains. 32 flowers were crossed. All of them started their ovarian development, and the modes of their development could be arranged into four groups.

Group	No. of flower	Behaviors of ovary
1	14	Dropped within 14 days after pollination.
2	2	Did not withered within 10 days after pollination, but the growth stopped, and dropped by 20th day after pollination.
3	13	Well continued the growth, but dropped by 20th day after pollination.
4	3	Finally reached to mature fruit stage, but produced the abortive seeds.

As shown above, three flowers produced finally the seeds, but these seeds did not contain both any embryo and any endosperm, and therefore the next generation could not be examined. It seems to be noteworthy that the shape of the seeds is very different from that of the seeds produced by the selfing of *L. Henryi* (Fig. 2.)

*L. Henryi* × *L. speciosum* Nearly 100 per cent of the pollen grains could germinate on the artificial media, and the pollen tubes grew to the length of twenty or thirty times of the longer diameter of the pollen grains. Among 8 flowers crossed, 2 flowers were dropped by 20th day after pollination, and the remains were lost by the accidents.

In this combination, the bud-pollination and the delayed-pollination were both examined. Following the assumption proposed by Yasuda (1948), though mechanisms concerning to the cross-sterility are quite obscure, it seems probable to exist a substance, which is produced in the ovary, moves upwards to the style and interferes the growth of pollen tubes in the style and/or ovary. Bud-pollination was made 4 days before flowering, and the delayed-pollination was made 4 days after flowering. The ovarian development, however, could not be observed in both cases, and the ovaries were all dropped by 10th day after pollination. Thus, it can be said that the rise and fall in quantity of such a substance, if exists, during the time from 4 days before flowering to 4 days after flowering, did not occurred.

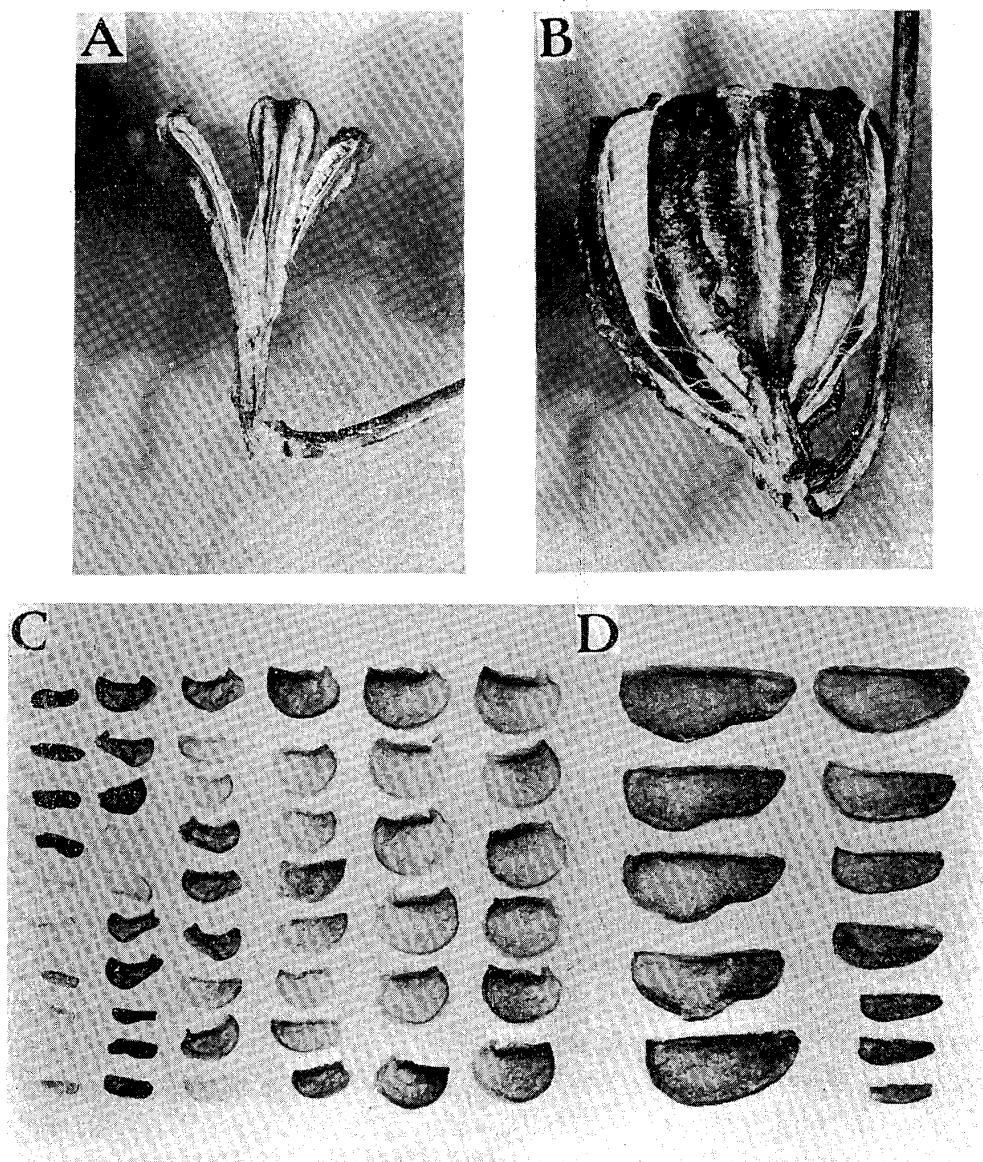


Fig. 2. Fruits and seeds produced by the cross of *L. Henryi* with *L. auratum*, and by the selfing of *L. Henryi*. A: Fruit, produced by the cross. B: Fruit, produced by the selfing. Thickening growth is very poor in the case of the cross. C: Seeds, produced by the cross. D: Seeds, produced by the selfing. Number of seeds produced by the cross is smaller than that produced by the selfing.

*L. Henryi* × *L. longiflorum* About 100 per cent of the pollen grains germinated on the artificial media, and their tube growth reached to the length of ten to twenty-five times of the longer diameter of the pollen grains. 7 flowers were crossed, and all of them were dropped within 10 days after pollination. Because the number of flowers used was small, the details of the pollen tube behaviors could not be traced.

*L. Henryi* selfing 100 per cent of the pollen grains could germinate on the artificial media, and their tube growth reached to the length of ten times of the longer diameter of the pollen grains. 15 flowers were

selfed, and all of them could start their ovarian growth, and finally produced perfectly matured fruits contained the fully matured, fertile seeds (Fig. 2).

*Growth rates of pollen tube* The pollen tube behaviors of the *Lilium* species used here are summarized in Fig. 3. There can not be observed a marked difference between the growth rates of *L. auratum* and *L. speciosum*. As to the starting time of the growth of pollen tube,

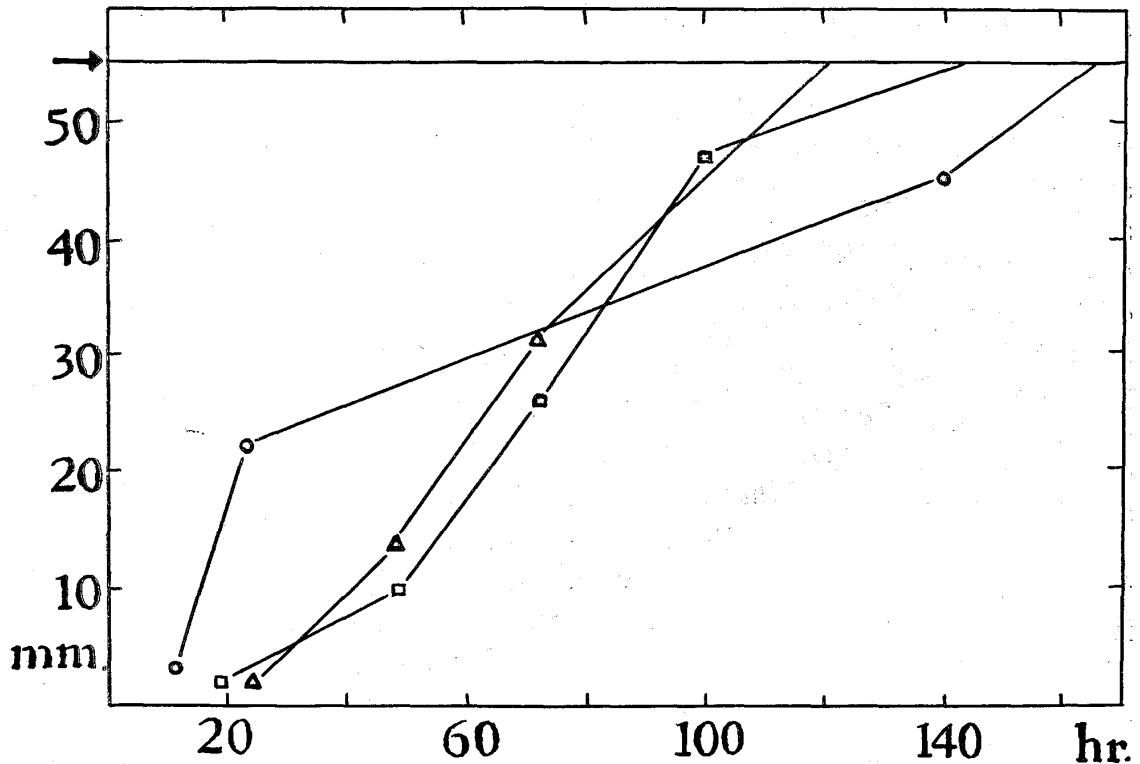


Fig. 3. Growth curves of pollen-tubes of some *Lilium* species in the styles of *L. Henryi*. —△— *L. auratum*, —□— *L. speciosum*, —○— *L. Henryi*.

however, that of *L. Henryi* is earlier to considerable extent than those of *L. auratum* and *L. speciosum*. And it is somewhat interesting to point out that the growth rate of the pollen tube of *L. Henryi* in its own style is very increased comparing with that on artificial media, while the growth rates of pollen tube of other species in the style of *L.*

Table 1. Comparison of the pollen tube length on artificial media with those in the style of *L. Henryi*. 24 hours after sowing.

Species of pollen	Growth length (mm)	
	on the artificial media	in the style
<i>L. Henryi</i>	1.4	23
<i>L. auratum</i>	9.6	2
<i>L. speciosum</i>	4.2	3

*Henryi* are more decreased than those on artificial media (Table 1).

From the facts above mentioned, it may be said that the existence of enhancement effects in the stigma and/or the style of *L. Henryi* to its own pollen tube growth may act rather suppressive to the pollen tube growth of other species. Though the nature of this tube growth regulating system still remains undetected, the preliminary experiments of Niizeki, Kawamata and Nakano (unpublished) suggested that the quantity, i. e. the concentration, of such an operating factor in this system is more substantial than the quality.

Marking mainly to the floral morphology, it is used to divide the genus *Lilium* into four sections. This grouping seems to be rather artificial one (e. g. Woodcock and Stearn, 1950, p. 114), but the better system still has not been proposed. Sexual isolating mechanism is one of the factors for the maintenance of the species, and the degree of inter-specific sterility can be accepted as an indicator of the stage in speciation reached at present. Therefore, the analysis of this phenomenon may throw some light upon the problem of species differentiation. Performing numerous interspecific crosses, Shimizu (1953) proposed a working hypothesis concerning to the inter-specific affinities in the genus *Lilium*. He suggested that the affinities among the members of *Lilium* may be determined by the kinds of flower pigments, such as anthocyanins, carotenes and flavons, and are rather indifferent to intersectional relationships in the current treatment. Since his determination of the degree of inter-specific affinities was based on the difference whether the fruit-set or seed-set is able or unable, the further analysis of pollen tube behaviors in the combinations between any species would be useful. The number of combinations observed here was exceedingly small, and insufficient to verify the Shimizu's hypothesis.

The female reproductive organ, the pistil, is composed of three main parts i. e. the stigma, the style and the ovary, and the inhibition of pollen tube growth can be expected to occur in any of these sites. Contrary to the expectation, the sites of inhibition observed in the present study are limited in two, the stigmatic (*L. maculatum*) and the ovarian (*L. auratum* and *L. speciosum*) inhibition. According to the further observations, however, the growth of pollen tubes of *L. Hansonii* clearly stops not only in its own style but also in the style of *L. Henryi* (Niizeki and Suzuki, in preparation), and that of *L. tigrinum* may be expected to exert a resembling behavior (Niizeki and Iwamura, 1960). These are examples of stylar inhibition, which can not be observed in the present study.

Moreover, the further cytological observations on the cross of *L.*

*Henryi* with *L. auratum* (Niizeki and Okazaki, in preparation) indicate that the inability of cytogamy between the cytoplasm of pollen tube and of embryo sac may be a cause of the cross-sterility. This example is quite different qualitatively from those mentioned above.

In the crosses using *L. Henryi* as a female plant, the existence of various modes of pollen tube behaviors were thus pointed out. And the author would like to use the following terms for the convenience to the grouping of the modes of inter-specific sterility: intergameto-sporophytic inhibition (including the stigmatic, stylar and ovarian inhibition) and intergameto-gametophytic inhibition (including the inability of cytogamy and karyogamy between male and female gametophyte). These two phenomena can be happened independently with each other, but the experimental data for the establishment of their physiological mechanisms or evolutionary meanings are quite scanty. The works are just began, and the results will be published elsewhere.

### Summary

The results of crosses, using *L. Henryi* as female plant and *L. maculatum*, *auratum*, *speciosum*, *longiflorum* and *Henryi* as male plants, were reported. Special attentions were paid to the pollen tube behaviors, and these were summarized in Fig. 3. Stigmatic inhibition was observed in the cross of *L. Henryi* × *L. maculatum*. The pollen tubes reached the ovarian parts in the crosses of *L. Henryi* × *L. auratum*, and *L. Henryi* × *speciosum*, but the fertile seed-set was unsuccessful.

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