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Hybridization of Liliaceae: Overcoming Self-incompatibility and Incongruity

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Abstract. **Using the irradiated mentor-pollen or the irradiated pioneer-pollen technique, self-incompatible pollinations of *Lilium longiflorum* 'White Europe' Thunb. produced more seed than a single application of self-incompatible pollen at 18°C; however, at temperatures of 22° and 26° these techniques did not consistently produce more seed than single self-incompatible pollinations. Application of these methods to overcome incongruity in *L. l.* 'White Europe' x *L.* hybrid 'Enchantment' produced a few embryos.**

For the lily breeder, the genetic variability within the genus *Lilium* provides many possibilities for hybridization. Exploitation of this genetic variability is frequently hindered because of gametophytic self-incompatability and incongruity. Incongruity refers to the incompatibility between species (3).

One approach in circumventing gametophytic incompatible and incongruous barriers is to create conditions in the style similar to those encountered by compatible pollen tubes. Using a pollen mixture (compatible, incompatible, or incongruous) is one means of achieving this objective. In theory, the compatible pollen tubes will engender a favorable environment for growth of the incompatible or incongruous pollen tubes (6). Kunishige et al. (4) successfully used mixed pollen to overcome the incongruity between *L. speciosum* and *L. henryi*.

Stettler (7) first reported the use of irradiated compatible pollen in mixed pollen to successfully make incongruous crosses within the genus *Populus*. He proposed that the irradiated compatible or mentor pollen was genetically inert but still provided the necessary stimulus for incongruous pollen to accomplish fertilization.

Visser et al. (9) reported that pollinating apple blossoms twice with compatible pollen at various time intervals produced more seed than a single pollination. Visser et al. termed the initial pollen "pioneer pollen" and suggested that it enhanced the efficacy of the 2nd pollen.

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Van Tuyl et al. (8) investigated the feasibility of using irradiated mentor-pollen (irradiated pollen applied immediately before the incompatible or incongruous pollen) and irradiated pioneer-pollen (irradiated pollen applied several hours before incompatible or incongruous pollen) to overcome incompatibility and incongruity in *Lilium*. Their initial results were promising, but, because of the preliminary nature of their trials, additional research was warranted. The purpose of our study was to investigate further the possibility of overcoming self-incompatibility and incongruity in *L. longiflorum* by the irradiated mentor-pollen and irradiated pioneer-pollen procedures. Two experiments were performed; the 1st study investigated both the self-incompatible incongruous reactions whereas the 2nd study dealt only with the self-incompatible reaction.

In the 1st experiment, 90 *L. longiflorum* 'White Europe' plants were used as seed parents; 45 plants were placed in each of 2 phytotron houses and maintained at a day/night (D/N) temperature of 22° or 26°C. Flowers of the seed parents were emasculated prior to anthesis.

As a compatible pollen parent, *L. longiflorum* 'White American' was used, whereas the incongruous pollen parent was Asiatic *L.* hybrid 'Enchantment'. Pollen of 'White American' was exposed to 100 krads of gamma irradiation in order to render it genetically inert. The irradiated pollen then was applied either as mentor pollen, i.e., immediately before a 2nd pollen ['White Europe' (incompatible) or 'Enchantment' (incongruous)] or as pioneer pollen, i.e., 6 hr before the 2nd pollen. All pollen was stored in a desiccator at room temperature (25°C) and rehydrated for 24 hr at 5° prior to use. All pollen was checked *in vitro* for germination on the day it was used. Ten treatments with about 8 flowers per treatment (Table 1) were applied at random to the available flowers from 12 Apr. through 5 May.

Seed pods were harvested at maturity, dried, and sorted on an illuminated glass sheet in order to eliminate any seed which contained vestigal embryos. Only relatively heavy seed were counted since previous experience had shown these to be viable. Pods produced by incongruous pollinations were harvested about 45 days after pollination. Ovules were excised and placed on an agar-solidified Murashige and Skoog medium (5), pH 5.0, supplemented with 20 g/liter sucrose and 10^{-2} mg/liter naphthaleneacetic acid.

The 2nd experiment was an elaboration of the self-incompatible aspect of experiment one. Time intervals of 8, 24, and 72 hr were allowed to elapse between pollen applications of the irradiated pioneer-pollen procedure. The (D/N) greenhouse temperature was 18°C for this experiment. Treatments were applied from 30 Aug. through 10 Sept (Table 2).

In vitro germination percentages of all pollen except 'Enchantment' were about 50% and thus sufficient to ensure *in vivo* germination under compatible conditions. Germination percentages of 'Enchantment' pollen varied from 13% to 23%.

No seed developed from flowers pollinated with irradiated pollen in either experiment. Apparently, this pollen was not capable of fertilization and did not stimulate haploid parthenogenesis or parthenogenetic diploidy (6) (Tables 1 and 2). Several parthenocarpic fruit developed from flowers pollinated with irradiated mentor or pioneer pollen as previously reported by Van Tuyl et al. (8).

Flowers pollinated with only compatible pollen or compatible pollen in combination with irradiated mentor or pioneer pollen produced the greatest number of seed in both experiments. The number of seed produced when irradiated mentor or pioneer pollen was applied in combination with compatible pollen was comparable to that obtained when only compatible pollen was applied (Tables 1 and 2). This similarity suggested that the irradiated mentor or pioneer pollen tubes did not compete with the compatible pollen tubes, either in the style or in the process of ovule penetration.

Several flowers treated with self-incompatible pollen produced seed at 22° and 26°C in the 1st experiment (Table 1). No seed was produced in the 2nd experiment when plants were self-pollinated at 18° (Table 2). Seed production in experiment one at 22° and 26° might have resulted from high temperature inactivation of the self-incompatible reaction. Ascher and Peloquin (2) attributed loss of self-incompatibility in lily at 31° and 39° to a breakdown of a postulated pollen-tube inhibitor factor. Van Tuyl et al. (8) also reported some seed set of self-pollinated flowers of 'White Europe' at 23° and 26°.

The application of irradiated mentor or pioneer pollen plus self-incompatible pollen at a temperature of 18°C produced seed, whereas a single application of self-incompatible pollen did not (Table 2). At 22° and 26°, however, a single application of self-incompatible

Table 1. Number of *L. longiflorum* flowers pollinated, number of pods containing seed, total number of seed, and the range of number of seed found in pods of each treatment at 22° and 26°C.

Treatment[z] no. and description	22°C				26°C			
	No. flowers pollinated	Pods with seeds	Total seed	Range of seed/pod	No. flowers pollinated	Pods with seed	Total seed	Range of seed/pod
1-C	8	8	1994	202–323	7	6	528	8–153
2-S	8	3	132	5–117	8	5	14	1–5
3-C_r	8	0	0	---	8	0	0	---
4-I	8	0	0	---	8	0	0	---
5-C_r+C	8	8	1835	208–220	8	8	1070	63–201
6-C_r+S	8	1	55	---	8	1	2	---
7-C_r+I	8	0	0	---	7	2	3[y]	---
8-C_r/C	8	8	1967	213–300	8	8	929	36–176
9-C_r/S	8	3	16	3–10	9	2	57	1–56
10-C_r/I	8	1	3[y]	---	9	0	0	---

[z]C = compatible pollen 'White American', S = self-incompatible pollen 'White Europe', I = incongruous pollen 'Enchantment', C_r = irradiated (100 krad) pollen 'White American', C_r+ _____= mentor pollen applied immediately before the 2nd pollen, and C_r/_____ = pioneer pollen applied 6 hr before a 2nd pollen.

[y] Ovules placed on a modified Murashige and Skoog medium.

Table 2. Number of *L. longiflorum* flowers pollinated, number of pods with seed, the total number of seed, and the range of number of seed found in the pods of each treatment at 18°C.

Treatment[z] no. and description	No. flowers pollinated	Pods with seeds	Total seed	Range of seed/pod
1-C	10	10	2278	114–297
2-S	10	0	0	---
3-C_r	10	0	0	---
4-C_r+C	10	10	2011	111–302
5-C_r+S	10	1	11	---
6-$C_r/_8$C	10	10	1995	169–254
7-$C_r/_8$S	10	1	9	---
8-$C_r/_{24}$C	10	10	2115	134–257
9-$C_r/_{24}$S	10	2	25	8–17
10-$C_r/_{72}$C	10	10	1190	18–271
11-$C_r/_{72}$S	10	0	0	---

[z] C = compatible pollen 'White American', S = self-incompatible pollen 'White Europe', C_r = irradiated (100 krad) pollen 'White American', C_r + _____= mentor pollen applied immediately before a 2nd pollen, and C_r/_____ = pioneer pollen applied 8, 24, or 72 hr before a 2nd pollen.

pollen was as effective for seed production as the irradiated mentor-pollen or the irradiated pioneer-pollen technique (Table 1). The amount of seed developed when irradiated mentor or pioneer pollen was used with incompatible pollen at 22° and 26° (experiment one) was not consistently greater than a single application of incompatible pollen. The seed from pollinations with irradiated mentor or pioneer plus incompatible pollen therefore might have resulted from stimulation of pollen tube growth by the irradiated mentor or pioneer pollen, or temperature inactivation of the self-incompatible reaction, or both (Table 1). No seed production resulted from single applications of self-incompatible pollen at 18°C. In contrast, the irradiated mentor- or the irradiated pioneer-pollen techniques produced seed at the same temperature (Table 2). These results are consistent with the concept of irradiated mentor or pioneer pollen inducing favorable conditions for incompatible pollen tube growth.

Increased time between application of the irradiated pioneer pollen and the self-incompatible pollen did not increase seed set in experiment 2. In fact, at the 72-hr interval, the least amount of seed was produced. This decrease occurred in both compatible and self-incompatible crosses and probably was caused by reduced flower receptivity.

Flowers pollinated with incongruous pollen in experiment one produced no seed, suggesting that fertilization had not occurred or that embryos had aborted (Table 1).

A seed pod which contained 3 embryos developed from a flower pollinated with irradiated pioneer and incongruous pollen at 22°C (Table 1). A total of 3 embryos developed from 7 flowers pollinated with irradiated mentor plus incongruous pollen at 26°C (Table 1). These results are considered as supporting circumvention of incongruity. Plants which were produced are being grown for hybrid confirmation. Possibly additional seed could have resulted with improved viability of the incongruous pollen from 'Enchantment'.

Our results support the irradiated mentor-pollen and the irradiated pioneer-pollen techniques as means to circumvent incongruity in L. *l.* 'White Europe' x *L.* hybrid 'Enchantment' crosses at 22° and 26°; however, at these same temperatures, these techniques did not produce better seed set than a single application of self-incompatible pollen. At 18° these methods were more successful than a single application of self-incompatible pollen. An avenue for future research to circumvent gametophytic self-incompatibility and incongruity in *Lilium* might be to incorporate the irradiated mentor- or pioneer-pollen techniques with the intra-stylar pollination technique (1).

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