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Temperature effects on self incompatibility in *Lilium longiflorum*

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Summary. Detached pistils of the clonal variety, *Lilium longiflorum* 'Arai No. 5', were submerged before pollination in 50 °C water for 0, 1, 2, 3, 4, 5, 6 or 7 min and then immediately compatibly and incompatibly pollinated. Incompatibility, as indicated by pollen tube length after 48 h at 23.5 °C, was eliminated by a 1–2 min submersion while compatibility was removed by a 4–5 min one. The 'window' of incubation temperatures at which incompatible and compatible pollen tubes are clearly differentiated occurred between 15 and 30 °C.

Key words: Self incompatibility – Compatibility – Pollen tube growth – Heat treatment – Aging – *Lilium longiflorum* – Seed set

Introduction

Exposure of pistils to an elevated temperature for varying times overcomes self incompatibility in plants, resulting in increased pollen tube penetration of the style and, in some species, self seed set (review: De Nettancourt 1977). However, excess exposure to heat can remove this increase in compatibility.

Depending on the species, plant breeders and geneticists have used temperature regimes of 24–40 °C applied to the whole plant before pollination for periods of as little as 12 h at the higher temperatures, to many days at the lower temperatures, to overcome both gametophytic (G) and sporophytic (S) self incompatibility (Lewis 1942, Hecht 1964, *Oenothera;* Campbell and Ascher 1972, *Nemesia* (G); Drewlow et al. 1971, Ronald and Ascher 1975, *Chrysanthemum* (S); El Murabaa 1957, *Raphanus* (S); Straub 1958, Flaschenriem and Ascher 1978, 1980a, b, van Herpen 1981, van Herpen and Linskens 1981, *Petunia* (G); Nasrallah and Wallace 1968, Johnson 1971, *Brassica* (S); Leffel 1963, *Trifolium* (G); and Makarem and Ascher 1977, *Ageratum* (S)). Excess heat (a constant 24 °C during flowering in *Brassica* to 24 h of 45 °C in *Ageratum*) reduces self seed set levels to zero, probably from pollen abortion and other adverse effects on the plant.

In plants with a gametophytic type of incompatibility characterized by inhibition of pollen tubes in the style (Linskens 1973, 1975, 1983), the incompatibility reaction in pistils attached to or detached from the plant can be removed by submersion in 48–50 °C for 2.5 to 6 min (Bali 1963, Bali and Hecht 1965 on *Oenothera rhombipetala;* Hecht et al. 1967, Hopper and Peloquin 1976 on *Lilium longiflorum* (G)). In *Oenothera organensis,* where self pollen tube inhibition occurs in the stigma, a submersion in 50 °C water for 5 min allows self pollen tubes to grow much longer although no self seed is set. Submersion in 60 °C water allowed only compatible and not incompatible pollen to germinate. Necrotic darkening allowing no pollen germination occurred after a 5 min submersion in 55–57 °C water in one season, but occurred at 53 °C in the next season (Hecht 1964).

However, in *Lilium,* where incompatible pollen tubes usually only grow to 80 mm in a 100 mm style (Ascher and Peloquin 1970), incompatible pollen tubes can be made to grow as compatible with seed set resulting, although seed is not obtained in the quantities resulting from a compatible pollination (Hopper et al. 1967). In *Lilium,* submersion for longer than 5–6 min in 50 °C water causes both compatible and incompatible pollen tubes to grow only as incompatible ones, and at even longer submersion times, limits pollen tube growth to the stigma: the pollen tubes stopping before entering the stylar canal whose cells show much necrosis (Hopper et al. 1967).

Hopper and Peloquin (1976) have shown in *Lilium* that each minute of pre-pollination stylar heat treatment at 49 °C, up to 3 min, causes a 9 mm increase in self pollen tube length grown at 24 °C for 48 h. By 3 min, the normal difference between incompatible and compatible pollen tubes has been eliminated.

Since heat treatment is effective when applied to the style before pollination (Hopper and Peloquin 1976; Townsend 1965, 1966; Campbell and Ascher 1972), heat must act on the stylar contribution to self incompatibility, compatibility and pollen tube support. The heat treatment is irreversible since a delay of 63 h between heat treatment and pollination in

Lilium does not result in a return of self incompatibility to the style. Also, heat applied to styles from buds can remove the self-incompatibility reaction, as in *Lilium*, when applied to styles 39 h before anthesis (Hopper and Peloquin 1976), or in the case of *Nemesia strumosa*, when applied for 24 h at 34 °C nine days before anthesis (Campbell and Ascher 1972). Although the heat treatment effect is not affected by delayed pollination in *Lilium*, a delay of 24 h between heat treatment and pollination in *Oenothera* gives even longer self pollen tubes (Hecht 1964).

The irreversibility of the heat treatment, in addition to observations that the flowers of a clone are more heat sensitive when developed before anthesis in a hot greenhouse, that older flowers are more heat susceptible, and that different clones of one species differ in their sensitivity to heat, has led to the hypothesis that there is a complex interaction of heat, cultivar and environment affecting incompatible and compatible pollen tube growth. Also, the progressive removal of incompatibility, compatibility and then all stylar pollen tube growth by increasing the amounts of heat applied parallels the happenings in an aging style (Ascher and Peloquin 1966 a). Pollen tube growth in lily styles varies with age, with older styles losing their capacity as a screen of self pollen tubes some time before they lose their capacity to support any pollen tube growth. But the age of the style at which these events occur varies with the cultivars that senesce faster and in flowers that develop under hot greenhouse conditions.

Because the clone of *Lilium longiflorum*, 'Arai No. 5', was found to be very susceptible to heat removal of incompatibility, detailed experiments were begun to document its response. This paper reports the effect of both varying lengths of time of 50 °C treatment and incubation temperatures on incompatible and compatible pollen tube growth in *Lilium longiflorum* 'Arai No. 5'.

Materials and methods

Plants of *Lilium longiflorum* Thunb. 'Arai No. 5' (from Japan) and 'Mount Everest' (from Oregon, USA) were grown in a 17 °C greenhouse, and given 12 h of additional light from November to March using 400 watt Philips mercury vapor lamps. From late January to late July, flowers were cut from the plant early, by 10 a.m., and placed in jars of water in a laboratory for 24 h under fluorescent light and a temperature cycle of 12 h 15 °C and 12 h 21 °C.

Stylar preparation, incubation and storage

Pistils from 'Arai No. 5' flowers were cut out with a piece of ovary attached, treated, copiously pollinated, laid on a moistened filter paper in a 150 mm diameter glass petri dish and incubated in the dark at 23.3 °C for 48 h. At the end of the incubation period, the ovary was snapped off and cold (4 °C) 1% aniline blue dye (E. Merck) dissolved in water was injected into the pistils through the stigma until a drop of dye appeared at the ovarian end. The pistils were then stored at 4 °C until determination of pollen tube length.

Pollen tube measurement

The pistil was opened along its length using the tip of a half-shield-shaped dissecting needle, crushed at various places along its length with the flat edge of the needle and pried open using two of the needles to reveal the dark blue pollen tubes against an undyed background. The two longest pollen tubes, one in each opposed half of the style, were measured to the nearest mm with a dissecting microscope.

Pollen source

Selfings of 'Arai No. 5' styles were done using the flower from which the style had come. For cross-pollinations, pollen was either 'Nellie White' pollen, kindly provided by Dr. P. D. Ascher of the University of Minnesota (USA), and stored dessicated at 4 °C and brought to room temperature 1 h before use; or a pollen mixture collected from 1 day old flowers of 'Mt. Everest'. All sources of pollen had 99–100% pollen stainability when used.

Heat-shock experiments

A. Detached pistils

'Arai No. 5' pistils with a piece of ovary attached were submerged in 50 ± 0.2 °C deionized, distilled water under a wire screen, for lengths of time from 0 to 7 min. They were left for 30–90 min before pollination, pollinated, and subsequently incubated for pollen tube growth.

Three experiments were run:

1. Six pistils of ages day of anthesis (A), and 1 and 2 days old were heated for 5 min and selfed intraclonally.
2. Thirty-two pistils of ages 1, 2, and 3 days old were heated for 0, 1, 2, 3, 4, 5, 6 and 7 min and intra-clonally selfed.
3. Ninety-six pistils of ages A, 1, 2 and 3 days old were heated for 1, 2, 3, 4, 5, 6 or 7 min, and the pistils left non-pollinated, own-flower selfed, or crossed to 'Nellie White'.

B. Attached pistils

Tepals and stamens were removed from 1 or 2 day old 'Arai No. 5' flowers, and the pistil submerged in 50 °C water to the top of the ovary for 0, 1, 2, 3, 4, 5, 6 or 7 min, and the stigma own-flower selfed, or crossed with 'Nellie White' pollen. Untreated pistils were also crossed with 'Mt. Everest' pollen.

The plants were brought each day from the greenhouse into the laboratory for heat treatment. The plants were scored for pod set, and the seeds from the pods counted and classed into embryo- and non-embryo-containing. Of the ones that had embryos, the ones having enough endosperm to give germination were also determined. After removal of seeds, pod weight and length, and total seed weight were measured. There were three replications using 10 plants having 47 flowers.

Incubation experiment

'Arai No. 5' pistils were intraclonally selfed and incubated for 24, 48 or 72 h at 15, 20, 25 or 30 °C. Some styles were crossed with 'Nellie White' pollen and incubated at the four temperatures for 48 h. Pollen tube length was measured.

Results

The effects of heat treatment have been investigated in isolated and attached styles using the behaviour of the pollen tubes and their stainability as parameters.

1 Heat shock experiments

A. Detached pistils

Pollen tube length after self pollination in heat-treated pistils was 56 mm, compared with 86 mm in non-heat-treated pistils. Stylar canal cells in the heat-treated pistils stained deeply, indicating much damage. Non-heated selfed pistils had 67 mm long pollen tubes. Two minutes of heat treatment caused the longest self pollen tube growth, 83 mm (Fig. 1). Four and 5 min of heat gave a pollen tube length halfway between the self length in control pistils and the 2 min treatment. The 6 and 7 min treatments produced pollen tube lengths equal to the 0 treatment. After 48 h incubation, pistils treated with 4, 5, 6 and 7 min of heat were slimy, indicating tissue breakdown.

Within 1.5 h of treatment, pistils heated for 7 min showed a browning of the stigmas as did some of the pistils given 4, 5 and 6 min of heat. One to 2 h after heat treatment, only non-treated stigmas were coated with stigmatic exudate. Self pollen tubes grew as compatible ones after 1 or 2 min of heat treatment (Fig. 2), but treatments longer than that caused pollen tubes to again grow as selfs. Heat treatment had no effect on cross pollen tubes until 4 and 5 min of heat treatment caused them to grow to the same length as self pollen tubes which were then growing at selfed lengths. Six and 7 min heat treatments caused a further decline in length so that the cross tubes were shorter than the selfs.

Variability of tube behaviour. The lengths of cross pollen tubes were less variable in pistils given 1, 2 or 3 min of heat treatment (Fig. 2), more variable in non-treated pistils, and even more variable in pistils given 4, 5, 6 or 7 min of 50 °C.

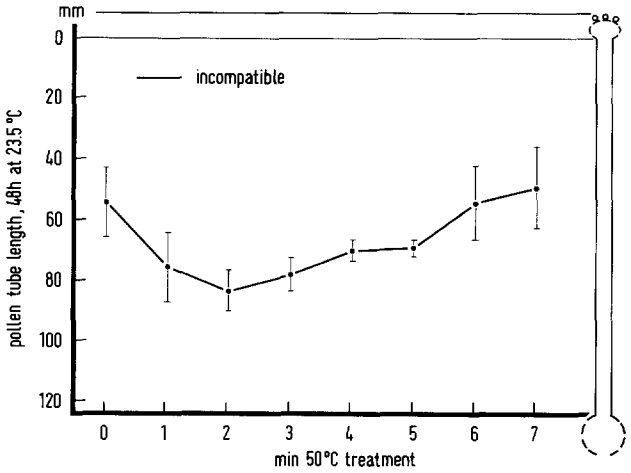

Fig. 1. Pistils of *Lilium longiflorum* 'Arai No. 5' were submerged for varying lengths of time in 50 °C deionized distilled water, self pollinated, and incubated to develop pollen tubes growth at 23.5 °C. Standard deviation is shown

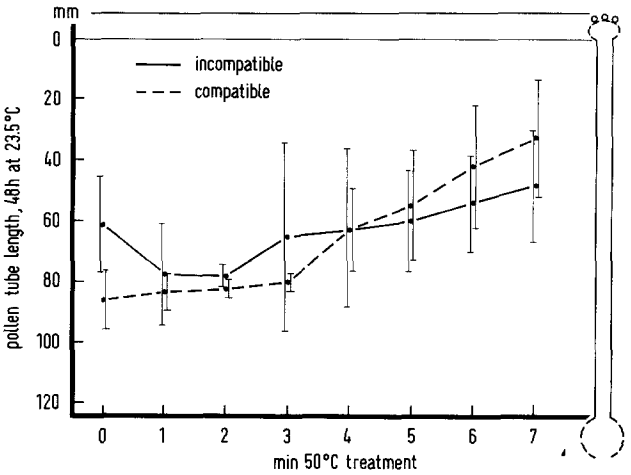

Fig. 2. As in Fig. 1, except that half of the pistils were compatibly pollinated with *Lilium longiflorum* 'Nellie White'. Average of measurement in 96 pistils

For self pollen tubes, 2 min of heat gave the least variable results, with 1 or 3 min also giving consistent compatible lengths in most of the pistils. Four, 5, 6 and 7 min of heat treatment resulted in a more variable response, as also seen in crossed styles, except that more tubes seemed to have a compatible growth while the exceptions were an incompatible or shorter growth.

Changing stainability. Stylar canal cells in non-treated pistils were not dyed by aniline blue dye. Heat treatment caused staining of the stylar canal cells. In older pistils, staining increased with more heat, but the pattern of its gradual appearance was not uniform. At first, there was an overall light blue, or a few scattered dark blue cells, mostly in the stigma, in the middle of the style, or at the base. The dark blue cells appeared first in the deeper parts of the canal where the distance to the epidermis is the thinnest. The first large patches of dark blue were found in pistils from 3 day old flowers at 2 min of heat treatment. Deep blue staining involving 100% of the stylar canal cells did not occur until 5, 6 and 7 min of heat treatment, and that only in the oldest pistils used, with stylar canal cells of 2 day old pistils turning all dark blue at 6 min of heat treatment. Young pistils, even at 7 min of heat treatment, had stylar canal cells that were non-stained or had only scattered patches of dark blue.

B. Attached pistils

Not all the 'Nellie White' pollinations set a pod (Table 1) with 0, 1 and 2 min heat treatment. The seed pod after the 2 min treatment was smaller than that of the control or the 1 min heat treatment. Heat treatment of the pistils for 3 min or more caused no pod set after

Table 1. Pod set and seed set data for 1 and 2 day old *Lilium longiflorum* 'Arai No. 5' flowers selfed and crossed after various times of submersion of the attached stigma-style in 50 °C deionized-distilled water. Self pollen was from other 'Arai No. 5' plants (A) or from the same plant (self). Cross pollen was from 'Nellie White' (NW) or 'Mt. Everest' (ME)

Min of heat	Pollen	No. fls poll	No. pods set	Total seeds	Total with embryo	Total germinable	$\bar{X}$ length	% with embryos	% germinable
0	self	2	0	–	–	–	–	–	–
	A	1	1	462	20	3	40	4.3	2.8
	NW	3	2	832	228	198	48	34.6	23.8
	ME	3	3	1,223	664	607	50	54.3	49.6
1	self	2	0	–	–	–	–	–	–
	NW	2	1	427	70	54	48	16.4	12.6
2	self	4	0	–	–	–	–	–	–
	NW	4	2	638	68	45	36	10.7	7.1
3	self	3	0	–	–	–	–	–	–
	NW	3	0	–	–	–	–	–	–
4–7	self	2/time	0/time	–	–	–	–	–	–
	NW	2/time	0/time	–	–	–	–	–	–

cross pollination with 'Nellie White' pollen. Increasing the duration of heat from 0 to 3 min reduced the 'Nellie White' germinable seed percentage from 24% at 0 min, to 16% at 1 min, 10% at 2 min and 0% at 3 min. Own-flower selfed flowers set no seed pod. An intracultivar self on a control style gave a pod with three germinable seeds in 462 seeds, 20 of which had embryos.

2 Incubation experiment

In 48 h, compatible pollen tubes grew longer than incompatibles at 20 and 25 °C (Fig. 3). Compatible pollen tubes grown 48 h were the same length as incompatible ones grown at 15 °C, an incompatible length; and were again the same length as incompatibles grown at 30 °C, a compatible length. The growth rates for incompatible tubes at 15, 20 and 25 °C were approximately the same at 24, 48 and 72 h of incubation. Except for the incompatible tubes growing at 30 °C (which had reached the end of the style sometime before completion of the 72 h of incubation), incompatible pollen tubes showed an increased growth rate between 48 and 72 h of pollen tube growth.

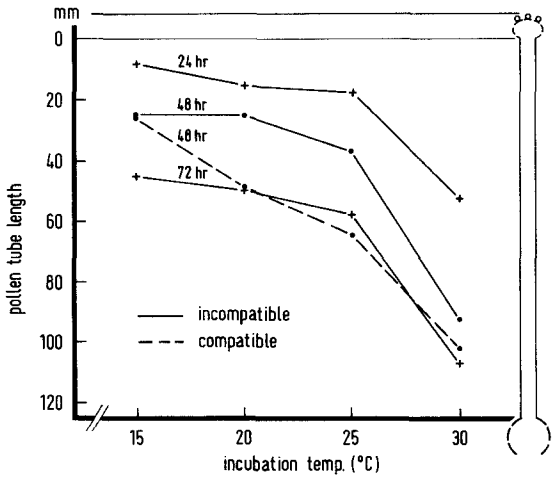

Fig. 3. Pistils of *Lilium longiflorum* 'Arai No. 5' were incompatibly selfed or compatibly pollinated with 'Nellie White' pollen, incubated for various times at various temperatures, and the pollen tube length measured. Average of measurement in 80 pistils

Discussion

The first experiment indicated that 5 min in 50 °C water removed both incompatibility and compatibility from lily pistils. The experiments using heat shock treatment from 0 to 7 min in 50 °C revealed that the stylar incompatibility reaction and stylar compatibility were very temperature sensitive in pistils of *Lilium longiflorum* 'Arai No. 5'. These results suggest that the stylar incompatibility reaction and stylar ability to support compatible growth can be gradually removed by applying higher temperature and/or duration of heat treatment, respectively. Such a removal of the incompatibility barrier is not an abrupt either/or situation. Compatibility, here, is removed by as little as 3–4 min of heat treatment at 50 °C.

The stylar incompatibility reaction of most *Lilium longiflorum* cultivars is lost after a 5–6 min submersion in 50 °C water (Hopper et al. 1967). Ascher (1975) found compatibility to be lost after as little as 6 min of 50 °C in cultivar 'Ace'.

Greater susceptibility to heat such as the removal of self incompatibility during 3 min of 49 °C reported by Hopper and Peloquin (1976) in 'Ace' was thought to be a result of the preanthesis development of the flowers in a greenhouse having daylight hours hotter than usual.

A cultivar particularly susceptible to a high heat treatment should probably have short-lived flowers. Ascher and Peloquin (1966a) noted that the incompatibility reaction in Easter lily is reduced in pistils that are older, and that this loss is quicker when the flowers senesce more rapidly in hot weather. 'Arai No. 5' flowers in the greenhouse in January and February are quite short-lived, becoming flaccid and starting to collapse 3–4 days after opening. They were fully collapsed at 5–6 days and browning at 6–7 days; flower-part abscission

started at 8 days. Senescence was a little slower in the laboratory, with flaccidity and collapse occurring at day 5, full collapse and browning at 7 days, and flower abscission at 11 days. Ascher and Peloquin (1966a) reported that tepal folding in aging Easter lily flowers may occur as early as day 5 in hot weather, but in cooler weather may not occur for 10 or more days. There were also differences between the clones, 'Ace' lasting longer than 'Croft', with 'Georgia' apparently being intermediate. If tepal folding for Ascher and Peloquin (1966a) is the same as full collapse, then flowers of 'Arai No. 5' are as short-lived as if they had developed in a very hot greenhouse.

Ascher (1975) showed that for flowers stored at 22–23 °C, 6 min of 50 °C removes all self incompatibility from pistils at anthesis to 4-days old. However, in older pistils, the same heat treatment causes both compatible and incompatible pollen tubes to be of identical length, but they fall to incompatible length 1–2 days sooner than compatibles normally do in aging styles. For flowers stored at 4–7 °C, 6 min of 50 °C causes compatibles and incompatibles to grow as incompatibles by day 7, 6–7 days sooner than compatibles would normally.

Hopper et al. (1967) reported that when temperature conditioning of the flowers in a hot greenhouse made the pistils more susceptible to heat removal of all ability to support pollen tube growth, necrosis of the stylar canal cells also occurred at a lower combination of heat and length of treatment. The association of necrosis with stylar inability to support pollen tube growth was an indication of too high temperatures. They suggested that a time/temperature combination should be found for lily at which only a little necrosis would occur and, therefore, at which many self pollen tubes would find their way to the ovary. If such was not found, then the use of heat treatment to overcome incompatibility in pistils left on the plant would be limited as a breeding tool to obtain self seed set.

General necrosis of 'Arai No. 5' stylar canal cells as indicated by staining with aniline blue dye did not occur as a general rule until after compatible growth was no longer possible. Yet, 'Arai No. 5' pistils were never so thoroughly necrosed that only stigmatic growth occurred, as reported by Hopper et al. (1967), indicating that the pollen tubes stopped just as they would have entered the stylar canal. These pistils, therefore, while more susceptible to removal of self incompatibility, were not more susceptible to complete removal of all ability to support stylar pollen tube growth.

Although they should have traversed the style as fast as did compatible, self pollen tubes in styles treated 1 and 2 min at 50 °C set no seed in heat treatments on the plant. Hopper et al. (1967), using 50 °C for 6 min, found for 'Arai' that 5 of 11 pollinations gave fruit set. Seed set was low, being only from 6 to 114 seeds/capsule while normal seed set is 75–300 seeds/capsule after a cross pollination. Since 55 °C for 6 min caused severe necrosis of stylar canal cells in their research, they believed that stylar cell necrosis might explain their low seed set since few pollen tubes would then have reached the ovary.

Cell necrosis is unlikely as a reason for the lack of seeds in 'Arai No. 5' since 3 min and more of 50 °C caused cross pollination to set no fruit either. Also, necrotic staining for these 1 and 2 day old styles should not appear until 5, 6 and 7 min of heat. The following may be possible explanations for this lack of seed: A. Heat treatment does not remove the self incompatibility reaction but the self pollen tubes by 48 h at 23.5 °C have reached an inhibited length, a length no different than compatible length. This is unlikely for incompatible pollen tubes would have to have grown at a faster rate and compatibles grown slower, all because of a heat treatment applied to the pistil sometime before pollination. B. Heat treatment allows only a few self pollen tubes to get through, and it is not correlated with visible canal cell necrosis. And, those numbers that reach the ovary are too few to make the ovary develop, especially in that time of year when techniques that should overcome interspecific incompatibility also yield no seed in lily.

Environmental circumstances that condition 'Arai No. 5' stylar self incompatibility and self compatibility to be more quickly removed also condition the plant to have its 'window' of temperatures that gives the greatest differentiation between compatible and incompatible pollen tubes during 48 h of pollen tube growth shifted to lower temperatures (Fig. 4). Interestingly, the incompatible pollen tube growth rate at 20 and 25 °C increased between 48 and 72 h after pollination instead of declining as might be expected if they were approaching an inhibited length between those times. Ascher and Peloquin (1970) reported that 'Arai' pollen

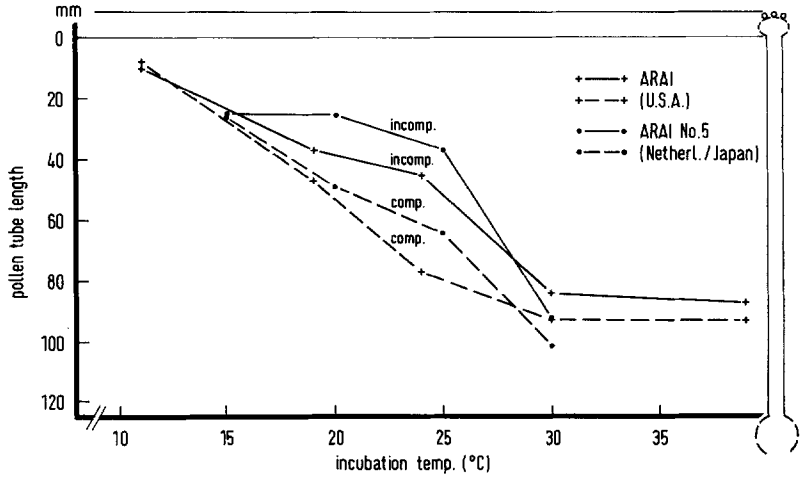

Fig. 4. A pattern of the data from Fig. 3 plotted against data on *Lilium longiflorum* 'Arai' derived from Ascher and Peloquin (1970)

tubes slowed down at 19 °C in later times of incubation, between 48 and 72 h. Also, incompatible pollen tubes here have the same growth rate at 15, 20 and 25 °C.

The foregoing would seem to give this picture of what is going on in *Lilium:* the Easter lily apparently has the ability to adapt to whatever reasonably conducive environment it is growing in so that its self-incompatibility reaction is maintained intact and no self pollinations can result in seed. Therefore, when growing in winter in an environment of 17 °C, the plant has an intact self incompatibility reaction that occurs between 15 to 30 °C. However, later on in the late spring, when temperature conditions are higher on the average, then the plant still has a tight self-incompatibility reaction but now its 'window' of incompatiblity has shifted upward and narrowed so that it now occurs between 20 and 30 °C. This theory will be tested in further research.

At the same time, the plant is more temperature sensitive when grown cool under much artificial light, and is much less so when grown hot under much sunlight. Also, the rates at which incompatible pollen tubes grow is the same in the first instance from 15 to 25 °C so that the affect of the change in incubation temperature is only on compatible pollen tubes. This research concurs with Ascher and Peloquin (1966 b) in their findings, from incubation of lily styles at different temperatures, that the similar growth of compatible and incompatible pollen tubes at low incubation temperatures is due to the effect of heat on the pollen tube, while the effect of the high temperature that removes the self incompatibility is due to its affect on stylar metabolism.

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