# Genetic Control of Isozymes in Lilium longiflorum Thunb

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# Genetic Control of Isozymes in *Lilium longiflorum* Thunb.

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Inheritance and linkage relationships of polymorphic isozymes in *Lilium longiflorum* were demonstrated. Eleven enzyme systems were stably resolved by starch gel electrophoresis. Five of them exhibited polymorphisms, and eight loci with 20 alleles were determined by segregation patterns of offspring obtained from six artificial crosses with horticultural cultivars and wild strains. Contingency chi–square analyses of independent assortment for 64 pairs of loci indicated two linked pairs, *Aat–2/6Pgd–2* (recombination fraction, R=0.34±0.07) and *Fest–2/6Pgd–1* (R=0.29±0.08). Resolved enzyme systems and allozymes could be very efficient for estimating genetic diversity among natural populations in *L. longiflorum*.

## INTRODUCTION

*Lilium longiflorum* Thunb. is a bulbous species naturally distributing in subtropical islands in an arc–arrayed archipelago running approximately 1300 km distance from Ryukyu to Taiwan (Wilson, 1925; Shimizu, 1987). Despite of such local endemism, the species enjoys great popularity in the world as an ornamental resource, particularly for religious usage as indicated by its English name 'Easter lily' (Miller, 1993; Jefferson–Brown and Howland, 1995).

In the light of conservation biology, island endemics are more highly susceptible to extinction than continental ones (Frankham, 1996; Riesberg and Swensen, 1996). Thus, attentions for such factors affecting extinction as ecological, demographic characteristics and genetic diversity are essential for sustainable use of the insular biological resources. However, very few studies on natural populations of *L. longiflorum* have been conducted to date (e.g., Shii, 1983; Wen and Hsiao, 1999).

Allozymes are very powerful tools not only for fields of plant breeding (Weeden, 1989) but also for estimation of genetic diversity (Brown and Weir, 1983) and mating systems (Ritland, 1983), and for evolutionary interpretation in natural plant populations (Crawford, 1989, 1990), even in recent years, when several new types of genetic markers such as RFLP, RAPD, AFLP and so forth have been developed. In the present study, we clarified genetic natures for stably–resolved enzyme systems in *L. longiflorum*, in advance to estimate genetic diversity and evolutionary trends of the species in our future studies.

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**Table 1.** Cross combinations for segregation analysis of allozyme patterns.

| Progeny group No. | Parentage | | |
|---|---|---|---|
| 1 | Georgia | × | Gelria |
| 2 | Georgia | × | Hinomoto |
| 3 | Georgia | × | KU9808 |
| 4 | Snow Queen | × | Georgia |
| 5 | Snow Queen | × | KU9809 |
| 6 | TO9501 | × | Snow Queen |

## MATERIALS AND METHODS

Six progeny groups were generated by control crosses for this study using four horticultural cultivars, 'Gelria', 'Georgia', 'Hinomoto', 'Snow Queen', and two and one individuals from natural populations of Kume Shima (KU9808, KU9809) and Tokuno Shima (TO9501) islands, respectively, in the Ryukyu Archipelago (Table 1).

Enzymes were extracted from immature leaves of each parent and four– to six–month old seedlings of the progenies. Approximately 200 mg samples of immature leaf were homogenized in cooled mortals with a pestle with 2 ml of the Tris–HCl grinding buffer (Soltis *et al.*, 1983), and a sprinkle of polyvinylpolypyrrolidone and sea sand. Crude enzyme extracts were soaked up by filter paper wicks (Whatman No. 3, 11×3 mm), and immediately used for horizontal starch gel electrophoreses described by Wendel and Weeden (1989). Two combinations of gel and electrode buffers in the procedures by Wendel and Weeden (1989) were used to resolve 11 enzymes. Aspartate aminotransferase (AAT), catarase (CAT), diaphorase (DIA), glucose–6–glutamate dehydrogenase (GDH), phosphate isomerase (GPI), and malic enzyme (ME) were resolved using System 6, and fluorescent esterase (FEST), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), phosphogluconate dehydrogenase (6PGD), and phosphoglucomutase (PGM) were determined using System 2. Staining protocols were also carried out according to the method of Wendel and Weeden (1989), except for a modification for FEST by dilution of the substrate with 1/20th volume of acetone.

Inheritance of enzyme loci was postulated on the basis of observed segregations. For single–locus segregations chi–square values were calculated to test goodness–of–fit to expected ratios. Nonrandom joint segregations between loci were tested by chi–square tests of independence. In case where evidence of linkage was detected, recombination fractions and their standard errors were estimated by maximum–likelihood formulas (Allard, 1956).

## RESULTS AND DISCUSSION

Some regions of electrophoretic banding patterns ME, DIA, FEST and MDH were poorly resolved and then omitted from data analysis (Figs. 1, 2). The other regions for all

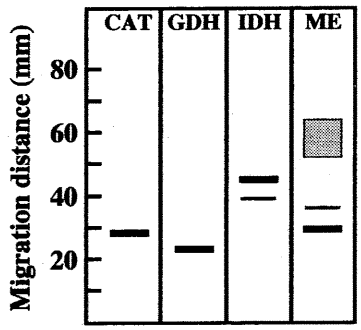


**Fig. 1.** Schematic representation of banding patterns for four enzyme systems in which variation was not observed among parents used for artificial crosses in *Lilium longiflorum*.

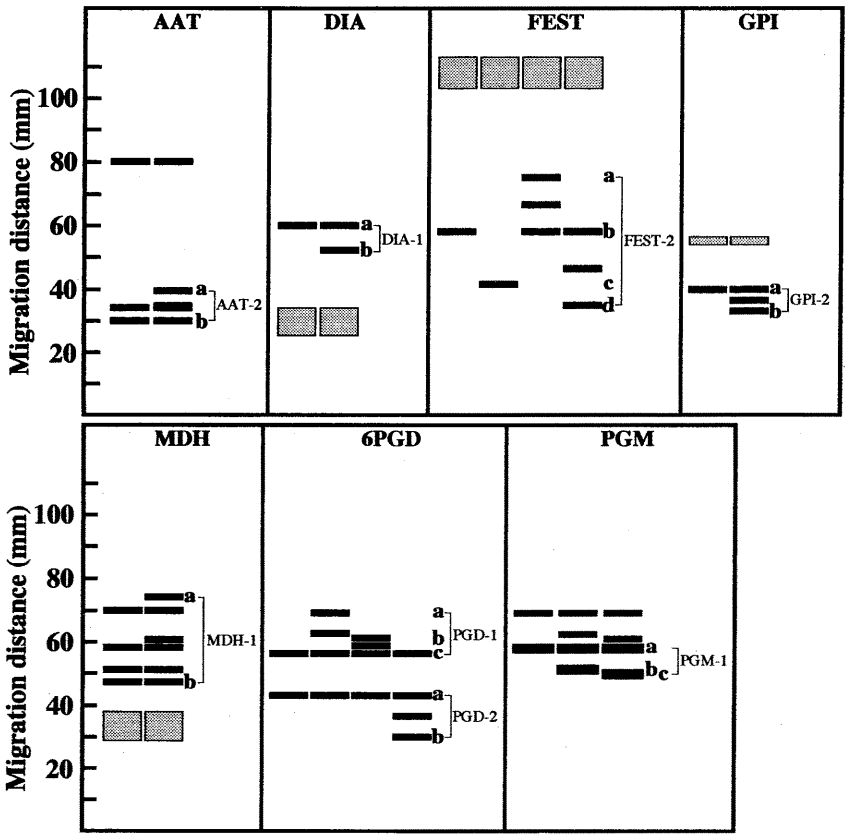


**Fig. 2.** Representative illustration of allozyme variation among parents used for artificial crosses in *Lilium longiflorum*. Zones correspond to eight postulated loci and location of alleles are shown in right of each enzyme system. Hatched bands represent areas of poor resolution.

enzymes were consistently resolved. No polymorphisms were detected for four of 11 enzymes, i.e., CAT, GDH, IDH, and ME, in all parents used for control crosses (Fig. 1). The remaining seven enzymes showed polymorphisms. We postulated eight loci from segregated enzyme phenotypes observed for parents and progeny groups. Proposed regions supposed to be governed by a single locus and allelic band locations of the each locus within starch gels are illustrated in Fig. 2.

**Aspartate aminotransferase (AAT)**

Two active zones appeared from 30 to 39 mm and in 80 mm migration distance (Fig. 2). Two phenotypes were observed in the slower-migrating zone for parents, consisting of two or three bands. Three bands located in an approximately equal distance, and the intermediate band of the three was more deeply stained. Suppose that the faster-migrating band of the double-banded phenotype is an invariant product by a different single locus, the segregation of the phenotype observed in progeny No. 5 fitted the expected 1:1 ratio (Table 2). Thus, it is highly likely that the polymorphic zone observed is controlled at least by two loci, invariant and variant one (*Aat-2*), the latter which produces a dimeric enzyme.

**Diaphorase (DIA)**

Two zones appeared though resolution of the slower-migrating zone from 27 to 33 mm was very poor (Fig. 2). Within the stably-resolved, faster-migrating zone from 52 to 60 mm, a single- or a double-banded phenotype was observed in parents, and segregating phenotypes for three and two progeny groups, respectively, fitted the expected 1:2:1 and 1:1 ratio (Table 2). This indicates that zone DIA-2 is governed by a single locus of a monomeric enzyme.

**Fluorescent esterase (FEST)**

A relatively wide zone from 35 to 75 mm in migration distance was consistently resolved, containing each two types of single- and triple-banded phenotypes within parents, while the more anodal zone from 103 to 113 mm was too poorly resolved to detect phenotypes (Fig. 2). Segregation patterns of the resolved region named FEST-2 were consistent with a single locus governing a dimeric molecule (Table 2) and segregating four alleles (Fig. 2).

**Glucose-6-phosphate isomerase (GPI)**

One single- and one triple-banded phenotype were detected from 33 to 40 mm migration distance for parents (Fig. 2). Segregation data for five progeny groups conformed to 1:1 or 1:2:1 expectations for a single locus (Table 2). These results indicate that the enzyme of this region, GPI-2, is dimeric with a mode of Mendelian inheritance.

**Malate dehydrogenase (MDH)**

Banding patterns for MDH were rather complex, with four or six deeply stained bands together with some faint bands of low resolution in the zone migrated more slowly (Fig. 2). Additional two bands of the six-banded phenotype appeared in the most anodal and an intermediate position within the deeply staining region. Banding phenotypes and

**Table 2.** Goodness-of-fit tests for single locus segregation patterns at eight loci.

| Locus | Progeny group No.[z] | Prospected genotypes | Progeny genotypes (N[y]) | Expected ratio | $\chi^2$ | P |
|---|---|---|---|---|---|---|
| *Aat-2* | 5 | bb×ab | ab(20):bb(30) | 1:1 | 1.62 | 0.20 |
| *Dia-1* | 1 | ab×ab | aa(17):ab(36):bb(22) | 1:2:1 | 0.79 | 0.67 |
| | 2 | ab×ab | aa(9):ab(27):bb(14) | 1:2:1 | 1.32 | 0.52 |
| | 3 | ab×ab | aa(17):ab(44):bb(14) | 1:2:1 | 2.49 | 0.29 |
| | 4 | aa×ab | aa(55):ab(45) | 1:1 | 0.81 | 0.37 |
| | 5 | aa×ab | aa(23):ab(27) | 1:1 | 0.18 | 0.67 |
| *Fest-2* | 1 | ab×bd | ab(16):ad(11):bb(26):bd(22) | 1:1:1:1 | 6.97 | 0.07 |
| | 2 | ab×ab | aa(11):ab(24):bb(15) | 1:2:1 | 0.72 | 0.70 |
| | 3 | ab×bb | ab(43):bb(32) | 1:1 | 1.33 | 0.25 |
| | 4 | bd×ab | ab(34):ad(26):bb(23):bd(16) | 1:1:1:1 | 6.74 | 0.08 |
| | 5 | bd×bb | bb(23): bd(27) | 1:1 | 0.18 | 0.67 |
| | 6 | cc×bd | bc(22):cd(26) | 1:1 | 0.19 | 0.67 |
| *Gpi-2* | 1 | ab×aa | aa(36): ab(39) | 1:1 | 0.05 | 0.82 |
| | 2 | ab×ab | aa(15):ab(27):bb(8) | 1:2:1 | 2.28 | 0.32 |
| | 3 | ab×ab | aa(19):ab(38):bb(18) | 1:2:1 | 0.04 | 0.98 |
| | 4 | aa×ab | aa(43):ab(57) | 1:1 | 1.69 | 0.19 |
| | 5 | aa×ab | aa(25):ab(25) | 1:1 | 0 | 1 |
| *Mdh-1* | 1 | ab×bb | ab(34):bb(41) | 1:1 | 0.48 | 0.49 |
| | 2 | ab×bb | ab(23):bb(27) | 1:1 | 0.18 | 0.67 |
| | 3 | ab×bb | ab(42):bb(33) | 1:1 | 0.85 | 0.36 |
| | 4 | ab×ab | aa(25):ab(46):bb(29) | 1:2:1 | 0.96 | 0.62 |
| | 5 | ab×bb | ab(27):bb(23) | 1:1 | 0.18 | 0.67 |
| | 6 | bb×ab | ab(19):bb(31) | 1:1 | 2.42 | 0.12 |
| *6Pgd-1* | 1 | bc×bc | bb(24):bc(36):cc(15) | 1:2:1 | 2.28 | 0.32 |
| | 2 | bc×cc | bc(30):cc(20) | 1:1 | 1.62 | 0.20 |
| | 3 | bc×cc | bc(36):cc(39) | 1:1 | 0.05 | 0.82 |
| | 4 | cc×bc | bc(47):cc(53) | 1:1 | 0.25 | 0.62 |
| | 6 | ac×cc | ac(20):cc(30) | 1:1 | 1.62 | 0.20 |
| *6Pgd-2* | 3 | aa×ab | aa(26):ab(49) | 1:1 | 6.45 | 0.01 |
| | 5 | aa×ab | aa(25):ab(25) | 1:1 | 0 | 1 |
| *Pgm-1* | 3 | aa×ac | aa(38):ac(37) | 1:1 | 0.00 | 1.00 |
| | 4 | ac×aa | aa(52): ac(48) | 1:1 | 0.09 | 0.76 |
| | 5 | ac×ab | aa(13):ab(10):ac(15):bc(12) | 1:1:1:1 | 1.04 | 0.79 |
| | 6 | ac×ac | aa(12):ac(31):cc(7) | 1:2:1 | 3.88 | 0.14 |

[z]See Table 1.
[y]Number of seedlings observed.

chi-square analysis to expected 1:1 or 1:2:1 segregation for six progenies from different crosses proved that the fastest- and the slowest-migrating bands within the variant region, MDH-1, are controlled by two alleles under a simple Mendelian manner of a dimeric enzyme system (Table 2).

**Phosphogluconate dehydrogenase (6PGD)**

Gels stained for 6PGD had two zones of activity (Fig. 2). One or three band(s) was observed for each zone, which was designated as 6PGD-1 and 6PGD-2. This indicates

that enzyme products of the regions are dimeric molecules. Tests of goodness-of-fit to 1:1 or 1:2:1 ratio for five and two progeny groups for 6PGD-1 and 6PGD-2, respectively, indicate that the region is controlled by a single locus under a mode of Mendelian inheritance, though the segregation for *6Pgd-2* in one progeny group (No. 3) was extremely deviated from the expected ratio 1:1 (Table 2).

**Phosphoglucomutase (PGM)**

A single region activity was observed for PGM (Fig. 2). A deeply stained band was always accompanied by an additional weakly stained band in a distance of 11 mm to anodal direction and a pair of the bands inherited together into progenies. The same type of PGM banding phenotypes was reported in eggplant (Isshiki *et al.*, 1994) and asparagus (Ozaki *et al.*, 2000), in which the additional bands were neglected for analysis. Suppose that each of a deeply stained band is a primary product by a single allele, PGM phenotypes for parents were interpreted as a single- or double-banded phenotype. The region PGM-1 was confirmed to be inherited in a simple Mendelian manner and to show the same mode of inheritance as monomeric enzymes by chi-square analysis for progenies from four controlled crosses (Table 2).

**Linkage analyses**

The results of contingency chi-square analyses of independent assortment for all pairs of jointly segregating allozyme loci are presented in Table 3. Based on significant (P=0.05) deviation from expected joint segregation ratios, evidence for linkage was detected in three of 64 pairs of loci tested, *Aat-2/6Pgd-2* for progeny group No. 5 and *Fest-2/6Pgd-1* for Nos. 2 and 3, with recombination fractions of $0.34 \pm 0.07$, $0.26 \pm 0.09$, $0.32 \pm 0.08$, respectively. The linkage test of *Aat-2/6Pgd-2* was performed in only single progeny group, No. 5, and the remaining two of four progeny groups tested for locus pair *Fest-2/6Pgd-1* did not exhibited significant evidence of linkage.

**Conclusion**

Stably-resolved 11 enzyme systems including eight loci in a simple Mendelian mode of inheritance will be available as a tool for estimating genetic diversity of *L. longiflorum*, we are caring for.

**Table 3.** Chi-square analysis of independent assortment for jointly segregating isozyme loci.[z]

| | *Aat-2* | *Dia-1* | *Fest-2* | *Gpi-2* | *Mdh-1* | *6Pgd-1* | *6Pgd-2* | *Pgm-1* |
|---|---|---|---|---|---|---|---|---|
| *Aat-2* | | N | na | N | na | na | R | N |
| *Dia-1* | 5 | | N | N | N | N | N | N |
| *Fest-2* | - | 1,2,3,4 | | N | N | N | na | N |
| *Gpi-2* | 5 | 1,2,3,4,5 | 1,2,3,4 | | N | R | N | N |
| *Mdh-1* | - | 1,2,3,4 | 1,2,3,4,5,6 | 1,2,3,4 | | N | na | N |
| *6Pgd-1* | - | 1,2,3,4 | 1,2,3,4 | 1,2,3,4 | 1,2,3,4 | | na | N |
| *6Pgd-2* | 5 | 3,5 | - | 3,5 | - | - | | N |
| *Pgm-1* | 5 | 3,5 | 4,5,6 | 3,5 | 4,5,6 | 6 | 3,5 | |

[z]N indicates that there was no evidence of linkage; R designates possible linkage pairs; na denotes pairs of loci for which no appropriately segregating progeny group was available; The number below the diagonal indicates the progeny group in Table 1 with gene pair genotypes allowing for analysis of linkage.

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