

Evolution of Early Flowering Ability in *Lilium formosanum* from its Progenitor *L. longiflorum*

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ABSTRACT

Attention to the evolutionary background of 'early flowering ability', i.e. extraordinary shorter period from seed germination to the first flowering in *Lilium formosanum*, could have great potential for reducing costs in commercial production of lilies. We clarified the phylogenetic relationship and geographic divergence of the early flowering ability and its related life history traits using seedling populations from natural populations of *L. formosanum* and its progenitor species, *L. longiflorum*. Based on the data obtained, we propose a hypothesis for evolution of the early flowering ability in *L. formosanum*.

Keywords: bulb dormancy, flowering time, geographic divergence, life history

Abbreviations: ABA, abscisic acid; AFLP, amplified fragment length polymorphism; DNA, deoxyribonucleic acid; QTL, quantitative trait loci; SSR, simple sequence repeat

CONTENTS

INTRODUCTION.....	21
CULTIVATION HISTORY.....	21
TAXONOMIC CLASSIFICATION.....	22
PHYLOGENY AND ORIGIN.....	22
INTERSPECIFIC ISOLATION MECHANISMS.....	23
VARIATION OF THE EARLY FLOWERING ABILITY.....	24
EVOLUTION OF THE EARLY FLOWERING ABILITY.....	25
THE PROJECT TEAM.....	26
ACKNOWLEDGEMENTS.....	26
REFERENCES.....	26

INTRODUCTION

Reduction in the period from seed germination to anthesis in perennial crops is very important in agriculture for getting flowers and fruits with less cultivation cost and for rotating the breeding cycle rapidly (Fortanier 1973; Wakana *et al.* 2005). Regrettably, however, this 'early flowering ability' has been found to be rather rare in perennial plants (Soost 1987; Wilkins and Anderson 2007) since photosynthetic resources captured within a perennial plant individual are predominantly allocated into storage organs until they reach the threshold size for the reproductive stage.

Lilies are geophytes that develop scaly, imbricated bulb(s) in the soil (McRae 1998). As the bulb is a storage organ, a certain time period for vegetative growth (including juvenile and adult phase) is prerequisite for flowering (reproductive phase) (Fortanier 1973). The length can be up to as long as six years in the case of *Narcissus* under cultivation, and in the case of most lilies, it can be from one to five years to grow to reproductive phase with 5 to 12 cm in circumference of the bulb.

Lilium formosanum Wallace, endemic to the mainland of Taiwan, is exceptional within geophytes as it can complete the vegetative period in less than 1 year (Wilson 1925;

McRae 1998; Hiramatsu 2002a; Anderson 2012a, 2012b). How did this exceptional species evolve under natural selection with such a truncated juvenility period?

L. longiflorum Thunb., a popular species in ornamental flower industry of the world, is genetically the closest relative of *L. formosanum* (Dubouzet and Shinoda 1999; Nishikawa *et al.* 1999). Our experimental approach always proceeded by comparing *L. formosanum* with *L. longiflorum* to reveal historical and ecophysiological factors affecting occurrence of the extremely early flowering trait.

In this review article, phylogeny, origin, interspecific reproductive isolation mechanisms and variation of the early flowering ability are compared with previously recognized cultivation history and taxonomic classification of the study species. We then put forward a hypothesis that early flowering ability of *L. formosanum* was established by evolutionary change in two major life history traits, namely, bulb dormancy and flowering season.

CULTIVATION HISTORY

Species belonging to the genus *Lilium* really distinguish themselves as diverse and attractive plant resources in world flower industries. They are bulbous plants of the Lili-

aceae. Approximately 100 species are distributed mainly in the temperate zones of the Northern Hemisphere, including Asia, Europe, and North America (McRae 1998). Fifty-nine (~60%) of them are distributed in Asia. Japan is inhabited by 15 species, of which seven are endemic (Okazaki 1996). Most species distributed in Japan are so attractive in their appearance that those were introduced abroad as early as the 18th century by European plant taxonomists and/or horticulturalists, through their 'plant hunting' activities for Japanese plant resources. Then, the introduced Japanese lilies drew a great deal of attention in the Western world (Shimizu 1971).

As for our study species, world economical use of *L. longiflorum* as a breeding resource compared favorably with that of *L. formosanum* in the early-days of horticulture. The scientific name '*longiflorum*' was given by Swedish plant taxonomist, Carl Peter Thunberg in 1794. He studied the Japanese flora in the end of the 18th century. The name represents the morphology of a long trumpet-like corolla. Living bulbs of the species were introduced to the Netherlands by Philipp Franz von Siebold, who was a German medical doctor and a primary author of 'Flora Japonica' (Siebold and Zuccarini 1835). This is considered to be the first record of exporting *L. longiflorum* overseas from Japan.

The species became quite popular in the European countries soon after introduction into the Netherlands (Shimizu 1971). In the second half of the 19th century, it was introduced to the island of Bermuda in the Atlantic Ocean and grown in great numbers until its cultivation was terminated by epidemic virus disease (Miller 1993). Subsequently, Japan supplied the majority of *L. longiflorum* bulbs for the U.S. market until World War II, with the highest annual export of approximately 30 million bulbs in 1928 (Ohkawa 2005). Okino-erabu Island, one of the islands in the Ryukyu Archipelago, where the species is indigenous, has been the center of bulb production.

Today, *L. longiflorum* enjoys great popularity in the world as an ornamental plant, particularly for religious use as indicated by its English name 'Easter lily' (Miller 1993; Jefferson-Brown and Howland 1995; Matsuo 2012). The major countries where bulbs of *L. longiflorum* are produced are the USA, the Netherlands and Israel (Miller 1993). In Japan, 95% of *L. longiflorum* production is occupied by one cultivar 'Hinomoto' (Matsukawa 1995), which seems to be established from a natural accession in the southern Ryukyu Archipelago (Sakazono *et al.* 2009).

Horticultural use of *L. formosanum* itself was rather limited, despite that its early flowering ability can be an attractive uncommon character within the genus *Lilium*, seemingly due to the early days of the commercial bulb production system that used to depend on vegetative production. Exceptionally, the selected line with wider leaves and pure white flowers was economically cultivated in Japan after the introduction of *L. formosanum* in 1924, and this line was replaced by an interspecific hybrid between *L. formosanum* and *L. longiflorum* in a little while (Shimizu 1971). The hybrid is now taxonomically named as *L. × formolongi*.

The first *L. × formolongi* was produced by Susumu Nishimura, a Japanese breeder, in 1939 (Shimizu 1971). He registered the first cultivar 'Nishimura Teppou' in 1951. Since then, intensive breeding of *L. × formolongi* has been advanced to bring forth many excellent cultivars in Japan and recently in Korea (Rhee *et al.* 2005).

The hybrids combine the outstanding characteristics from each of the parental species; i.e., the early flowering ability from *L. formosanum* and the elegant appearance from *L. longiflorum*. The former character inherited from *L. formosanum* allows cut flower production within one year from sowing the sexually propagated seeds. As a result, cultivation of *L. × formolongi* can put the virus disease problem, which has been serious in asexually propagated *L. longiflorum*, almost out of concern at least within the first growing season.

In the most popular cultivation system of *L. × formo-*

longi in Japan, seeds are sown in mid-autumn to early winter, chiefly around December (Okazaki 1996). Young seedlings are cultivated under greenhouse heated in moderate temperature to grow until they develop five to six scaly leaves. They are, then, transplanted in the soil in April to May. Cut flowers are harvested from August to October, the period when flowers of *L. longiflorum* are difficult to produce. Fundamental research on the extreme early flowering trait is expected to contribute to further development of the bulbous flower industry.

TAXONOMIC CLASSIFICATION

The most authoritative classification of whole the genus in the earlier taxonomic studies was that of Harold F. Comber, who published a revised classification of the genus *Lilium* in 1949 (Comber 1949). Comber had years of experience in growing most of *Lilium* species from seed. Besides the flower characteristics, to which major priority was given in the previous classifications (e.g., Wilson 1925), Comber (1949) used seed morphology, germination type and other vegetative features such as arrangement of leaves, form and growth habit of bulbs, to categorize seven sections within the genus. Both *L. longiflorum* and *L. formosanum* were placed in one of the two subsections within section *Leucolirion*; the section is characterized by the trumpet-shaped flower.

Close relationship between the two study species has been suggested from cytological and genetic evidence. A C-banded karyotype was very similar between *L. longiflorum* and *L. formosanum* and their karyotypes were dissimilar to those of *L. regale* and *L. sulphureum* in the other subsection of section *Leucolirion* (Smyth *et al.* 1989). Moreover, when *L. formosanum* was crossed with *L. longiflorum*, fertile hybrids are produced, which were consequently developed into major horticultural cultivars of *L. × formolongi* by Japanese breeders (Shimizu 1987; Okazaki 1996), *cf.* see **CULTIVATION HISTORY**.

Classification of *Lilium* species has been reconstructed using molecular data more recently (Dubouzet and Shinoda 1999; Nishikawa *et al.* 1999), during the progression of our research project. Both studies confirmed very close relationships between *L. longiflorum* and *L. formosanum*. Details of the phylogenetic relationship between the two species, however, remains unresolved until our research project due to the very small number of samples (presumably one for each species) and the lack of genetic markers for estimating variation within the species.

PHYLOGENY AND ORIGIN

Conclusive phylogenetic relationships between *L. formosanum* and *L. longiflorum* were first demonstrated from the comparative data of population genetic structure in the two species (Hiramatsu *et al.* 2001a). The degrees of allozyme variability and divergence for *L. longiflorum* were very high as insular endemic species, indicating a relatively longtime persistence of the present widespread distribution across many Ryukyu and Taiwan islands in this phenotypically little-diverged species. By contrast, *L. formosanum* exhibited lower variability and divergence than did *L. longiflorum*, and was genetically close to the southwestern peripheral populations of *L. longiflorum* with a genetic distance (*GD*) of 0.022 as its lowest value. The results demonstrate that *L. formosanum* is highly likely a recent derivative of *L. longiflorum* in a southwestern area of the archipelago. Combined with other biological and insular geohistorical information, it seems that *L. longiflorum* was established around the end of the Pliocene when the current distribution area was still a continuous part of the ancient Asian continent, and *L. formosanum* was derived from southwestern populations of *L. longiflorum* around the late Pleistocene when the mainland of Taiwan was completely separated from the adjacent islands and the main continent.

INTERSPECIFIC ISOLATION MECHANISMS

Since speciation results from the development of reproductive isolation mechanisms which prevent gene flow between populations (Futuyma 1997), there is a possibility that reproductive isolation mechanisms preventing gene flow are developed between the currently recognized species. Reproductive isolation mechanisms in plants are classified into temporal, ecological, and floral isolation as pre-mating mechanisms, and pollen-pistil incompatibility, inviability, sterility, and hybrid breakdown or incongruity as post-mating ones (see Judd *et al.* 1999 as a review). Considering that reproductive isolation mechanisms are generally a by-product of adaptive differentiation in allopatric populations under natural selection (Futuyma 1997), they may be developed in relation to the development of species-specific life history traits.

Though interspecific fertile hybrids can be established simply by aid of artificial pollination between *L. formosanum* and *L. longiflorum*, the case has never been generalized from the wide crosses among various lineages covering diversity within each species. Information on the other isolation mechanisms between the two species, moreover, has never been assembled. Those situations brought us to the investigation of isolating status between the study species.

The two species are undoubtedly insular endemics. ‘Ryukyu’, where *L. longiflorum* is natively grown, is the medieval dynasty name of the region with hundreds of subtropical islands located in southwest Japan. The region is now called governmentally ‘Okinawa’, and geographically ‘the Ryukyu Archipelago (or the Nansei Islands)’. The southwest end of the archipelago borders on the mainland of Taiwan, where *L. formosanum* is distributed.

The first distinguished description on the geographic distribution, habitat and morphology of the study species was published in 1925 by Ernest H. Wilson (Wilson 1925) entitled as a monograph ‘The Lilies of Eastern Asia’. Unlike the preceding taxonomic works, Wilson observed many populations growing in the wild during his twenty years’ field works from 1899 to 1918 in East Asia, and recognized 40 species indigenous in Eastern Asia. A part of his classification within the genus is not phylogenetically reliable today (Nishikawa *et al.* 1999). His description on species’ wild status is, however, quite trustworthy and informative, as has been quoted frequently by many researchers.

The two study species seems to be distinct by their morphologies and their geographic distribution ranges according to his descriptions as follows:

Lilium formosanum – “This graceful Lily is characterized by its long, narrow leaves, its flowers with all six segments keeled with vinous purple, by its papillose pubescent nectariferous-furrow and its obscurely papillose filaments. These characteristics, and also the bulb and usually scabrid stem, readily distinguish it from *L. longiflorum* ... This Formosan Lily is widespread on the island and is the only trumpet-flowered species known to grow there. It is, perhaps, most common in the north where in the north-east corner of Giran prefecture it is abundant, especially near the sea. In northern Formosa there is much volcanic rock and on this and on sandstone it is equally plentiful. ... On the subordinate ranges west of Morrison up to 10,000 feet altitude this Lily is by no means uncommon in grassy places. It is never found in forests and always prefers open grassy places.”

Lilium longiflorum – “It may be well to emphasize the fact that it (*L. longiflorum*) is an endemic Liukiu (sic Ryukyu) plant and that it is known in a wild state elsewhere. ... I saw this Lily flowering wild in pockets in the coral rock by the sea. It appears to be a maritime species and, unlike most Lilies, a limestone plant.”

Above-mentioned Wilson’s descriptions demonstrate that the two species are morphologically distinct and geographically isolated between the mainland of Taiwan and the Ryukyu Archipelago. However, several botanists repor-

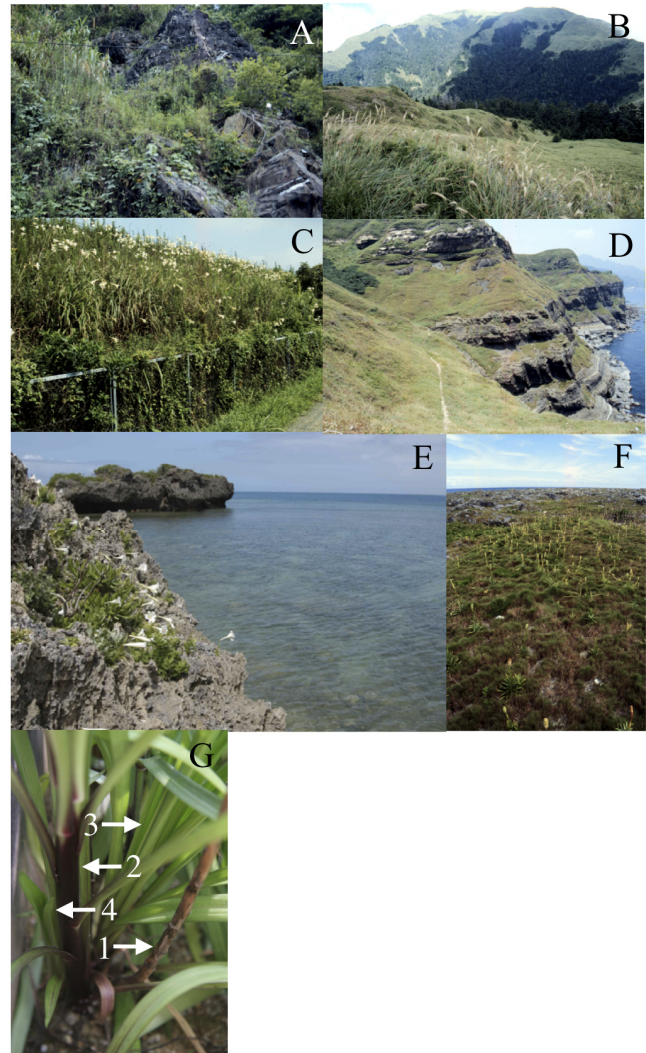


Fig. 1 Typical natural habitats and growing appearance of *Lilium formosanum* and *Lilium longiflorum*. (A) Steep, fragile rocky habitat of *L. formosanum* in Paolai, Taiwan. (B) Grassy habitat of *L. formosanum* developing on highland at an altitude of 3,000m in Hohuanshan, Taiwan. (C) Naturalized flowering population of *L. formosanum* in an artificially often-disturbed grassy vegetation developing along the expressway margin in Fukuoka, Japan. (D) Landscape of a grassy habitat of *L. longiflorum* developing along the coastal cliff in Pitouchiao, Taiwan. (E) Seaside flowering *L. longiflorum* population on limestone in Ishigaki-jima Island of the Ryukyu Archipelago, Japan. (F) Fruiting *L. longiflorum* population on the well-lit grassy field dominated by *Zoysia pacifica* in Kikai-jima Island of the Ryukyu Archipelago, Japan. (G) Multiple-shoot formation in a potted seedling plant of the lowland ecotype of *Lilium formosanum* experimentally grown in a heated greenhouse approximately between 10 and 20°C during the winter season. White arrows indicate shoots differentiated from August of the first year to March of the second year with numbers indicating the order of shoot formation.

ted *L. longiflorum* populations on the mainland of Taiwan (Shii 1983; Wen and Hsiao 1999). Wen and Hsiao (1999) treated those plant materials as *L. longiflorum* var. *scabrum* according to classification by a Japanese botanical taxonomist, G. Masamune, without showing any morphological basis. The measurement of the length/width ratio of stem leaves developed under the same cultivating condition revealed that plants from the northeastern peninsula, Pitouchiao in the mainland of Taiwan are morphologically very similar to *L. longiflorum* plants from the Ryukyu Archipelago, while they are distinctive to *L. formosanum* plants from the inland in the mainland of Taiwan (Hiramatsu *et al.* 2001c). The fact is the crucial evidence that *L. longiflorum* is also distributed on the mainland of Taiwan where the *L. formosanum* is distributed, and reproductive isolation under natural condition is never established by island separation,

Table 1 Capsule set and seed production per capsule in artificial reciprocal pollination among four local populations of *Lilium longiflorum* and *L. formosanum*.

Female parent	Successful capsule set (%) / Amount of seed production per capsule (g) using male parent indicated below			
	L-KM	L-PI	F-WU	F-PA
L-KM	100 (n=20) / 0.96	80 (n=20) / 0.71	0 (n=20) / 0.00	0 (n=20) / 0.00
L-PI	60 (n=20) / 0.15	97 (n=30) / 1.33	0 (n=20) / 0.00	0 (n=20) / 0.00
F-WU	72 (n=18) / 0.22	96 (n=22) / 1.40	100 (n=18) / 1.27	95 (n=19) / 2.26
F-PA	44 (n=18) / 0.22	95 (n=20) / 1.02	100 (n=20) / 1.57	100 (n=16) / 1.97

The population L-KM and L-PI are *L. longiflorum* populations of Kume-jima Island in the Ryukyu Archipelago, Japan and Pitouchio in the mainland of Taiwan, respectively. The population F-WU and F-PA are *L. formosanum* populations of Wulai and Paolai in the mainland of Taiwan, respectively. Outcrosses were performed using different individuals within each population.

i.e., geographic isolation.

The evidence that island formation is not functional for the isolation mechanism gives us a question which isolation mechanism contributed critically to differentiation between *L. formosanum* and *L. longiflorum*. We were able to answer this question from the ecological difference between the two species as follows.

Populations of the species differed in preference for the environments of surrounding habitats (Hiramatsu *et al.* 2001c). Those of *L. formosanum* were found in the inland areas with frequent natural and artificial disturbance (Fig. 1A-C), whereas those of *L. longiflorum* in the coastal areas with nutritional, saline and pH stresses (Fig. 1D-F). Thus, the two species were never sympatric in the mainland of Taiwan. The habitat preference may be attributed to differentiation in adaptive strategies between the species. Furthermore, flowering period was also largely differentiated between them. Most populations of *L. formosanum* flowered between July and August although one population located in the southeastern part of Taiwan flowered from April to May, the period which partly overlaps with that of Taiwanese *L. longiflorum* (Hiramatsu *et al.* 2001c). The results indicate that the combination of relatively distinct differences in habitat preference and flowering time is potentially additively functioning as pre-mating reproductive barriers under the natural environments, though each of the reproductive barriers is not complete one. These ecological differences may have strongly restricted the gene flow between the species, and, thereby, promoted speciation.

By contrast, artificial hybridization was successful in setting fruits and mature seeds only when *L. formosanum* was pollinated with *L. longiflorum*, although it was not successful in the reverse interspecific cross (Table 1). Seed germination rates were comparable with those in crosses within each parental species. There is unilateral interspecific cross compatibility and post-mating reproductive isolation does not completely work between the two species (Hiramatsu *et al.* 2001c). This has consequently allowed production of interspecific hybrid cultivars, *L. × formolongi* (Shimizu 1971), as explained in CULTIVATION HISTORY.

VARIATION OF THE EARLY FLOWERING ABILITY

Researches on flowering physiology have been devoted to the *L. longiflorum* for the purpose of development in its forcing cultivation. The species requires a cool-moist period for inducing and hastening flowering (see Miller 1993 as a review). Dipping thermal treatment approximately at 45°C for 1 h has been also proven to be available for forcing (Imanishi 2002). It was also proven that a cold requirement for flowering can be partly replaced by the long day treatment below the critical temperature of 21°C (Weiler and Langhans 1968). They were, however, always concerned with the bulbs of a few leading cultivars (e.g., 'Ace', 'Nellie White', 'Hinomoto') during the growth period over the threshold size of flowering and neglected within-species variation. In contrast to flowering physiology of *L. longiflorum*, moreover, that of *L. formosanum* has been little studied; it was only reported by Shii (1983) who just described without data that prechilled plants of *L. formosanum* could be forced to flower within 12 weeks, and long-day treat-

ment enhanced flower initiation of the pre-chilled plants. The case was reconfirmed by Anderson (2012b). In short, most of the previous researches on *L. formosanum* and *L. longiflorum* examined a limited number of genotypes to describe the intraspecific variation.

A species often connotes morphological, ecophysiological and genetic variation among populations resulting either from adaptive changes or from changes by chance, namely 'genetic drift' (Futuyma 1997). Hence, to document the patterns of the geographic variation in various traits for a species and its related species is an important step toward understanding speciation.

Variation in ecophysiological traits of natural plant populations often shows more or less a geographically gradient pattern along latitude and altitude as a result of a combination between natural selection under different climates and restricted gene flow among populations, as the plants are relatively limited in their mobile ability (Judd *et al.* 1999). Large number of studies comparing various ecotypes from differing latitudes and altitudes in the same experimental field (common garden experiments) have proved that the traits are genetically determined (Chapin and Chapin 1981; Reinartz 1984; Lacy 1986; Li *et al.* 1998; Weber and Schmid 1998; Olsson and Agren 2002). The traits included number of days to anthesis, plant biomass, relative growth rate (RGR = (dW/dt)/W, where W = individual weight), phenological growth pattern, and they are often significantly correlated with each other.

As already mentioned, the study species are insular endemics. *Lilium formosanum* is indigenous within the mainland of Taiwan, while *L. longiflorum* occurs along the arc-arrayed archipelago lying over an approximately 1,300 km distance from southwest to northeast and may be exposed to a latitudinal climatic gradient significant for generating intraspecific adaptive changes. The archipelago, furthermore, consists of 'continental' islands that was once an eastern part of the Eurasian Continent and often encountered geographic fluctuation during the archipelago formation (Kizaki and Oshiro 1977; Kimura 1996). Under the climatic gradients and geographic history, the two species were expected to show remarkable variation in many traits among populations within each species. We posited that plant materials extensively collected through the entire distribution of the study species should be essential for understanding the evolution of two species and in particular the origin of extremely early flowering ability in *L. formosanum*.

In order to answer the fundamental question whether the character of the extreme early flowering ability is really specific to *L. formosanum*, intra- and inter-specific variation in the frequency of flowering individuals were investigated for the first-year seedlings grown from different seed sources of the study species under the same cultivating conditions in an unheated greenhouse (Hiramatsu *et al.* 2002). Seasonal change and allocation pattern of annual net biomass production were also compared among these plants to clarify whether 1) the trait is corresponded to the early flowering ability, and 2) the observed variation in the traits for each species is interpreted in terms of differentiation in adaptive strategies along altitudinal environmental gradients among local populations.

Flowering rate and net annual biomass for three low-

Table 2 Comparison of morphological and ecophysiological characteristics diverged between the lowland ecotype of *Lilium formosanum* and the Taiwan, Ryukyu ecotype of *L. longiflorum*.

Characteristics	Lowland ecotype of <i>L. formosanum</i>	Taiwan ecotype of <i>L. longiflorum</i>	Ryukyu ecotype of <i>L. longiflorum</i>
Phylogenetic relationship	Derivative species	Progenitor species	
Leaf length / width	15~53 (narrow)	6~15 (wide)	4~18 (wide)
Ability of first-year flowering under cultivating condition	88~100%	26%	0~14%
Annual net biomass production under cultivating condition	High	Low	Very low to none
Dormancy	25~29 gDM	8gDM	2~6 gDM
Flowering time in native distribution	High	Lower medium	Low to very low
	Little or weak	Little or weak	Moderate to strong
	July to August	March to April	March to May

land populations of *L. formosanum* exceeded those for four *L. longiflorum* populations at least by 3.3 times (89~100% vs. 0~26% for flowering rate, 24.9~28.9 g vs. 2.1~7.6 g dry weight for net annual biomass) (Hiramatsu *et al.* 2002). Within *L. longiflorum*, it was noticeable that the values tended to decrease from the southernmost population to the northernmost population with latitudinal gradient pattern. The lowest degrees of flowering rate and net production in the northernmost *L. longiflorum* population were associated with high frequency of individuals that obtained little net biomass production during spring to summer, indicating a deep bulb dormancy status. It seems that the variations of the growth habits within *L. longiflorum* reflect region-specific adaptive strategy different among populations with relatively wide latitude distribution in the archipelago. Extreme high annual productivity and ability of early sexual reproduction in *L. formosanum* is likely a unique trait developed under lowland subtropical climates in relation to the loss of bulb dormancy of southernmost population of *L. longiflorum*. The trait undoubtedly allows naturalization of *L. formosanum* from Taiwan to Japan (Hiramatsu *et al.* 2001a), South Africa (Walter 1983) and Australia (Warner *et al.* 2006).

EVOLUTION OF THE EARLY FLOWERING ABILITY

On the basis of the progenitor-derivative relationship of the two species, it is considered that the traits specific to *L. formosanum* evolved from *L. longiflorum* more or less in association with the early flowering ability. The characteristics diverged between the lowland ecotypes of *L. formosanum* and the Taiwanese ecotypes of *L. longiflorum* in **Table 2** allow us to hold the hypothesis that there are at least two possible evolutionary traits controlling the extreme precocious flowering ability specific to the lowland ecotypes of *L. formosanum*.

First, the degree of bulb (not seed) dormancy is likely to a genetic trait varying among local ecotypes of the two species in correlation with the precocious flowering traits as explained in **VARIATION OF THE EARLY FLOWERING ABILITY**. Dormancy has been considered as a strategy to survive unfavorable conditions such as cold and dry climates in higher latitudinal regions, accompanied by bulb formation and endogenous ABA increase in case of bulbous plants (Okubo 2000). Difference in the present lowest winter temperature between the northernmost and southernmost region within the combined geographic distribution of *L. longiflorum* and *L. formosanum* is approximately 12°C. Such a remarkable climatic gradient along latitude seems to allow quantitative divergence in the degree of bulb dormancy among local populations. Due to deeper bulb dormancy, the northern ecotypes of *L. longiflorum* may be imposed to grow more slowly or stop growing below threshold plant size for flowering, and eventually may develop less or no flower stalk during the first year growth. By contrast, the sequential multiple flower stalk formation in lowland ecotypes of *L. formosanum* (**Fig. 1G**; Hiramatsu *et al.* 2006) can be interpreted as non- or very weak dormant growth of bulbous plants similar to the sequential scaly leaf formation as observed in *Amaryllis* (*Hippeastrum*) (Rees 1985) and Dutch iris (*Iris × hollandica*) (Okubo and Uemoto 1981)

under favorable cultivating conditions. The growth pattern of *L. formosanum* is a very special case because most *Liliaceae* species usually show bolting once a year at their adult stage under natural conditions. It can be, thus, concluded that no or very weak bulb dormancy and thermal stimulation, respectively, are the strategy and environmental condition essential for developing the ability of early onset of flowering.

Despite the general recognition that dormancy is an important trait for adapting to unfavorable conditions, surprisingly, the fact that genetic variation in dormancy status can be a trigger of speciation is not well-documented. The two species, which exhibit intra- and interspecific variation in growth response related to dormancy along latitudinal and altitudinal gradient, can provide a good opportunity to understand how dormancy influenced species diversification in plants.

Moreover, the considerable difference in early flowering rate of first-year seedlings between the lowland ecotypes of *L. formosanum* (89~100%) and the Taiwanese ecotype of *L. longiflorum* (26%) (**Table 2**; Hiramatsu *et al.* 2002) should be explained by another factor, because the year-round thermal conditions are not so different between the places where these ecotypes are growing. This brings us attention to the differentiation in flowering season between the species as another factor regulating the ability of extremely early sexual reproduction in *L. formosanum*.

Flowering period for the lowland ecotypes of *L. formosanum* with the highest ability of early flowering and for the Taiwanese ecotypes of the *L. longiflorum* with lower ability of early flowering, respectively, is July to August (summer) and March to April (spring) under natural conditions of their habitats (**Table 2**; Hiramatsu *et al.* 2001c). The similar periodical difference in flowering season was expressed constantly from the second year seedling growth in our field experiment on 33°N, where the seedlings of the lowland *L. formosanum* ecotypes flowered in July to August, whereas those of the Taiwanese *L. longiflorum* ecotype flowered in May to June (**Fig. 2**). This fact indicates that flowering season is genetically differentiated between the ecotypes and eventually, vegetative growth period before the flowering season in the slightly early flowering Taiwanese ecotype of *L. longiflorum* is considerably shorter than that in the extremely early flowering lowland ecotype of *L. formosanum*. It can be, thus, hypothesized that the former ecotypes are not able to grow over the threshold plant size for flowering until their proper flowering season. Assuming that some weak dormant individuals in the Taiwanese ecotype of *L. longiflorum* reach the threshold plant size thereafter, the fact that some flowering individuals of these ecotypes during the first year bloomed from July to September (**Fig. 2**) is not surprising. This untimely bolting and flowering is seemingly the same case as summer sprouting observed very occasionally in wild and cultivated *L. longiflorum* (Van Tuyl 1985). The hypothesis described here could be experimentally verified by observing segregation in the extreme early flowering trait and flowering season among the progenies between the summer-flowering lowland ecotype of *L. formosanum* and the spring-flowering Taiwanese ecotype of *L. longiflorum* under the environment providing non-dormant or very weak dormant growth.

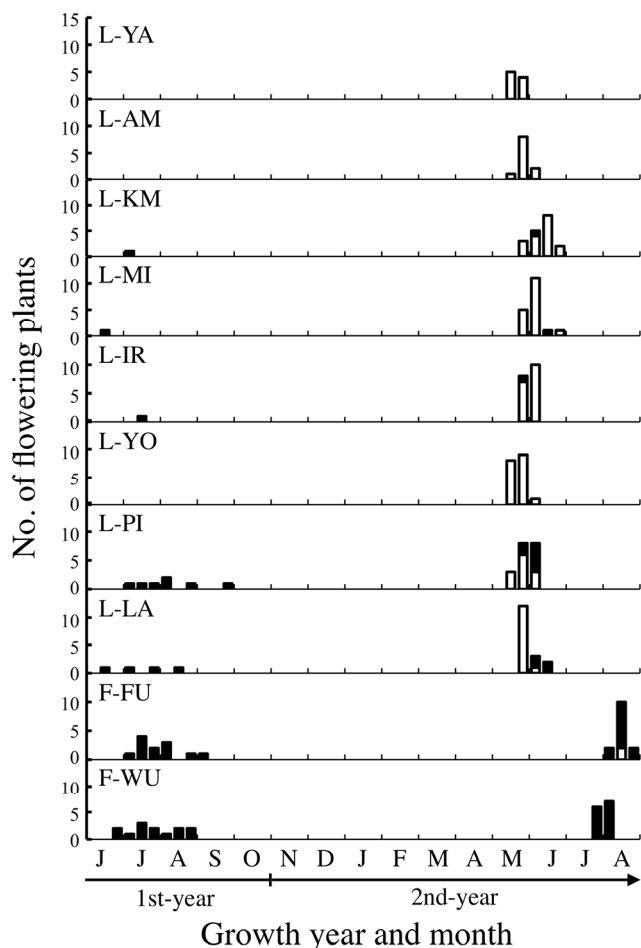


Fig. 2 Frequency histograms of flowering plants experimentally established from seeds of natural *Lilium longiflorum* and *L. formosanum* populations from November, 2005 under an unheated greenhouse condition in Fukuoka, Japan (33°N). Closed (■) and open (□) histograms represent individuals with flowering and without flowering in the first year, respectively. Seeds were originated from natural *L. longiflorum* populations in Yaku-shima (L-YA), Amami-Oshima (L-AM), Kume-jima (L-KM) island of northern Ryukyu, Miyako-jima (L-MI), Iriomote-jima (L-IR), Yonaguni-jima (L-YO) island of southern Ryukyu, Japan, Pitou-chiao (L-PI), Lan-Yu Island (L-LA), Taiwan, a naturalized *L. formosanum* population in Fukuoka, Japan (F-FU), and a natural *L. formosanum* population in Wulai (F-WU), Taiwan.

Genetic analysis for the extreme early flowering ability will be useful not only for improving breeding efficiency in *Lilium* but also for understanding its evolutionary background. Shortening the growth cycle up to the first reproductive phase and frequently alternating generations in *L. formosanum* with the extreme early flowering trait can be interpreted as the evolutionary shift in life history similar to that from perennials to annuals or annualized perennials for adaptation to disturbed habitats often observed in the cosmopolitan plant species with wide geographic distribution (Ehrendorfer 1970; Wilkins and Anderson 2007). Genetic basis of such evolutionary shifts has been little known until today; i.e., Hu *et al.* (2003) have found that two major QTLs regulating the shift from perennial to annual are closely corresponded between genus *Oryza* and *Sorghum* using F₂ populations from a cross between the species with the representative life histories for each genus. As for *L. formosanum* and *L. longiflorum*, genetic variation in the early flowering ability is distinct from very high to low (Table 2; Hiramatsu *et al.* 2002) and fortunately, interspecific F₁ is fertile to produce F₂ progeny. It is, thus, expected that a population established from the cross between distinct genotypes with contrasting eco-physiological traits from each species provides us with important information on inheritance mode of traits regarding the extreme early

flowering and presence of QTLs significantly contributable to the traits. For this purpose, combinational use of allozyme (Hiramatsu *et al.* 2001b), AFLP (Saruwatari *et al.* 2007) and SSR (Sakazono *et al.* 2010) analyses of nuclear DNA may be the effective genetic markers detecting such QTLs, since genome size of *Lilium* is largest among the angiosperms (2C = more than 67 pg) (Siljak-Yakovlev *et al.* 2003; Soltis *et al.* 2003).

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