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New Insight into *Lilium brownii* var. *colchesteri*

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ABSTRACT

Lilium brownii var. *colchesteri* has been widely cultivated for long time by its perfect flower shape with its colour arrangement and fragrance. However, it did not receive enough attention in recent lily research programs, and information on the history and culture is lacking. An overall research project on this species including breeding, flowering control, propagation, virus-free bulb production, flower pigment and scent along with the surveys of old literature and arts to clarify the introduction history of the species into Europe and Japan, has been conducted. The major results are: 1) *L. brownii* var. *colchesterii* was probably introduced in about 1600 from Korea to Fukuoka, 2) there was confusion of the species nomenclature of this species in Europe at the time of introduction, 3) all individuals of our present collection in Japan and Korea are clones, 4) F_1 hybrids of *L. formosanum* × *L. brownii* var. *colchesteri* obtained through cut-style pollination and ovary slice culture methods showed the early flowering traits of *L. formosanum*, but the flower shape and colour were similar to those of the pollen parent, 5) F_2 seedlings were obtained from self-pollination of F_1 through ovary-slice culture, 6) control of flowering was successful by temperature treatments, 7) an *in vitro* propagation procedure was established, 8) virus-free bulblets were obtained by a combination of meristem tip culture and chemotherapy, and 9) pigments that characterize the flower colour were identified.

Keywords: *breeding*, flowering control, *L. formosanum*, pigment, propagation, scent, virus-free
Abbreviations: AFLP, amplified fragment length polymorphism; **BA**, benzyladenine; **CMV**, *Cucumber mosaic virus*; **CT**, cumulative temperature; **GC-MS**; gas chromatography-mass spectrometry; **IAA**, indole-3-acetic acid; **MS**, Murashige and Skoog; **NAA**, naphthaleneacetic acid; **LMoV**, *Lily mottle virus*; **LSV**, *Lily symptomless virus*; **RHS**, Royal Horticultural Society; **RT-PCR**, reverse transcription polymerase chain reaction

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INTRODUCTION

"…. the loveliest of all lilies – relying for effect not upon brilliant colour but on a quiet scheme of ivory white and purplish maroon, punctuated by heavy anthers of rich russet. It is the perfect form of the flower that makes it priceless…" (Sir Herbert Maxwell, cited by Coats 1970). It is *Lilium brownii* F. E. Brown ex Miellez (or *L. brownii* F. E.

Received: 30 November, 2010. Accepted: 20 December, 2012.

Invited Review

Fig. 1 A flower of *L. brownii* var. *colchesteri* on 9 June 2005 in Fukuoka. Photo by H. Okubo in Journal of the Faculty of Agriculture, Kyushu University 61, 145-163 (2006) (with kind permission).

Fig. 2 Discoloring of *L. brownii* var. *colchesteri* flowers, at opening (left) and on the next day (right) at 20°C. Photo by H. Okubo.

Brown ex Miellez var. *colchesteri* Wilson – it is unclear to which Sir Maxwell referred). Besides the abundance of gorgeously and beautifully flowering *Lilium* species endemic or native to Japan such as *L. auratum*, *L. japonicum*, *L. speciosum*, and *L. longiflorum*, the alien species of *L. brownii* var. *colchesteri* (**Fig. 1**), native to China, has been also praised for ornamental purposes for many years after its introduction into Japan in the year of around 1600 (Okubo, 2006, 2008). The Chinese species is still an important crop cultivated as a table delicacy or for pharmaceutical purposes, but not for as ornamental use in its mother country.

Lilium brownii var. *colchesteri* has unique characters that are not found in other *Lilium* species. Its colour shifting traits at flowering (**Fig. 2**), its noble and tender fragrance and its perfect loveliness have tempted us. This is the first reason for starting to study this lily. The species is susceptible to viruses and probably or partly by which it is disappearing in Japan now. This is the second reason. There is another special motive of giving spotlight on this species. "Hakata" of the standard Japanese name of the species "Hakata Yuri" (Hakata lily) is the old city name of Fukuoka City.

The research project consists of various aspects; breeding, physiology, propagation, pathology, and identification of pigments and scent compounds along with the survey of old literature and arts to clarify the introduction history of the species into Europe and Japan, which started in 2003.

CLASSIFICATION AND DISTRIBUTION

Botanical classification

Lilium brownii var. *colchesteri* and *L. brownii* are classified into the *Leucolirion* section according to the classification of *Lilium* by Wilson in four sections based on flower shapes (Wilson 1925) or into the section *Archelirion* according to Comber's classification in seven sections based on many other characters such as seed germination manner, leaf arrangement, bulb scale and seed characters, bulb shape and habit as well as flower shapes (Comber 1949). Recent molecular studies based on nucleotide se-quence variations in the internal transcribed spacer regions of 18S-25S nuclear ribosomal DNA reveled that *L. brownii* is closely related to *L. formosanum* Wallace and *L. longiflorum* Thunb. of *Leucolirion* (Nishikawa *et al*. 2001).

The commonly accepted synonym of *L. brownii* var. *colchesteri* is *L. brownii* var. *viridulum* Baker. "Hakata lily" corresponds only to *L. brownii* var. *colchesteri*, not to *L. brownii*. Another variety, var. *australe*, from southern China, is known as Hong Kong lily (McRae 1998) for which information is scarce. Probably, "Brown's lily" is the most common English name rather than "Hong Kong lily", and it seems to cover both *L. brownii* and its variety *colchesteri* now, although it might have been for *L. brownii* only at first in Europe in spite that the introduction of *L. brownii* var. *colchesteri* was earlier than that of *L. brownii* (see **HISTORY**).

Lilium brownii var. *colchesteri* and *L. brownii*

Typical characters that distinguish *L. brownii* var. *clochesteri* from *L. brownii* are colour and scent of the flowers (Wilson 1925). Flowers inside the funnel are pale yellow on opening changing to white or nearly so, and those of reflexed apices of segments are cream-colour rapidly changing to pure white in *L. brownii* var. *colchesteri*, while *L. brownii* flowers are pure white. The flowers of *L. brownii* var. *colchesteri* are very fragrant, whereas those of *L. brownii* are odorless or slightly fragrant. The words "…, and its only fault is its almost total lack of scent" by Coats (1970) indicate that it was *L. brownii*, whereas those of "ivory white (not pure white)" by Maxwell cited by Coats (1970) suggest var. *colchesteri*.

Leaf shape is another indicator for the discrimination. Leaves of *L. brownii* are lanceolate or acuminate, and all of them are nearly of equal length, while those of *L. brownii* var. *colchesteri* are usually broadest above the middle, although Wilson (1925) recognized that they are narrow-lanceolate on several specimen. They are largest about middle-height of the stem, and much reduced in size below the flowers in var. *colchesteri*.

Wallace (1932) considered *L. brownii* to be a hybrid between *L. philippinense* var. *formosanum* (= *L. formosanum*) and *L. brownii* var. *colchesteri.* His opinion was, however, not supported by karyotype analysis (Kumazawa and Kimura 1949) even though both are 2n=24.

Distribution areas

Lilium brownii var. *colchesteri* is distributed in the Chinese provinces Hebei, Shanxi, Henan, Shaanxi, Hubei, Hunan, Jiangxi, Anhui, and Zhejiang, while *L. brownii* is spread in Guangdong, Guanxi, Hunan, Hubei, Jiangxi, Anhui, Fujian, Zhejiang, Sichuan, Yunnan, Guizhou, Gansu, and Henan (Liang 1980). The former is the more common wild form (Wilson 1925) and is spread rather north than the latter. The two overlap in six provinces, but there is no information available whether there is habitat segregation and sexual isolation or they co-exist and have chances of natural hybridization.

There are records that *L. brownii* also grows wild in Kachin, Myanmar (Kress *et al*. 2003) and in Ha Giang, Vietnam (Thao *et al*. 2009). According to Jefferson-Brown and Howland (1995), however, the type of *L. brownii* with purplish buds and unscented flowers has not been found growing in the wild. Reexamination of the species and varieties found at different places seems necessary.

Major production area of *Lilium brownii* var. *colchesteri* for edible bulbs in China is Hunan Province (pers. obs. and interviews by Okubo in Shaoyan, Hunan in June-July 2004). Presently, *L. brownii* var. *colchesteri* is grown in small

numbers in Iki Island (Nagasaki Prefecture), Fukuoka, Gifu and Kyoto Prefectures in Japan, among which the plants in Fukuoka are probably from asexually propagated bulbs in Gifu Prefecture. In contrast to the disappearing situation in Japan it is widely cultivated in Korea, not commercially but by personal interest mostly in private home gardens for ornamental purposes. No commercial markets for an edible or ornamental plant have been established in Korea.

We have no information whether either one is popularly cultivated in other areas of the world at present. *Lilium brownii* sent by Wilson to England and America died after a year or two due to its weakness that causes it to be a difficult lily (Wilson 1925), whereas it is said that it is not a difficult lily to cultivate in England (Anonymous 1912). We have collected cultivated *L. brownii* var. *colchesteri* bulbs in several places (*ca*. 38°N) near the demilitarized zone of Korea, where the average temperature in January is around -5°C. According to the information from our colleagues, members of the project team, in China (X. M. Geng and F. X. Luo, pers. comm.), *L. brownii* var. *colchesteri* was found growing in Dangdong (*ca*. 40°N), Liaoning Province, just north of the border between China and North Korea. The Province is not included in the list of distribution areas of var. *colchesteri* by Liang (1980). The river dividing the countries freezes in winter. The variety *colchesteri* seems to be hardy to cold.

Genetic variation within the variety *colchesteri*

Genetic variation within the species of *L. brownii* var. *colchesteri* among the populations in Japan and Korea has been investigated with 302 amplified fragment length polymorphism (AFLP) markers (Saruwatari *et al*. 2007). Nineteen individuals in four prefectures in Japan, 19 in two provinces in Korea (about 200 km distance between them) and one in China (the source is unknown) were analyzed. One *L. longiflorum* and one *L. formosanum*, both in Taiwan, were as out-group species. In this study, ten plants of clonally propagated *L. longiflorum* 'Hinomoto' were used to establish a threshold value of the genetic similarity for identifying clones in *L. brownii* var. *colchesteri*. Threshold values for identification of clones have been proposed in AFLP fingerprinting as 0.98 in poplar (*Populus nigra*) (Arens *et al*. 1998), from 0.96 to 1.00 in *Populus nigra* subsp. *butulifolia* (Winfield *et al*. 1998), 0.983 in *Salix exigua* (Douhovnikoff and Dodd 2003), or 0.974 in *Sequoia sempervirens* (Douhovnikoff *et al*. 2004), all being between 0.96 and 0.98. Considering these with the results with *L. longiflorum*, the threshold value of around 0.97 was adopted in this study.

Average values of the similarity within Japanese and within Korean accessions were 0.9779 and 0.9813, respectively, and the value between Japanese and Korean accessions was 0.9788, all being higher than the threshold value. When one Chinese accession was included in the analysis, the values decreased to 0.9477, lower than 0.97. Individuals of *L. brownii* var. *colchesteri* in Japan and Korea investigated in this study are, therefore, considered to be clones, and the results support the ideas that the introduction of the species into Japan was through Korea. Further surveys with more Chinese accessions are desired.

HISTORY

In China

Lilium brownii var. *colchesteri* has been known since early times in China. A set of two Chinese letters pronounced "Pai-Ho" corresponds to *L. brownii* var. *colchesteri*. Probably it first appeared in the book "Shen-Nong-Ben-Cao-Jing" written by Tao between 453 and 536 AD (Tao 453-536), in which this lily is considered as a natural medicine. Wu (1848) considers that a plant described by a different letter in an ancient book "Nan-Du-Fu" written in 78-140 AD is *L. brownii* var. *colchesteri*. The illustration of the lily (var. *colchesteri*) probably first appeared in the book "Jiu-Huang-Ben-Cao" by Zhu (1406). The book is the description with figures of >400 wild and cultivated plant species that can be consumed for diet in case of starvation.

Lilium brownii is expressed by adding a letter pronounced "Yeh" as "Yeh-Pai-Ho" (Liang 1980).

In Europe

It seems there was some confusion in the introduction history of the species into Europe. According to Elwes (1880), *L. brownii* was introduced in 1804 by Captain Kirkpatrick of the East-India Company's service, and has been sparingly cultivated in England ever since. The Latin name *brownii* was first given to the plant in 1841 by Miellez after a nurseryman F. E. Brown of Slough, England and brought to notice about 1838.

Later, Wilson (1925) described separately the introduction history of *L. brownii* and its variety *colchesteri* in Europe. *Lilium brownii* is said to have been introduced into England about 1835 probably by the British India Company from Canton, China, and to have first flowered with F. E. Brown at Slough, near Windsor, in 1837. It was introduced into Belgium and Holland in 1838, and in 1841, made its public debut at a Horticultural Exposition held in Lille, France.

Before the introduction of *L. brownii*, *L. brownii* var. *colchesteri* is authentically known to have reached England from the Faté Gardens, Canton, China, being sent by William Kerr to Kew in 1804. This was misnamed *L. japonicum* by Aiton in 1811. The plant, for example, flowered in Liverpool Botanic Garden and figured by Bury (1831) under the name of *L. japonicum* is not true *L. japonicum* (**Fig. 3**). Okubo had a chance to see this figure at the exhibition of "Five Hundred Year Old Treasures from the World of Botanical Art" which was held by Royal Horticultural Society (RHS) to commemorate its bicentenary in 2005. He is convinced that it is *L. brownii* var. *colchesteri* by its leaf and flower shapes and flower colors in agreement with Wilson's description (Wilson 1925). But, the anthers in the figure are reddish and round, while those of our *L. brownii* var. *colchesteri* collection are red brown and oblong. In *L. brownii* var. *colchesteri*, the difference in colour of the pollen seems to be correlated with that of the flower (Wilson 1925). The variation in the size of the anthers is extraordinary and their shape has no significance (Wilson 1925). Also, *L. japonicum* figured and described in 1813's Curtis's Botanical Magazine (Anonymous 1813) is not true *L. japonicum*, but it looks *L. brownii* var. *colchestri*. It is also explained that it was the import from China by the Directors of East-India Company in 1804. The true *L. japonicum* is endemic to Japan and first appeared in Europe in "Flora Japonica" published in 1784 by C. P. Thunberg. The live plant was first introduced by Kramer to England in about 1871 and first flowered in 1873. It is commonly called Sasa lily, Japanese lily, Japanese pink lily or Kramer's lily in English.

Planchon gave the name *L. odorum* Planchon to the present *L. brownii* var. *colchesteri* in 1853. A lithograph of a lily plant in an article by Planchon and Van Houtte (1853-1854) was printed under the names of both *L. japonicaum* and *L. odorum*. However, curiously, in the earlier volume of the same journal, and one of the authors being the same, Lemaire and Van Houtte (1845) described that the denomination of the lily is contested; *L. japonicum* vs. *L. brownii*, and they adopted the latter.

The plant introduced in 1804 is now considered as a variety of that in 1835, and Wilson named it *L. brownii* var. *colchesteri* Wilson.

In the 22 October 1870 issue of "The Gardeners' Chronicle and Agricultural Gazette" published in London, there is an advertisement of *L. brownii* bulbs for sale by a Dutch company in Haarlem at 6 shilling each and £3 and 10 shillings per dozen (Anonymous 1870). It is amazingly expensive (it is said that the average yearly income of a labor in

Fig. 3 *Lilium brownii* var. *colchesteri* figured by Bury (1831) under the name of *L. japonicum*. Image courtesy Missouri Botanical Garden. http://www.botanicus.org (free download).

Fig. 4 Design of lily and court-cow-carriage patterns on a "Noh" costume (1568-1603) in National Museum, Tokyo. Image: TNM Image Archives. Source: http://TnmArchives.jp/ (with kind permission).

Fig. 5 "Peonies and lilies" (attributed to Tawaraya Sotatsu, 1568-1615). Freer Gallery of Art, Smithsonian Institution, Washington, D. C.: Gift of Charles Lang Freer, F1898.56 (with kind permission).

England in 1880 was around £50). Elwes (1880) says that *L. brownii* has never become at all common in England, but he has seen cultivation on the continent, where it is largely grown by Dutch and Belgian nurserymen, and at Berlin, where it is counted by thousands.

In Japan

History of *L. brownii* var. *colchesteri* in Japan was discussed based on the survey of the old fine arts, fabrics and literature (Okubo 2006, 2008). The oldest literature in which the word "Hakata Yuri" (Hakata lily) appeared is a book of Haiku (short poem having lines of only five, seven and five syllables) poems edited in 1633 by Matsue (1633).

Design of lily and court-cow-carriage patterns on a "Noh" (an old Japan's theatrical art of music, dance and drama) costume (1568-1603) in National Museum, Tokyo (**Fig. 4**) or a painting entitled "Peonies and lilies" (attributed to Tawaraya Sotatsu, 1568-1615) in Freer Gallery of Art, Smithsonian Institution, Washington, D. C. (**Fig. 5**) is the oldest drawing of *L. brownii* var. *colchesteri* in Japan. There is a controversy whether the image on the costume is *L. brownii* var. *colchesteri* or *L. longiflorum* (Shimizu 1971) as it was drawn somewhat deformed. Two colors, white and yellow, were used to paint the flowers, and therefore, it must be the expression of the shifting colors of the *L. brownii* var. *colchesteri* flowers (Okubo 2006). Another supporting fact is that even though *L. longiflorum* is native to Japan, the first literature in which the species appeared is in 1695 in Japan (Ito 1695). It is more than 90 years later than the estimated year when *L. brownii* var. *colchesteri* was introduced into Japan from China, maybe due to the distribution areas of *L. longiflorum* (Ryukyu Islands) being remote from the mainland of Japan. As for the painting of Tawaraya Sotatsu, the identification is easy by the difference in leaf shapes between the two species.

The lily, probably the bulb, was gifted by a Chinese

man to a Japanese woman named "Taé" in Hakata (Fukuoka City), and spread to various places (Tsuda and Tsuda 1765), although it is unknown when it was. It was first called "Taé-yuri" thereby, but finally it has gained the Japanese name of "Hakata Yuri" after its place of source in Japan. The word "Hakata Yuri" frequently appeared in Haiku and Ikebana (flower arrangement) books and fine art masterpieces as well as in horticultural books throughout Yedo period (1603-1867), indicating the public popularity of the plant in the country.

It is considerable from these facts that the lily was first introduced into Hakata (Fukuoka), Japan in the era of Azuchimomoyama (1568-1603). There is no evidence of the introduction of *L. brownii* into Japan.

BREEDING

Less attention seems to have been given to this species for breeding purposes. There are old reports that *L. brownii chloraster* (not *colchesteri*) was used as one of the parents of the hybrid *L. kewensis* (now lost) with the pollen of *L. henryi* (Anonymous 1906; Thomas 1906). There may be, however, the confusion with *L. leucanthum* var. *chloraster* in these two articles where *chloraster* and *leucanthum* were regarded as the varieties of *L. brownii*. According to Wilson (Wilson 1925), the bulbs of *L. leucanthum* var. *chloraster* were first sent to Kew in 1888-1889, and first flowered in 1891.

Lilium brownii was, probably for the first time, used in breeding to establish Centifolium hybrids, later renamed as Olympic hybrids, at the Oregon Bulb Farms, USA by Jan de Graaff and his collaborators who initiated their lily breeding program about 1940 (Jefferson-Brown and Howland 1995; Benschop *et al*. 2010). It is also said that none of the three varieties, *brownii*, *colchesteri* (*viridulum*) and *australe*, was used in hybridizing at Oregon Bulb Farms (McRae 1998). We are not sure which is correct. Interspecific crosses of *L. brownii* with *L. longiflorum* (Van Tuyl 1980) and with *L. formosanum* (by Zalivski) were also reported by McRae (1998), but it does not seem that cultivars with the species-specific characters of *L. brownii* or var. *colchesteri* have ever been distributed.

Breeding of lily cultivars with virus resistance, one of the most important breeding objectives, has been conducted for many years. Another strategy to avoid virus disease damage is to breed cultivars that can be propagated by seeds and can flower in relatively short period before they are infected and/or heavily damaged. Short juvenility can also lessen the chances of the attacks by other pathogens such as *Fusarium*, etc.

Lilium formosanum is natively distributed in the mainland Taiwan vertically from sea level to 3,500 m (Yang 2000). Based on isozyme diversity, Hiramatsu *et al*. (2001) found that *L. formosanum* is a derivative species from *L. longiflorum* in its southern distribution area in Ryukyu Archipelago. Cultivars of interspecific hybrid of *L. formosanum* × *L. longiflorum* (= *L.* × *formolongi*) have been developed to incorporate into the year round cultivation system of the Easter lily in Japan and they are in the market in summer season when *L. longiflorum* cultivars are less available (Imanishi 2005).

Lilium formosanum reaches anthesis within 12 months after seed sowing (Hiramatsu *et al*. 2002) unlike other *Lilium* species that take several years to start flowering from seeds (Shimizu 1971). The traits can be used for a breeding purpose to avoid possible virus transmission through conventional propagation method of scaling. The trials to introduce the characters of precocious flowering as well as those of multiple flower stalks of *L. formosanum* have been successfully conducted in the crosses with pollen parents of *L. auratum*, *L. speciosum*, *L. regale*, 'Lollypop', 'Pink Tiger' and 'Zaza' of Asiatic hybrids and 'Le Reve', 'Marco Polo' of Oriental hybrids and 'African Queen' of Trumpet hybrids with the aid of cut-style pollination and ovary-slice culture (Saruwatari *et al*. 2008). Introducing the 'precocious flowering' ability into *L. brownii* var. *clochesteri* has been also studied to establish new cultivars. The progress (Saruwatari 2009; Hai *et al*. 2010, 2012) of the breeding project until now is summarized below.

Interspecific hybrids between *L. formosanum* and *L. brownii* var. *colchesteri*

Lilium formosanum, being naturalized in Fukuoka Prefecture, Japan and *L. brownii* var. *colchesteri* in Iki Island, Nagasaki Prefecture, Japan and in Chungcheongnam-do and Kangwon-do Provinces, Korea were used. Techniques of cut-style pollination (Asano and Myodo 1977; Van Tuyl *et al*. 1991) and ovary-slice culture (Hayashi *et al*. 1986; Kanoh *et al*. 1998) were the aids in reciprocal crosses between the two species.

Either normal or cut-style pollination gave 100% capsule set in the crosses of *L. formosanum* × *L. brownii* var. *colchesteri*, whereas seeds did not contain mature embryos. In the reciprocal crosses, no capsules set. Ovules from *L. brownii* var. *colchesteri* × *L. formosanum* did not germinate, but 36 out of 2,280 ovules from the reciprocal *L. formosanum* × *L. brownii* var. *colchesteri* germinated by the methods of cut-style pollination and ovary slice culture. Eight plants survived after acclimation in a greenhouse. The earliest flowering occurred 606 days after pollination (not from seed sowing) to flowering. They had both the maternal- and paternal-specific genes in two isozyme loci.

Leaves of *L. formosanum* are liner to narrow oblong-lanceolate, whereas those of *L. brownii* var. *colchesteri* are oblanceolate. Those of F_1 progenies were intermediate with variations. Flower shape of *L. formosanum* is narrow funnel and that of *L. brownii* var. *colchesteri* is funnel. All the progenies had funnel-shaped flowers as in *L. brownii* var. *colchesteri*. Anther colour of *L. formosanum*, *L. brownii* var. *colchesteri* and their progenies was yellow, brown and brown, respectively. Flower colour at anthesis in the F_1 was yellow and it changed to white on the next day exactly as that of *L. brownii* var. *colchesteri*. However, the progenies were rarely or only slightly fragrant.

Self- and backcross-pollination of the F_1 progenies

F_2 seedlings were obtained from self-pollination of the F_1 through ovary-slice culture. Seeds with mature embryo were also obtained in the backcrosses of *L. formosanum* × F_1, but not in those of *L. brownii* var. *colchesteri* × F_1. No seeds, however, germinated.

Further development of the techniques and materials is required. Introducing different accession from other areas, non-clone accession, may be the hope for success.

CONTROL OF FLOWERING

In China, cultivation of *L. brownii* var. *colchesteri* in China is done in open fields, and the cultivation cycle is once a year. In Shaoyan, Hunan Province, bulbs planted in October grow in April – June, and all the flower buds are cut before flower opening in July to promote bulb growth. There is no flowering control given, since the production is for fresh and dried vegetables and for pharmaceutical purposes, and there is no market for cut flowers. There are records of cultivation of the species in Japan from the 18th centuries, and commercial production was taken place for bulb export until the World War II (Matsuzaki 1935). However, no attempts for forcing or retarding culture of the species have been taken before. The species has a potential to be used for cut flowers as well as for breeding materials because of its elegant fragrance and noble combination of perianth and anther colours. Control of flowering for year-round cultivation was established by Tsuchiya *et al*. (2006). They studied the effects of temperatures on the growth and flowering, and attempted forcing and retarding cultures. The details are described below.

It flowers outdoors in early June in Fukuoka (33.40°N,

130.30°E), 7-10 days earlier than *L. longiflorum*.

Effects of cold duration

Bulbs were stored in moist peat moss at room temperature after harvest in August 2004. They were planted in soil in plastic pots on 8 October, 5 November, 19 November and 3 December after 10°C storage for 0, 4, 6 and 8 weeks, respectively, and grown in a greenhouse where the minimum temperature was 12°C. Sprouting rates of the cold treated bulbs were >90% irrespective of its duration, whereas those of uncooled bulbs were 50%. The bulbs cooled for 4, 6 and 8 weeks took respectively 170, 138 and 144 days to flower, while no flowering was observed in the untreated bulbs. Days to sprouting and anthesis decreased with increased duration of cold. The number of leaves was not affected by cold treatment. The most effective temperature range and duration for flowering of *L. longiflorum* is reported to be 1.5-7°C for 6 weeks (Stuart 1954). *L. brownii* var. *clochesteri* seems to have a similar cold requirement to *L. longiflorum* for flowering.

Effects of growth temperature

Bulbs were packed in wet peat moss at 10°C on 9 November 2004 after harvest. They were then treated at 5°C on 3 December for 30 days and grown in soil-filled plastic pots at 15, 20 and 25°C in phytotron rooms of the Biotron Institute, Kyushu University. The plants that did not flower at 15°C were grown at 20°C after 1 April.

Longer stems and higher leaf number were observed at anthesis after cold treatment at 5°C rather than at 10°C. Days to anthesis were 95, 71 and 56 at 15, 20 and 25°C, respectively, and therefore, the cumulative temperatures (CTs) (growth temperature (°C) × days to anthesis from planting) were around 1,420°C-days at any temperature, of which 310°C-days was from planting to sprouting and 1,110°C-days from sprouting to flowering. Days and CT to anthesis in *L. longiflorum* 'Georgia' grown at 25/20°C (day/night) were 88 days and 1,980°C-days, respectively (Matsukawa *et al*. 1968). As *L. brownii* var. *colchesteri* flowered in 56 days at 25°C and 71 days at 20°C, it would flower in 64 days at 25/20°C (day/night) by calculation. The flowering was 24 days earlier and the CT was about 600°C-days lower in *L. brownii* var. *colchesteri* than in *L. longiflorum*. The results imply the possibility of extra-forcing of this species.

Forcing culture

Bulbs of November 2004 harvest were treated in moist peat moss at 8°C on 24 November and planted in a greenhouse on 28 December and 5 and 13 January 2005, according to root development during the storage. On 5 April when the first flowering occurred, all the flower buds were removed, and newly developed bulbs were harvested on 31 May. A hot water treatment at 45°C for 30 min was given on 1 June and the bulbs were stored at 25°C until the start of cold treatment. The cold treatment at 10°C for the first week and at 5°C for the next seven weeks (total eight weeks) started in approximately every one week from 3 June to 9 September. Some bulbs received a six or seven week cold treatment on 1 June. After the cold treatment, the bulbs were planted at 20 or 25°C.

Flowering started on 18 September and continued until 17 November without interruption of non-flowering period longer than one week. The CT ranged from 1,370 to 1,655°C-days with the average of 1,488°C-days. Eight weeks cold treatment at 5°C is more favorable than six weeks treatment to allow forcing more effective. This trial also proves the possibility of the cut flower harvest twice a year.

Retarding culture

Bulbs harvested in November 2004 were stored in moistened peat moss at 10°C on 9 November, 5°C on 3 December, and -2°C on 15 December. They were planted each week from 24 June to 9 September, and grown at 15°C for the first week and then at 20°C. The bulbs at -2°C were transferred to 5°C one week prior to planting.

Dates of flowering were from 29 August to 17 November. Average CT was 1,210°C-days, of which 108°C-days was for sprouting and 1,102°C-days until flowering. The shorter CT for sprouting is probably due to that sprouting had started during the -2°C storage.

Lilium longiflorum flowers, if no market exists, are commonly placed in dark storage at 2-4°C after harvest, and up to one week of storage has little or no adverse effect on longevity after storage (Miller 1993). The method is also applicable to *L. brownii* var. *colchesteri*. Yellow color at anthesis fades out in one day at >15°C, but remains for 5-7 days at 5°C.

In conclusion, year-round flowering of *L. brownii* var. *colchesteri* is possible by combination of forcing and retarding techniques as described above, although the experiments for winter to spring flowering have not been carried out. The most contributing factor to control flowering is considered to be temperature. Flowering dates can be predicted by the calculation of CT. Minimum size of flowering bulbs was about >20 g in all the above experiments.

PROPAGATION

Seeds

Since *L. brownii* var. *colchesteri* is self-incompatible (Shimizu 1971) and one clone is present in our present Japanese and Korean collections (Saruwatari *et al*. 2007), no seeds of the plant have been obtained yet in the cross trials. Overcoming self-incompatibility in *L. longiflorum* by heat (Ascher and Peloquin 1970; Campbell and Linskens 1984; Hiratsuka *et al*. 1989) or cytokinin treatment (Matsubara 1973; Sakazono *et al*. 2010) is known. However, such trials have not yet been conducted in *L. brownii* var. *colchesteri*. McRae (1998) mentioned that *L. brownii* set seeds when crossed with var. *colchesteri* or with var. *australe*. It may be possible to obtain seeds by crossing different landraces or varieties.

Bulblets and bulbils

As in the most other *Lilium* species, bulblets are formed on the stem in soil in *L. brownii* var. *colchesteri*. Bulbils (aerial bulblets) do not develop in the upper axils of the leaves. However, they are formed in quantity on the axils near soil surface or on the underground stem (like a bulblet), but their tops appear above the soil surface (like a bulbil). They can be used for propagation.

Scaling

Scaling is the most common propagation method in commercial production to obtain flowering-size bulbs in many *Lilium* cultivars. Scale propagation was attempted in *L. brownii* var. *colchesteri* (H. Tsuchiya, unpublished). Scale segments of *L. brownii* var. *colchesteri* prepared from the bulbs harvested in October 2004 were put in plastic bags with moistened peat moss and kept at 15, 20, 25 or 30°C for 2 months, then transplanted to vermiculite in plastic boxes in glass rooms each at the same temperatures until 30 April 2005. The average number of bulblets produced per scale was 1.0-1.2 at any temperature.

Bulblets obtained through the scaling experiments at different temperatures received 5°C for 8 weeks on 1 April 2005, and were grown in a greenhouse starting 1 June. The bulblets obtained at 15°C sprouted without cold treatment, but those at 20, 25 and 30°C did not. All those nursed at any

temperature sprouted with the cold treatment, but the sprouting rates were around 20%. The higher the scaling temperature, the lower the sprouting rate after cold treatment. Nearly 100% sprouting from bulblets after cold temperature can be achieved in *L. longiflorum* and *L. speciosum*, though. It can be said that propagation efficiency by scaling is rather low in *L. brownii* var. *colchesteri*.

In vitro

In vitro propagation techniques were established by Masuda *et al*. (2009) through the experiments for eliminating virus diseases as described later in this article. The procedures are as follows:

Bulbs were cleaned under running tap water for one hour after harvest after removing damaged outer scales. Clean inner scales were excised and surface sterilized first in 70% (v/v) ethanol for 30 sec, then 1% sodium hypochlorite solution for 30 min, followed by three washes with sterilized distilled water. The separated entire scales were put in culture on Murashige and Skoog (MS) medium (Murashige and Skoog 1962) with 2.2 μM 6-benzyladenine (BA), 2.9 μM indole-3-acetic acid (IAA), 30 gl^{-1} sucrose solidified with 3 gl^{-1} gellan gum (pH 5.8 before autoclaving) and cultured at 25°C under continuous light. The bulblets formed on the base of the scales were removed and transplanted to 1/2 MS medium with 0.5 μM naphthaleneacetic acid (NAA), 30 gl^{-1} sucrose, 3 gl^{-1} gellan gum (pH 5.8 before autoclaving) for further growth. During this subculture, cold treatment at 4°C for 6 weeks was given for breaking dormancy (Tsuchiya *et al*. 2006). Then, they were transferred to the phytotron at 20°C. The developed bulblets produce a few leaves, and entered dormancy again. Several repeats of the sequence of the cold treatment and culture at 20°C are necessary to obtain bulbs large enough for planting in soil. Minimum flowering-size bulbs can be obtained in two years.

VIRUS-FREE BULB PRODUCTION

Symptoms of distorted and twisted growth of mosaic leaves and abnormal shape and color breaking in the flowers are often observed in *Lilium brownii* var. *colchesteri*, similar to those found in other *Lilium* species, which suggests virus infection of the species. It is considered to be one of the causes of decrease in the species population in Japan since all the individuals in Japan and Korea have long been clonally propagated (Saruwatari *et al*. 2007), but not through seeds. *Lily symptomless virus* (LSV; *Carlavirus*), *Lily mottle virus* (LMoV; *Potyvirus*) and *Cucumber mosaic virus* (CMV; *Cucumovirus*) are the major viruses in *Lilium* species in many countries (Asjes 2000). It is reported that the LSV frequently occurs with CMV or LMoV in cultivated lily plants, and the plants with complicated infection in these viruses are more seriously susceptible to other diseases (Derks and Asjes 1975; Derks 1995; Asjes 2000).

Elimination of viruses through meristem tip or callus culture alone or with chemotherapy is widely reported in other *Lilium* species (Allen and Anderson 1980; Allen *et al*. 1980; Blom-Barbhoorn and Van Aartrijk 1985; Ozaki *et al*. 1996; Xu *et al*. 2000; Nesi *et al*. 2009). Masuda *et al*. (2009) identified LSV, LMoV and CMV in *L. brownii* var. *colchesteri* by reverse transcription polymerase chain reaction (RT-PCR), and developed the techniques to propagate virus-free bulbs by combination of meristem tip culture and chemotherapy. It is summarized as follows: All the regenerated bulblets on MS medium with 2.2 μM BA, 2.9 μM IAA, 30 gl^{-1} sucrose and 3 gl^{-1} gellan gum (pH 5.8 before autoclaving) were infected by at least one of the three viruses after the first meristematic tip culture, of which 26.7% were by LSV, LMoV and CMV, 33.3% by LSV and LMoV, but 26.7% were by LSV only and 13.3% by LMoV only. LSV of the LSV-infected bulblets was successfully eliminated to obtain virus-free bulblets through a second meristematic tip culture with 50 μM 2,4-dioxohexa-hydro-1,3,5-triazine (DHT), an antiviral chemical, whereas LMoV was not from the LMoV-infected bulblets. These results are in agreement with the effect of Virazol (Ribavirin), another antiviral chemical, on LSV and LMoV elimination in *Lilium* x *parkmanii* hybrids and *L. longiflorum* (Cohen 1985; Xu and Niimi 1999). The virus-free bulblets were transferred for subculture at 25ºC on a 1/2 MS medium with 0.5 μM NAA, 30 gl^{-1} sucrose, 3 gl^{-1} gellan gum (pH 5.8 before autoclaving) without DHT, and then acclimated in a glass room at 20°C to grow to mature bulbs after dormancy break at 4°C for 6 weeks (Tsuchiya *et al*. 2006). The virus-free mature bulbs are now being used for various purposes in this project.

FLOWER PIGMENTS

Cyanidin-3-rutinoside as a major anthocyanin (Banba 1967) and several carotenoid pigments of β-carotene, cryptoxanthin, zeaxanthin, capsanthin, capsorubin and echinenone-like carotenoid (Banba 1968) were reported in the flowers of some *Lilium* species. Recently, cyanidin 3-*O*-β-rutinoside-7-*O*-β-glucoside and cyanidin 3-*O*-β-rutinoside were identified in Asiatic and Oriental hybrids (Nørbæk and Kondo 1999). Carotenoids in Asiatic hybrid lilies were antheraxanthin, (9Z)-violaxanthin, *cis*-lutein and violaxanthin in yellow flowers and capsanthin in red flowers (Yamagishi *et al*. 2010).

Flowers of *L. brownii* var. *colchesteri* show cream-yellow at flowering and soon fade to white in the next day (**Fig. 2**). The colour fading is temperature dependent. Content and composition of carotenoids and transcriptional changes of carotenoid cleavage deoxygenase 4 gene (*LbCCD4*) were investigated during anthesis (Thien *et al*. 2010; Hai *et al*. 2012). The major carotenoids making up the cream-yellow colour of the petal were lutein, zeaxanthin and β-carotene, among which zeaxanthin was the majority (about 50% of the total). Transcriptional level of *LbCCD4* gene determined by Quantitative RT-PCR increased and reached the maximum at 12 hrs after anthesis, followed by the decrease in total carotenoid content of the petal. Further investigation is now on the way.

Outer surface of the flowers are suffused, sometimes only slightly, with rose-purple similarly to that of *L. formosanum*. Their pigmentation seems to be by anthocyanins, but it has not been analyzed.

SCENT

It is quite difficult to exactly express scent/fragrance. Flowers of *L. brownii* var. *colchesteri* have a very graceful fragrance. The scent is different from that of *L. longiflorum*. It is also not really like that of Oriental hybrids such as 'Casa Blanca' that contains p-creosol and p-cresol as minor compounds causing unpleasant odor (Oyama-Okubo 2010).

Identification of the fragrant compounds in the species is now under way. Whole flower buds and flowers of each plant were covered with a plastic bag and the collected gas was analyzed by gas chromatography-mass spectrometry (GC-MS). At half opening, two major peaks, common to both species but higher in *L. brownii* var. *colchesteri* than in *L. longiflorum*, were detected (E. Yoshida and K. Shimizu, pers. comm.). Two other peaks specific in *L. brownii* var. *colchesteri* were found on the next day of full opening. These compounds may contribute to the fragrance of *L. brownii* var. *colchesteri*. Identification and chemical synthesis of the compounds may have the possibility of contribution to perfume industry.

Generally, *Lilium* species of which the flowers containing anthocyanins, *L. japonicum* and *L. speciosum*, for example, are rich in scent. Flowers of *L. brownii* var. *colchesteri*, however, contain carotenoids at anthesis (see **FLOWER PIGMENT**) and are fragrant as well. The increase in fragrance after flower opening in accordance with the fading of the petal color in the species suggests the possible relationship between the two aspects. Occurrence

and generation of carotenoid-derived aroma compounds in flower scents are known in many other plant species.

EPILOGUE

Revival of the Hakata lily in Fukuoka with its noble and delightful fragrance drifting all over the city in flowering season – is our dream. It may come true through this project or by further cooperation with many others in the future. It might be a bit local patriotism though. New cultivars incorporated in the global lily industry are, of course, expected. Give Hakata Lily a light. Give *Lilium brownii* var. *colchesteri* a chance.

THE PROJECT TEAM

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CONDOLENCES

The authors lament for H. Tsuchiya, a student member of the project team, who passed away so young by a sudden accident. He greatly contributed to the establishment of culture system for year-round flowering of Hakata lily in this project.

ACKNOWLEDGEMENTS

The project team is grateful to Dr. T. Matsukawa (Fukuoka Agricultural Research Center, retired), T. Hara (Fukuoka Flower Market, Fukuoka), H. Inokuchi (Hiro Co., Fukuoka), N. Fujito, E. Yamaguchi and T. Miyamoto (Fukuoka City Office), M. Inoue (S. I. M., Musashino, Tokyo), T. Yamada (Green Dynamics, Matsudo, Chiba) and many others for their cooperation and helps. A part of this project was financially supported by Fukuoka City.

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