Chapter 9
Lilium

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9.1 Some Basic Facts About the Genus *Lilium*

Genus *Lilium* has about 100 species distributed in the northern hemisphere extending up to Asian tropics (Latitude 10–60°). These perennial herbs have unsurpassed beauty and great commercial significance. *Lilium candidum* (Madonna lily) was used already as a cut flower in the middle Minoan IIIA–B period (ca. 1750–1675 BC) known to be of biblical importance. *Lilium longiflorum* is often used in Easter season as pot plant and known as "Easter lily" in the United States. In China, lilies have been cultivated for food and medicine for at least 2,000 years (Haw 1986). Today, lily hybrids are one of the most important cut flowers and pot plants of the worldwide horticultural bulb trade. Lily hybrids and species are also used as garden plants. In most cases, "lily species" are susceptible to various diseases and cultural problems that render them difficult to maintain in gardens. But, many species are attractive for gardeners in Europe and North America. More than 300 cultivars are registered per year and the accumulated number of registered lily cultivars is more than 9,400 (http://www.lilyregister.org). The important aim of lily breeding is to combine the delicate features of their wild relatives with disease resistance, hardiness, and year-round forcing ability in the cultivars. Lily cultivars can be divided into three classes based on uses: one for cut flowers in the greenhouse, another as pot plant, and further for garden cultivation. Eastern Asia and North America are centers of high diversity with about 60 and 21 species, respectively. The closest relatives to lilies are found in eastern Asia, where the genus *Lilium* originated, along with the genera *Fritillaria*, *Nomocharis* Franchet, *Notholirion* Wallich ex Boissier, and *Cardiocrinum* (Endicher) Lindley (Woodcock and Stearn 1950). Lighty (1968) reported that *Lilium* and *Nomocharis* are very closely related and some recent molecular classifications (Fay and Chase 2000) include *Nomocharis* in genus *Lilium*. The 21 species of *Lilium* that are native to North America are derived from Asian stock (Lighty 1968). *Lilium philadelphicum* and *L. catesbaei* are the only two North American lilies with erect flowers. These two species almost certainly represent a single introduction from Asia (Nishikawa et al. 1999; Hayashi and Kawano 2000). Most of the *Lilium* species possess $2n = 2x = 24$ chromosomes with the exception of *Lilium tigrinum*. Natural triploids in *L. tigrinum* are common in the habitat of Korea, where diploid plants predominate in the coastal area and the triploid plants predominate in the mountainous area. Most lilies are largely self-incompatible, and cross-pollination is required for seed set, however some species are confirmed as self-compatible.

Lily bulbs were used as a food and equally versatile as medicine, and the mashed bulbs were variously employed in the treatment of spider bites, cuts and bruises, fever, coughs, consumption, stomach ailments, and rheumatism. Contemporary medical use seems to be largely limited to *L. tigrinum*, which bulbs are used to treat a variety of internal discomforts including those associated with menstruation and menopause (Zhao et al. 1996; Zhang 2007).

Although there were previous attempts to classify *Lilium* species, the taxonomic work of Comber (1949) is by far the most comprehensive. Comber classified

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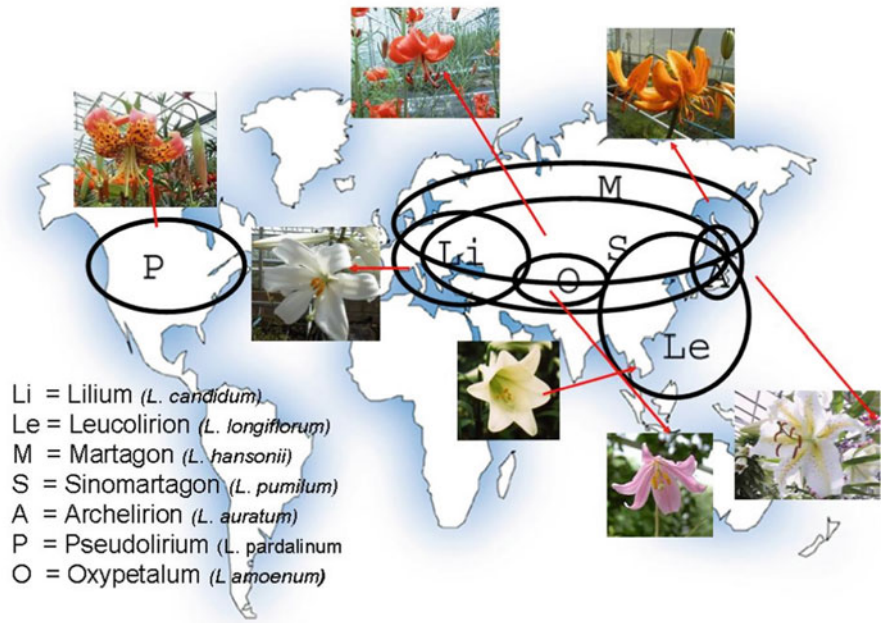

Fig. 9.1 The natural distribution of sections of the genus *Lilium* around the northern hemisphere with each section of a representative species

the genus into seven taxonomic sections, viz., *Martagon*, *Pseudolirium*, *Liriotypus*, *Archelirion*, *Sinomartagon Leucolirion*, and *Daurolirion*. This classification was based on 15 phenotypic characteristics. De Jong (1974) revised this classification: *Martagon*, *Pseudolirium*, *Lilium*, *Archelirion*, *Sinomartagon*, *Leucolirion*, and *Oxypetalum*. Besides this, information that is relevant to phylogenetic considerations has also emerged from the crossability data of species within and among different taxonomic sections (Lim et al. 2008) as well as meiotic studies (Sect. 1.1.8). In addition to the above approaches, molecular phylogeny of the genus *Lilium* has also been carried out on a limited scale (Nishikawa 2007). On basis of these data, a new classification should be proposed.

9.1.1 Characteristics of Classified Species

9.1.1.1 Section *Martagon*

Involved species: *L. tsingtauense*, *L. miquelianum*, *L. distichum*, *L. hansonii*, *L. martagon*, *L. medeloides*.

The species of the section *Martagon* are primitive in the genus *Lilium*, especially *L. hansonii*. Most *Martagon* species are native in Korea, Japan, China, Manchuria and Russia, and just have a restricted distribution in the countries and islands along the East Sea of Korea and Japan. Only *L. martagon* is widespread with its distribution area from East Russia to West Spain. Based on Lighty (1960), Korea and the adjacent area of Manchuria are very likely near the center of origin of the genus *Lilium*. China and Korea host *L. distichum* and *L. tsingtauence*. The Russian area around the Vladisvostok and up along the Amur River is also the home of *L. distichum*. *L. hansonii* habitats in the Ullung-Do Island of Korea and that is the only known area of native growth of this species. There, one finds the ancestral colonies of the wheeled lily of Japan, *L. medeoloides*. They all show a whorl of leaves near the middle of the stem plus a few scattered leaves above or below. Flowers of this section are nodding to upright, horizontal, and pendant with a range of 6–12 cm in diameter. They possess a range of perfume-like fragrances as in *L. distichum*, sweet-dung smelling in *L. hansonii*, and *L. tsingtauense* possess no smell. There is no clear criterion for discriminating the four highly related species: *L. distichum* and *L. medeoloides*, and *L. tsingtauense* and *L. miquelianum*.

Crosses with *Martagon* species within the *Martagon* section and with other sections produce quite different results. It is easy to cross and get seed set between *L. hansonii* as a female parent and *L. tsingtauense* (Martagon), and *L. cernuum* (Sino-martagon) as male parent. However, the reciprocal crossings showed almost no seed set and embryo formation. This may be highly related to the plant vigor, which is important for the fertilization and embryo growth as a female parent. Plant vigor of *L. cernuum* and *L. tsingtauense* is very weak and the flower size is also small. Many hybrids are made by crossing between *L. martagon* and other species, such as *L. hansonii*. Bridge-crossing can be made among three species: *L. martagon*, *L. hansonii*, and *L. tsingtauense*.

L. hansonii is very peculiar to have its only habitat in Ullung-Do Island, Korea. These plants are growing in a humus alkaline soil at the level of 200–300 m above sea level. A number of plants are seen in the habitats, which are well protected because digging of these is prohibited by Korean law. There are few plants that produce flowers with pure yellow colour without any spots. They possess strong odor, like sweet mixed with other unpleasant smells. All flowers showed pendant thick tepals with slightly unbalanced tepals like *L. distichum* found in other Korean region. Plants are vigorous and tall to reach about 1 m.

L. distichum, called Chosen lily (Chosen is one of the Korea dynasty), is native in China, Russia, and Korea. Lighty (1968) reported that *L. distichum* was found in northern Korea, but recently the author has found this in southern Korea around Mt. Dukyusan as well. This species has a side-facing dull orange-colored flower with one tepal pendant and rest of the five tepals ascending, showing asymmetrical shape of flowering. It has also fragrance but, mostly not so attractive, however, very few flowers posses a strong perfume-like fragrance unknown in other lilies.

L. medeoloides shows side- to down-facing orange-colored flowers with long flowering stems. It is mainly native to Japan above Shikoko through Hokkaido, Kamchatka, Sakhalin islands, and partly to the main land of Russia above Vladivostok. Baker (1871) stated in his synopsis that *L. medeoloides* are found in both Koreas.

9.1.1.2 Section *Pseudolirium*

Involved species:

2a. *L. bolanderi*, *L. columbianum*, *L. kelloggii*, *L. humboldii*, *L. rubescens*, *L. washingtonianum*
2b. *L. maritimum*, *L. nevadense*, *L. occidentale*, *L. pardalinum*, *L. parryi*, *L. parvum*, *L. roezlii*
2c. *L. canadense*, *L. grayi*, *L. iridollae*, *L. machuxii*, *L. michiganense*, *L. superbum*
2d. *L. catesbaei*, *L. philadelphicum*

North America is one of the centers of worldwide diversity of lily with about 21 species. Most of the species are distributed along the American West Coast (*L. bolanderi*, *L. columbianum*, *L. kelloggii*, *L. humboldii*, *L. rubescens*, *L. washingtonianum*, *L. maritimum*, *L. kalleyanum*, *L. occidentale*, *L. pardalinum*, *L. parryi*, *L. parvum*, *L. wigginsii*). A few species are native to eastern North America such as: *L. canadense, L. grayi*, *L. superbum*, *L. catesbaei*, *L. michiganense*, *L. michauxii*, *L. iridollae*. In natural condition, the species can be found from sea level (*L. columbianum*) to areas at high elevation of around 3,000 m (*L. parvum* and *L. parryi*). West Coast lilies can be divided into two main types – the "dry growers" and "wet growers" (Robinett and Robinett 1991). "Dry growers" usually can be located in areas of high seasonal rainfall, deep in soil that retains moisture. "Dry growers" can be found, at the edge of woodlands or scrubs. The following species can be included to this group: *L. bolanderi*, *L. columbianum*, *L. kelloggii*, *L. rubescens*, *L. washingtonianum.* "Wet growers" can be found in areas rich in water around the year: along the streams, in seeps, or in bog condition. This group comprises, *L. kelleyanum*, *L. maritimum*, *L. occidentale*, *L. pardalinum*, *L. parryii*, *L. parvum*, *L. wigginsii*.

Species of North American lily have a wide variation in their flower tepals' color (from white to peach, orange, yellow, pink, red, purple, scarlet), flower shape (Turk's cap, trumpet, bowl, bell-shaped) with or without spotting. Most of the species are characterized by nodding flowers with the exception of two species with erected flowers *L. philadelphicum* and *L. catesbaei*. Plants can reach a height from 30 cm (*L. catesbaei*) to 3 m (*L. superbum*). Leaves can be arranged in whorls or scattered on the stem. The bulbs of the North American species are radically asymmetrical, slowly growing, and scaly rhizomes.

A number of the successful hybridizations that have been done among species of *Pseudolirium* section created the group of American species hybrids. Successful crossing in this respect has been between: *L. pardalinum* and *L. humboldtii*, *L. kelloggii* and *L. parryi*, *L. pardalinum* and *L. bolanderi* (Fox 1974), as well as between *L. canadense* and *L. michiganense* (Wadekamper 1988). Hybrids were also obtained from crosses between *L. pardalinum* and oriental hybrids from the section *Archelirion*, while *L. canadense* has been successfully crossed with *L. longiflorum* (Fig. 9.2).

9.1.1.3 Section *Lilium*

Involved species: *L. candidum*, *L. carniolicum*, *L. chalcedonicum*, *L. monadelpum*, *L. polyphyllum*, *L. pomponium*, *L. pyrenaicum*, *L. albanium*, *L. ledebourii*, *L. kesselringianum*, *L. jankae*, *L. ponticum*, *L. ciliatum*, *L. akkusianum*, *L. bosniacum*, *L. szovitisanum*.

Species of the *Candidum* subsection are found in Europe and the Middle East. The species are important from the hybridizer's point of view. As *L. candidum* crosses readily, with *L. chalcedonicum* and *L. monadelphum*. *L. polyphyllum* in most of its characteristics is very close to *L. monadelphum* (Anurag 2007) *L. candidum* holds pleasant odor, whereas *L. pyrenaicum* produces an unpleasant one. Flowers of the species bloom during June–July. Interspecific crosses were made between *L. henryi* and *L. longiflorum* with *L. candidum* (Fig. 9.2).

9.1.1.4 Section *Archelirion*

Involved species: *L. auratum*, *L. brownii*, *L. japonicum*, *L. nobilissimum*, *L. rubellum*, *L. speciosum*.

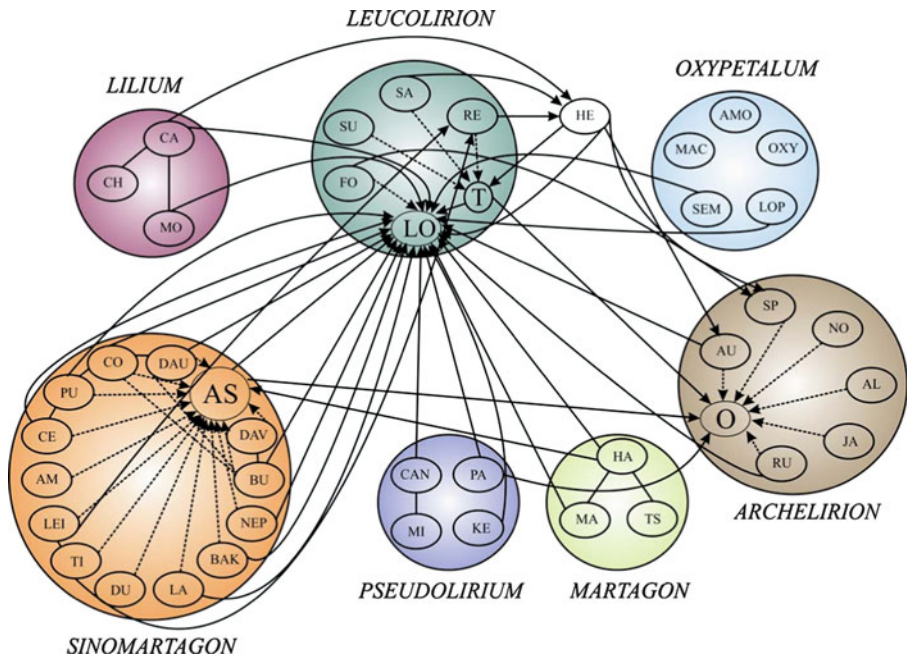

Fig. 9.2 *Crossing polygon* of the genus *Lilium* including all the successful crosses of species between different sections of the genus *Lilium*. In this figure, the connections between the Asiatic, Trumpet, and Oriental hybrid groups (*large ellipses*) are shown by *dotted lines*. In successful crosses between species (*small circles*) of different sections (*large circles*), the *arrows* point toward the female parent. Abbreviations: AL: *L. alexandrae*; AM: *L. amabile*; AMO: *L. amoenum*; AS: Asiatic hybrids; AU: *L. auratum*; BAK: *L. bakerianum*; BU: *L. bulbiferum;* CA: *L. candidum*; CAN: *L. canadense*; CE: *L. cernuum*; CH: *L. chalcedonicum*; CO: *L. concolor*; DAU: *L. dauricum;* DAV: *L. davidii*; DU: *L. duchartrei*; FO: *L. formosanum*; HA: *L. hansonii*; HE: *L. henryi*; JA: *L. japonicum*; KE: *L. kelloggii*, LA: *L. lankongense*; LEI: *L. leichtlinii*; LO: *L. longiflorum*; LOP: *L. lophophorum* MA: *L. martagon*; MAC: *L. macklinae* MI: *L. michiganense*; MO: *L. monadelphum*; NEP: *L. nepalense*; NO: *L. nobilissimum*; OXY: *L. oxypetalum*; O: Oriental hybrids; PA: *L. pardalinum*; PU: *L. pumilum*; RE: *L. regale*; RU: *L. rubellum*; SA: *L. sargentiae;* SEM: *L. sempervivoideum*; SP: *L. speciosum*; SU: *L. sulphureum*; T: trumpet hybrids, TI: *L. tigrinum*; TS: *L. tsingtauense*

The species of the section *Archelirion* are native to Japan with the exception of *L. speciosum,* which is also found in Taiwan and southeastern China (McRae 1998). In natural condition the species can be found on the margins of hillside woods and well-drained slopes (*L. auratum*), on the steep cliffs of the coast (*L. nobilissimum*) and in shaded and moist places in forest, grassy slopes (*L. speciosum*) in high mountain meadows (*L. rubellum*) and moist places (*L. japonicum).* *L. rubellum* and *L. nobilissimum* are threatened with extinction and are under special protection in Japan nowadays.

Species from the *Archelirion* Section range from early (*L. rubellum*, *L. auratum*) to late flowering (*L. nobilissimum*, *L. speciosum*), plants can reach a height from 30 cm (*L. rubellum*) and 50 cm (*L. nobilissimum*), to up to 2.5 m (*L auratum*). Strongly scented flowers are of white (*L. nobilissimum*, *L. japonicum*, *L. auratum*) or pink color (*L. rubellum*). The flowers of *L. speciosum* are white to pink in color with carmine spots and papillae; similarly flowers of *L. auratum* have gold radial markings, and orange spots. Most species of the section *Archelirion* are resistant to *Botrytis elliptica,* a pathogenic fungus that affects most of the lilies from other sections, but susceptible to *Fusarium* (Barba-Gonzalez et al. 2005a, b). In Japan, *L. auratum, L. speciosum, L. rubellum* and *L. nobilissimum* are cultivated for their edible bulbs, which are rich in starch.

A successful hybridization has been made between *L. rubellum* and *L. longiflorum* (section *Leucolirion*) where the former was used as a pollen donor. Hybrids were also obtained from reciprocal crosses between *L. nobilissimum* and *L. regale* (Section *Leucolirion*) (Obata et al. 2000). *L. auratum* has been successfully crossed with *L. henryi* and *L. longiflorum* and many modern oriental cultivars are derived in part from this species. *L. speciosum* has also been widely used for breeding, successful crosses have been made with other species of the Oriental section as well as with *L. henryi* and *L. alexandrae* (McRae 1998).

The hybridization among the species of the *Archelirion* section resulted in the Oriental hybrids (O-genome; Beattie and White 1993; McRae 1998). These hybrids nowadays form the most important group of cultivated lily hybrids, in spite of the fact that their forcing time is a few weeks longer than most of the Asiatic hybrids. Since 1990, around 2,000 cultivars have been registered (Leslie 1982–2005). Oriental hybrids have massive strongly scented flowers of white and pink color where the dark pink is dominant over white (Lim and Van Tuyl 2006). Flowers are side-wards or up-facing, and open flat while some of the petals may be bent back.

Oriental hybrids have been successfully crossed with *L. pardalinum* (Section *Pseudolirium*) and Asiatic hybrids (Section *Sinomartagon*) when used as female parent. One of special interest at present is to combine the resistance to *Fusarium oxysporum* and viral diseases from Asiatic hybrids with the resistance to *B. elliptica* from the Oriental hybrids into a new group of interspecific hybrids (Schenk 1990; Lim et al. 2000a). Crosses are also made between Oriental hybrids and *L. longiflorum*, and Trumpet hybrids.

9.1.1.5 Section *Sinomartagon*

Involved species:

5a. *L. dauricum*, *L. maculatum*, *L. wilsonii*, *L. bulbiferum*, *L. davidii*, *L. duchartrei*, *L. henryi*, *L. tigrinum*, *L. lankongense*, *L. leichtlinii*, *L. papilliferum*, *L. rosthornii*

5b. *L. amabile*, *L. callosum*, *L. cernuum*, *L. concolor*, *L. pumilum*, *L. fargesii*

5c. *L. aboricola*, *L. bakerianum*, *L. euxanthum*, *L. majoense*, *L. nepalense*, *L. orchraceum*, *L. paradoxum*, *L. poilanei*, *L. primulinum*, *L. sherifiae*, *L. souliei*, *L. stewartianum*, *L. taliense*, *L. wardii*

Many species involved in this section possess diverse phenotypic and physiological traits. Current Asiatic lily cultivars are derived from this section by anonymous crossings since 1890s. *L. bulbiferum*, *L. dauricum*, *L. tigrinum*, *L. amabile*, *L. cernuum*, *L. concolor*, among others have been widely used in hybridization being ancestors of the modern Asiatic hybrids. The tremendous diversity in phenotypical and physiological aspects in this section is, for example, shown in the flowers which are orange, pink, red, purple, yellow, white and green, and up-facing to downward-facing. The flower shape is bowl, flat, and recurved. Interspecific hybridizations among species and their hybrids made possible to release commercial hybrid cultivars. It encompasses large numbers of cultivar registration so far, but the number of new hybrids registered recently is decreased due to a decreasing economic importance of Asiatic hybrids.

China is the largest center of origin of this section, where more than 33 species are native. Seeds of this section show instant epigeal germination. *L. tigrinum* and *L. davidii* are edible lilies cultivated for cooking in China. *L. tigrinum* shows wide range of habitats in most areas of East Asia, distributed at 400–2,500 m elevation. This species shows diploid and triploid plants in the natural habitat of which diploids are mainly found on the seaside and triploids are mainly found in the mountainous area of Korea (Kim et al. 2006). It is very tolerant to the different environmental conditions in China, Korea, and Japan. *Lilium nepalense* is medium hardy and needs good protection against frost. It shows an affinity to *L. nobilissimum*, since an wonderful hybrid between these two species has been reported (McRae 1998). Nishikawa et al. (1999) compared nuclear ribosomal DNA (internal transcribed spacer region) of 55 species including *Nomocharis saluensis*. They found that *L. nepalense* together with *N. saluensis* showed great affinity to the subsection *Lilium*. *L. primulinum, L. nepalense*, *L. majoense*, and *L. poilanei* seem to be closely related species. *L. amoenum* is a rare species, which is considered to be on the borderline to the genus *Nomocharis* and closely related to *L. sempervivoideum* (which has more narrow leaves, in section *Oxypetalum*). *L. arboricola* seems to be related to *L. primulinum*, *L. lijiangense*, and *L. wardii*; however, the flowers are pure green without any other coloration. *L. bakerianum* shows diverse flower colors such as white, green, pink, yellow, or purple. Flower shapes are also very diverse in this section as recurved (*L. rosthornii*, *L. lankongense*, *L. leichtlinii* var *maximowiczii*, *L. amabile*, *L. pumilum*, *L. nepalense*, *L. taliense*), bell-shaped (*L. mackliniae*), lantern-shaped (*L. lophophorum*), Turk's cap-shaped (*L. arboricola*, *taliense*, *wardii*), and funnel-shaped (*L. majoense*, *L. nepalense*) flowers can be found. Some species are very rare and are classified by de Jong (1974) in *Oxypetalum* (*L. sempervivoideum*, *L. amoenum*, *L. henrici*, *L. lophophorum*, *L. mackliniae*). Some species are highly related in their morphology, for example, *L. leichtlinii* var. *maximowiczii* is similar to intermediate between *L. tigrinum* and *L. amabile*. *L. taliense*, *L. duchartrei*, and *L. lankongense* are closely related, whereby *L. duchartrei* bears its flowers in an umbel, the other two in racemes. Some species are stoloniferous (*L. bakerianum*, *L. lankongense*).

9.1.1.6 Section *Leucolirion*

Involved species:

6a. *L. leucantum*, *L. regale*, *L. sargentiae*, *L. sulphureum*, *L. Leucanthum*
6b. *L. fomosanum*, *L. longiflorum*, *L. neilgherrense*, *L. philippinense*, *L. wallichianum*, *L. wenshanense*

L. longiflorum Thunberg (Easter lily; Japan) is the common white lily of the florist trade. Many cultivars exist of which cultivars such as "Georgia" as cut flower and "Nellie White" as pot plant are among the most widely cultivated and commercially important lily. The plants of this section show a tubular flower shape with a white color and distinct fragrance. With *L. longiflorum* and *L. formosanum* Wallace (Formosa lily), interspecific hybrid "*L. formolongo*" are derived for annual seed lily, which is tall, has a vigorous growth habit and flowers at early stage. Other species, such as *L. philippinense* Baker (Philippine lily), are often tall and have a thin stem which is not an attractive characteristic for lily culture.

9.1.1.7 Section *Oxypetalum*

Involved species: *Lilium oxypetalum*, *L. amoenum*, *L. henrici*, *L. mackliniae*, *L. lophophorum*, *L. sempervivoideum*, for description see section *Sinomartagon*.

9.1.1.8 Crossability Throughout the Genus Lilium

Undoubtedly, the taxonomic classification based on phenotypic characteristics described above is a useful guide to delimit the species. But further refinement can be achieved through other methods such as interspecific hybridization as well as molecular phylogeny in order to determine species relationships. A crossing polygon (Fig. 9.2), which is constructed on the basis of species hybridizations carried out at Plant Research International, Wageningen University and Research Center, Netherlands can be used for this kind of refinements. Generally, the species within each section (included in each large circle) are easily crossable and the hybrids are fertile. On the contrary, hybridization between species of different sections (combinations indicated by arrows among large circles) is

very difficult to achieve because of pre- and post-fertilization barriers and high sterility of the hybrids. Nevertheless, through in vitro embryo, ovule or other rescue methods, successful intersectional species hybrids, shown in the crossing polygon, have been achieved. These interspecific hybrids have also been useful for assessing species relationships through the analysis of chromosome pairing during meiosis. Thus, the analyses of meiosis using genomic in situ hybridization (GISH) in the F_1 hybrids between Longiflorum × Asiatic, Asiatic × Oriental, *L. henryi* × *L. auratum*, among others, have provided valuable data regarding chromosome pairing relationships among different genomes (Lim et al. 2001a, b; Barba-Gonzalez et al. 2005a, b; Van Tuyl et al. 2005).

9.2 Conservation of *Lilium* Germplasm

As with other plant species, *Lilium* species are also facing the threat of genetic erosion, so more and more attention is being paid for conservation of *Lilium* germplasm as the genus represents one of the most important crops of cut flowers and pot plants worldwide which is also used as kind of vegetable and medicine in some places of the world. The conservation of lily germplasm is divided into two main strategies: in situ and ex situ.

9.2.1 Ex Situ Conservation

9.2.1.1 Cold Storage of Bulbs

Collections of bulb crops are usually preserved in the field or greenhouse by yearly planting, harvesting and storing of the bulbs, with high investments of labor, space, and risk of losses caused by diseases (Towill 1988; Withers 1991). Since many lily genotypes are unique and heterozygous, they should be preserved vegetatively as clones. By increasing the storage duration of the bulbs would make the maintenance of a field collection more efficient. Temperature is the most important factor in lily bulb storage. Normally, the lily bulbs are stored at 4°C. If a longer storage is required, the temperature must be decreased to −2°C and bulbs should be put in moist peat (Van der Salm and Van der Salm 1985; Beattie and White 1993). However, sometimes sprouts are damaged after bulb storage at −2°C (Boontjes 1983; Beattie and White 1993), and the regeneration ability of bulb scales will decrease after more than one year of storage and will be completely lost after 5 years (Bonnier et al. 1997). So increased freezing tolerance of lily would reduce the risk of freezing injury, and could make storage at a lower temperature than −2°C possible. Lower temperature (less than −2°C) would further minimize growth conditions and therefore bulbs could be stored for maximum time period. Freezing tolerance can be increased by: cold-acclimatization, abscisic acid treatment, partial dehydration, or by low atmospheric pressure (Halloy and Gonzalez 1993; Hincha 1994; Lang et al. 1994; Mantyla et al. 1995). Controlled atmospheric storage (CAS) and modified atmosphere packaging in closed bags (MA package) were also used to decrease the metabolism of clone material in bulb flowers (Prince et al. 1981, 1986; Bonnier et al. 1996). Ion leakage from lily bulbs measured by the electrical conductivity of external solution increased with damage caused by frost, heat or dehydration, and with viability loss during storage (Bonnier et al. 1992, 1994). Therefore, ion leakage could be used for measuring the viability and estimate maximum storage duration of lily bulbs.

9.2.1.2 Conservation in Gene Banks

The lily gene bank at Plant Research International has maintained several thousands of lily genotypes for more than 35 years. In gene banks, as a means of preserving genetic material, the storage of seed is the preferred method, but clone germplasm and recalcitrant seed species can also be kept in field plantings as field gene banks.

9.2.2 In Situ Conservation

9.2.2.1 Tissue Culture as In Vitro Gene Banks

An alternative method to preserve lily collections is conservation in vitro. In vitro stored collections need relatively small amounts of space, medium components can be used that minimize growth, plants can be

multiplied quickly, and there is often a possibility to eliminate viral diseases (Towill 1988; Withers 1991). Lily material could be stored more than 28 months at 25°C (Bonnier et al. 1997) before transfer to new medium is required. However, at each transfer event, there is a risk of contamination with microbial organisms (Withers 1991). Furthermore, the establishment of an in vitro collection is labor-intensive and genotypes may react differently under identical conditions (Towill 1988).

Slow growth increases the maximum storage duration. In vitro slow growth could be obtained by low temperature (−2°C), osmotic stress (Grout 1991; Withers 1991), or by a low concentration of nutrients (Engelmann 1991). However, the low temperature is commonly used to store lily bulbs (Beattie and White 1993; Bonnier and Van Tuyl 1997).

9.2.2.2 Cryopreservation in Liquid Nitrogen

Cryopreservation of lily meristems could be a suitable method for long-term preservation. Research by Bouman and De Klerk (1990) resulted in the survival of 8% of meristems of *L. speciosum*. By using the technique of vitrification, apical meristems from scale bulblets of *L. japonicum* had been successfully cryopreserved. The rate of shoot formation after cryopreservation was approximately 80% after 4 weeks. Later on, this vitrification method was also successfully applied to five other lily genotypes (Matsumoto et al. 1995). Although so many methods could be used for the preservation of lily germplasm, the existing challenge is to interface between the in situ and ex situ system. Until now, the two systems are more or less implemented independently by two different groups of people and institutions with a different basic conservation philosophy.

9.3 Role in Development of Cytogenetic Stocks and Their Utility

Because of the large size of their chromosomes (Bennett and Smith 1976), *Lilium* species have been used in cytological and cytogenetic studies for a long time. However, there has been very little interest in producing cytogenetic stocks such as tri- or monosomic series or alien addition or substitution lines as has been done in many other crop plants. There are a few main reasons for this. Lily is not a major leading crop when compared to wheat, maize, or tomato that are well known for such developments; secondly, the generation time in lily – from seed germination to seed production – exceeds two or more years. This obviously discourages the development of cytogenetic stocks in lily. Nevertheless, whenever *Lilium* species or cultivars are used in any significant investigation, the names of the species or cultivars are promptly mentioned so that they are available for verification when necessary. There are several examples of basic studies such as: chromosome identification, karyotype analysis (Stewart 1947), chiasma formation and crossing-over (Brown and Zohary 1955; Fogwill 1958), and time and duration of female meiosis (Bennett and Stern 1975), where the material used in the experiments is well documented. Later on, different banding pattern techniques such as C- and Q-banding pattern were used to study the chromosome structure of different *Lilium* spp. (Holm 1976; Von Kalm and Smyth 1984; Smyth et al. 1989). These banding patterns were also used to detect the nucleolar organizer regions (NORs) (Von Kalm and Smyth 1980, 1984; Smyth et al. 1989). Flow cytometry has been employed to estimate the genome size of different *Lilium* species (Van Tuyl and Boon 1997). Although systematic development of cytogenetic stocks is lacking in lily, there is evidence that alien additions and substitutions are possible in the case of *Lilium*.

In general, the karyotypes of individual plant species, but not their hybrids or progenies, are used for comparison of evolutionary trends, if any. But in the case of *Lilium* not only the individual species but also their hybrids and their progenies have been used for karyotype studies. Besides using conventional staining methods, DNA in situ hybridization has been used for distinguishing the chromosome constitution of the hybrids and the progenies. Obviously, these analyses have provided a great wealth of information on chromosome morphology as well as on homoeology and crossing-over relationships among the genomes of different species involved in the development of the most important lily cultivars. In this section, both conventional as well as molecular methods of karyotype analyses in the genus *Lilium* are described.

Among angiosperms, the species of the genus *Lilium* have the largest genomes with haploid DNA content (1C) ranging from 32.75 pg in *L. pyrenaicum* (Bennett 1972) to 47.90 pg in *L. canadense* (Zonneveld et al. 2005). The species are diploids with 24 chromosomes ($2n = 2x = 24$), in several species aneuploids were found having additional chromosomes or chromosome fragments (Stewart 1943). Tiger lily (*L. tigrinum*) is the only triploid species in the genus (Noda 1978; Kim et al. 2006). *Lilium* species represent interesting material for cytological study due to exceptionally large size of chromosomes, which ranges, in *L. longiflorum* from 18.1 μm to 34.4 μm (Lim et al. 2000, 2001a, b). There are many reports on chromosome morphology and karyotype analysis by using conventional cytological techniques (Stewart 1947; Darlington and Wylie 1995; Lighty 1960; Fedorov 1969; Marasek and Orlikowska 2003). However, chromosomes' morphology (length and centromere position) is highly conserved within and between species, therefore only a few chromosomes are recognizable on the basis of the above traits (Lim et al. 2001a, b; Marasek and Orlikowska 2003). Chromosomal markers in lily refer predominantly to the presence and the position of secondary constrictions (Uhring 1968; North and Wills 1969; Okazaki et al. 1994; Fernandez et al. 1996; Obata et al. 2000; Marasek and Orlikowska 2003). Chromosome morphology and the presence and position of secondary constriction have been also used to verify *Lilium* hybrids (Okazaki et al. 1994; Obata et al. 2000). In order to help in *Lilium* chromosome identification, banding techniques have been applied such as silver staining of nucleolar organizing regions (Ag-NORs) (Von Kalm and Smyth 1980; Smyth et al. 1991), staining of heterochromatin sections (C-bands) (Smyth et al. 1989; Smyth 1999), and fluorescent staining (Kongsuwan and Smyth 1977; Lim et al. 2001a, b; Marasek et al. 2005). Fluorescent in situ hybridization (FISH) is another technique producing chromosomal markers that show positions of specific genes thereby permitting chromosomal identification. The use of FISH with 5S rDNA and 25S rDNA probes has provided molecular cytogenetic markers for identification of somatic chromosomes in different *Lilium* species and has been used for verification of lily hybrids (Lim et al. 2001a, b; Marasek et al. 2004a, b). Genomic in situ hybridization (GISH) using labeled whole-genomic DNA enables unambiguous distinction between the different genomes in interspecific crosses, which makes it very useful for identifying plant hybrids and chromosome recombination. In the genus *Lilium*, GISH was successfully used for identification of parental chromosomes in hybrid (Lim et al. 2000a, b; Barba-Gonzalez et al. 2005a, b) and to trace recombination events in BC_1 and BC_2 progenies of LA and OA hybrids (Karlov et al. 1999; Lim et al. 2000a, b; Barba-Gonzalez et al. 2005a, b; Zhou et al. 2008). Based on the recombination sites identified through GISH cytological maps were constructed for four (L, O, and two times A in different backgrounds) lily genomes, which can be used as landmarks for assigning molecular markers or desirable genes to chromosomes of *Lilium* (Khan et al. 2009a). GISH has also been applied to reveal the restitution mechanisms (FDR – first division restitution, IMR – intermediate division restitution, SDR – second division restitution) that lead to different chromosome constitution in $2n$ gametes (Lim et al. 2001a, b, 2004; Barba-Gonzalez et al. 2005a, b; 2008).

Karyotype analysis of interspecific hybrids and their backcross progenies through GISH technique can provide highly useful information that cannot be obtained by conventional methods of analyses of chromosomes of species alone. In interspecific hybrids, the homoeologous chromosomes can be directly compared through GISH, as in the case of the F_1 hybrids of Longiflorum × Asiatic (LA), Oriental × Asiatic (OA), and *L. auratum* × *L. henryi* (AuH) (Lim et al. 2000a, b; Van Tuyl et al. 2002; Barba-Gonzalez et al. 2005a, b). In the backcross progenies, besides a direct comparison of homoeologous chromosomes of the parents, invaluable insight can be obtained on intergenomic crossing-over. For example, highly unequal distribution of crossovers among different pairs of homoeologous chromosomes in the BC progenies of LA and OA hybrids has been observed (Khan et al. 2009a). Analyses of karyotypes of triploid BC_1 progenies derived from the functioning of $2n$ gametes of LA and OA hybrids have been useful to determine the restitution mechanisms (viz., FDR and IMR), through which the sexual polyploid progenies originated. Furthermore, besides triploid BC progenies, in the case of LA hybrids, it has also been possible to produce diploid ($2n = 2x = 24$) BC_1 progenies through the use of haploid gametes from LA hybrids. Karyotype analysis of these diploid BC_1 progenies has yielded useful information on the extent of intergenomic crossing-over in the diploid LA hybrids (Khan et al. 2009b).

This investigation has conclusively established that, despite considerable genome differentiation between the karyotypes of different *Lilium* species of different taxonomic sections, the homoeologous chromosomes appear to be mostly homosequential as far as crossovers are concerned and they fully compensate for each other.

9.4 Role in Crop Improvement Through Traditional and Advanced Tools

Commercial cultivation of the three major groups of lilies, viz., Longiflorum, Asiatic and Oriental lilies, is practiced through vegetative propagation of bulbs. Also the parental wild species of *Lilium* from which these cultivars have originated reproduce through bulbs and thus are vegetatively propagated. There are certain constraints to breed such vegetatively propagated crops. These are: a high degree of heterozygosity, sterility of the hybrids and progenies, segregation for deleterious recessive genes and effort needed to produce large progeny populations that could facilitate selection. Some or all of these constraints may be relevant to lilies. Besides these limitations, if the final products, i.e., cultivars, are to be polyploids the breeding procedures have to be appropriately modified. Taking the above considerations into account, the possibilities of crop improvement in lily are highlighted in this section.

9.4.1 Overcoming Interspecific Crossing Barriers

Hybridization between the cultivars or species from different taxonomic sections in the genus *Lilium* is generally difficult and can succeed only through the use of special techniques. In the last decades, many different techniques have been developed to overcome pre-fertilization barriers. These include:

1. Cut-style method. This method has been utilized to produce interspecific hybrids in the genus *Lilium* (Myodo 1962; Van Tuyl et al. 1991), *Allium* (Doubouzet et al. 1994), and *Fritillaria* (Wietsma et al. 1994). This method comprises the deposition of pollen on the stylar surface after cutting the style with stigma (usually a millimeter above the ovary), and allowing pollen to circumvent stylar barriers, which normally inhibit pollen tube growth (Myodo 1962). A variation of this method is the stylar graft technique, in which pollen grains are deposited on a compatible stigma. After 1 day, the style of the pollen donor is cut 1–2 mm above the ovary and grafted on to the ovary of another plant. Style and stigma are joined in vivo using a piece of a straw filled with stigmatic exudate or are stuck together with only the exudate (Van Tuyl et al. 1991; Van Creij et al. 2000).
2. Pull-style method. This technique involves pulling out the receptive style, brushing its base with the desired pollen, and reinserting it into the ovary. It has been applied to overcome pre-fertilization barriers in the genus *Allium* (Doubouzet et al. 1994).
3. Intrastylar pollination. In this method, a compatible species' style is utilized as a pistil donor and it is pollinated on the stigma with pollen from another species. This has been utilized successfully to produce *Lilium*-interspecific hybrids (Asano and Myodo 1977a; Asano 1980).
4. Mentor pollen. This method consists of "inactivating" genetically by irradiation-compatible pollen (but it is still capable to germinate) and mix it in incongruent pollen. This method has been useful to overcome self-incompatibility but not in interspecific crosses in the genus *Lilium* (Van Tuyl et al. 1982). In the genus *Cucumis* when this method was utilized on interspecific crosses, embryo-like structures were developed but they were not able to germinate (Den Nijs and Oost 1980).
5. In vitro pollination. Several in vitro pollination methods have been developed in order to overcome pre-fertilization methods. These include:
   a. Stigmatic pollination. This method has been successful for the production of compatible embryos of lily and Nerine (Van Tuyl et al. 1992). It consists of normal pollination and the successive artificial cultivation of the entire pistil.
   b. Placental pollination. This method consists of performing a longitudinal cut in the ovary, exposing the ovules, and applying an abundant amount of pollen. These method has been utilized in the genus *Tulipa* (Van Creij 1997) and *Lilium* (Van Creij et al. 2000), with successful

penetration of the pollen tube into the ovaries, however, there was no embryo formation.

Once pre-fertilization barriers are overcome, hybrid embryo growth is restricted by post-fertilization barriers. Both embryo and endosperm have to develop an equilibrium for sharing nutrients in an undisturbed developmental process. In general, the first division of the zygote is delayed to favor the first division cycles of the endosperm cells. When the equilibrium in the development of the zygote and endosperm is disturbed, an abortion of the young embryo or disintegration of endosperm follows. This abortion can take place in various stages of development of the young seed. Depending on the stage of embryo abortion, various in vitro techniques can be applied to rescue the abortive embryo (Van Tuyl and De Jeu 1997).

In the last decades, the production of a wider number of interspecific hybrids was possible with the development of several in vitro methods that made possible to overcome the post-fertilization barriers, these methods include:

1. Ovary slice. This method has been applied mainly in the production of interspecific hybrids of the genus *Lilium* (Straathof et al. 1987; Kanoh et al. 1988; Van Tuyl et al. 1991; Arzate-Fernandez et al. 2006) and *Tulipa* (Van Creij et al. 2000). It can be applied when the maternal tissue does not have a negative effect on the development of the seeds (Van Tuyl and De Jeu 1997). It consists in slicing the ovary in placing it in vitro, where the seeds are allowed to grow until embryos can be dissected.
2. Ovary culture. This method has been utilized to produce interspecific hybrids in several genera, these include: *Allium* (Nomura et al. 2002), *Lilium* (Van Tuyl et al. 1982, 1991), *Nemesia* (Datson et al. 2006), and *Nerine* and *Tulipa* (Van Tuyl et al. 1990). This method consists of surface sterilization of ovaries and the excision of ovules and transfer to a substrate that allows them to either grow until the embryo can be excised or has germinated.
3. Embryo rescue. This method consists of surface sterilization of ovary and the excision of immature embryos out of the ovules. This method is one of the most effective in the production of interspecific hybrids, and it is utilized mainly when there is no endosperm in the seed and very small embryos are produced, which usually abort in early developmental stages (Myodo 1975; Asano and Myodo 1977b). This method has been applied in the production of interspecific and intergeneric hybrids of the genera *Allium* (Bino et al. 1989), *Alstroemeria* (Buitendijk et al. 1992, 1995), *Lilium* (Van Tuyl et al. 1991), *Pelargonium* (Denis-Peixoto et al. 2006), and *Primula* (Kato et al. 2001). One of the main problems in embryo culture in some cases is the size of the embryo itself; however, modifications to this method have been introduced that mitigate this. The ovaries are cut off into half and placed in liquid medium, in which the embryos are capable to germinate and the plantlets can be subsequently transferred to solid medium (Van Tuyl and De Jeu 1997). Using special pollination techniques, many interspecific hybrids have been produced in the genus *Lilium* and the examples are furnished in Table 9.1.

Whereas a number of interspecific hybrids have been produced through the use of special hybridization techniques, there is also a fairly long list of hybrids obtained between species within a section in the genus *Lilium* without special techniques as well (Table 9.2).

Until relatively recently, most of the cultivars in all the three major hybrid groups of lilies were diploid ($2n = 2x = 24$), and most importantly, they were *intrasectional* species hybrids such as Longiflorum (L), Asiatic (A), Oriental (O), and Trumpet (T), etc., in which closely related species were involved as parents. However, in recent years *intersectional* species hybrids such as LA, OT, and LO involving distantly related cultivars/species are most successfully cultivated (Lim et al. 2008). These are not only hybrids between the cultivars of different sections but also they are polyploids possessing distinctly differentiated genomes (i.e., allopolyploids). Obviously, these allopolyploid hybrids are most ideal for combining desirable horticultural traits available in the distantly related cultivars/species into new cultivars. A recent study of the LA group of the Dutch lily cultivars has shown that they are predominantly triploid ($2n = 3x = 36$) with one genome of L and two genomes of A (i.e., LAA) constitution (Zhou et al. 2008). The predominance of triploid cultivars also indicates that this particular ploidy level is the most ideal threshold for a successful cultivar. An important drawback of triploid cultivars is that they are not suitable for use in further breeding.

Table 9.1 Reports of successful intersectional crosses among different species of the genus *Lilium* after special pollination techniques and embryo rescue

Cross	References
L. alexandrae × *L. auratum*	McRae (1972)
L. alexandrae × *L. speciosum*	McRae (1972)
L. alexandrae × *L. rubellum*	McRae (1972)
L. alexandrae × *L. nobilissimum*	McRae (1978)
L. speciosum × *L. rubellum*	Matsumoto (1992)
(*L. auratum* × *L. japonicum*) × *L. rubellum*	McRae (1972)
L. nobilissimum × *L. henryi*	Asano (1982)
L. pyrenaicum × *L. pomponium*	North (1994)
L. henryi × *L. candidum*	Van Tuyl et al. (2002)
L. lankongense × *L. davidii*	Marshall (1983b)
L. lankongense × *L. cernuum*	Marshall (1983b)
L. lankongense × *L. leichtlinii*	Marshall (1983b)
L. lankongense × *L. duchartrei*	Marshall (1983b)
L. duchartrei × *L. davidii*	Marshall (1983b)
L. taliense × *L. davidii*	Marshall (1983b)
L. pumilum × *L. leichtlinii*	McRae and McRae (1985)
L. cernuum × *L. dauricum*	McRae and McRae (1979)
L. cernuum × *L. concolor*	McRae and McRae (1979)
L. tigrinum × *L. regale*	McRae (1991)
L. longiflorum × *L. cernuum*	Myodo and Asano (1977)
L. longiflorum × *L. henryi*	Myodo and Asano (1977)
L. formosanum × *L. speciosum*	Myodo and Asano (1977)
L. longiflorum × *L. dauricum*	Asano and Myodo (1980)
L. longiflorum × *L. pumilum*	Asano and Myodo (1980)
L. longiflorum × *L. brownii*	Van Tuyl (1980)
L. longiflorum × *L. martagon*	Van Creij et al. (1990b)
L. longiflorum × *L. rubellum*	Van Tuyl et al. (2000)
L. longiflorum × *L. Bulbiferum*	Van Tuyl et al. (2000)
L. longiflorum × *L. canadense*	Van Tuyl et al. (2000)
L. longiflorum × *L. concolor*	Van Tuyl et al. (2002)
L. longiflorum × *L. hansonii*	Van Tuyl et al. (2005)
L. longiflorum × *L. monadelphum*	Van Tuyl et al. (2005)
L. longiflorum × *L. kelloggii*	Van Tuyl et al. (2011)
L. longiflorum × *L. sempervivoideum*	Van Tuyl et al. (2011)
L. longiflorum × *L. pardalinum*	Van Tuyl et al. (2011)
L. longiflorum × *L. hansonii*	Van Tuyl et al. (2005)
L. longiflorum × *L. bakerianum*	Lim et al. (2008)
L. longiflorum × *L. hansonii*	Lim et al. (2008)
L. longiflorum × *L. lophophorum*	Wang et al. (2009)
L. regale × *L. leichtlinii*	Matsumoto (1992)

Therefore, starting from diploid intersectional cultivar/species hybrids (e.g., LA, OA), different breeding strategies can be envisaged including (1) breeding at the diploid level through the production of diploid BC progenies; (2) use of unreduced (2*n*) gametes to produce triploid cultivars (unilateral sexual polyploidization); (3) use of bilateral sexual polyploidization; and (4) use of somatically doubled allotetraploids.

9.4.2 Breeding at the Diploid Level

Hybrids between two diploid cultivars/species from two different taxonomic sections are, in almost all cases, completely sterile and they cannot be used as parents. Rarely however, such F_1 hybrids do produce normal haploid gametes and they can be used to generate diploid BC progenies as has been demonstrated

Table 9.2 Reports of successful intrasectional crosses without the help of special pollination techniques and embryo rescue among different species in the genus *Lilium*

Cross	Reference	Cross	Reference
L. speciosum × *L. auratum*	Yerex (1948)	*L. davidii* × *L. dauricum*	Marshall (1981)
L. speciosum × *L. japonicum*	Woodriff (1958)	*L. davidii* × *L. cernuum*	Marshall (1981)
L. speciosum × *L. rubellum*	Woodriff (1959)	*L. callosum* × *L. dauricum*	Marshall (1983a)
L. speciosum × *L. henryi*	Emsweller and Stuart (1948)	*L. davidii* × *L. wilsonii*	Marshall (1981)
L. speciosum × *L. nepalense*	Woodriff (1969)	*L. davidii* × *L. bulbiferum*	Marshall (1981)
L. auratum × *L. japonicum*	Pfeiffer (1952)	*L. davidii* × *L. leichtlinii*	Marshall (1981)
L. auratum × *L. nobilissimum*	Matsumoto (1992)	*L. davidii* × *L. amabile*	Marshall (1981)
L. sargentiae × *L. regale*	Yerex (1948)	*L. davidii* × *L. maculatum*	Slate (1968)
L. sargentiae × *L. henryi*	Yerex (1948)	*L. wilsonii* × *L. bulbiferum*	Marshall (1981)
L. henryi × *L. leucanthum*	Yerex (1948)	*L. wilsonii* × *L. dauricum*	Marshall (1981)
L. henryi × *L. regale*	Andel (1982)	*L. bulbiferum* × *L. dauricum*	Marshall (1981)
L. leucanthum × *L. sargentiae*	Yerex (1948)	*L. dauricum* × *L. maculatum*	Clas (1972)
L. henryi × *L. auratum*	Henningsen (1951)	*L. amabile* × *L. maculatum*	Fisher (1969)
L. hansonii × *L. martagon*	Lawrence (1950)	*L. candidum* × *L. chalcedonicum*	Lawrence (1949)
L. hansonii × *L. medeoloides*	Skinner (1949)	*L. tigrinum* × *L. amabile*	Knox-Finlay (1977)
L. martagon × *L. medeoloides*	Lawrence (1950)	*L. tigrinum* × *L. davidii*	Patterson (1955)
L. martagon × *L. tsingtauense*	Doak (1977)	*L. tigrinum* × *L. maculatum*	Slate (1968)
L. concolor × *L. callosum*	Slate (1968)		
L. concolor × *L. dauricum*	McRae (1978)	*L. longiflorum* × *L. formosanum*	Kline (1950)
L. concolor × *L. pumilum*	Preston (1948)	*L. parryi* × *L. kelloggii*	Walden (1962)
L. pumilum × *L. dauricum*	Marshall (1983a)	*L. parryi* × *L. pardalinum*	Kline (1948)
L. pumilum × *L. bulbiferum*	Marshall (1983a)	*L. bolanderi* × *L. kelloggii*	Beane (1957)
L. pumilum × *L. davidii*	Marshall (1983a)	*L. pardalinum* × *L. bolanderi*	Woodriff (1950)
L. pumilum × *L. amabile*	Marshall (1983a)	*L. canadense* × *L. parryi*	Showalter (1961)
L. pumilum × *L. cernuum*	Hager (1953)	*L. michiganense* × *L. canadense*	Wadekamper (1988)
L. callosum × *L. amabile*	Marshall (1983a)	*L. superbum* × *L. canadense*	Pfeiffer (1976)

in the case of LA hybrids (Khan et al. 2009b). This has opened up the prospect of breeding and selection at the diploid level after which the selected genotypes can be used to produce triploid cultivars. For this synthesis, either unilateral sexual polyploidization or diploid × (synthetic) tetraploid crossing can be used. This procedure is somewhat similar to breeding other vegetatively propagated autopolyploid crops like potato (Chase 1963; Mendiburu et al. 1974).

9.4.3 Use of Unilateral Sexual Polyploidization

Although F_1 hybrids between cultivars of different sections are mostly sterile, a small percentage of them can produce 2*n* gametes in reasonable frequencies. It is easy to detect such genotypes because the presence of larger pollen grains is a reliable indication. Detection of genotypes that produce 2*n* eggs is more difficult because it requires crossing with normal pollen and testing whether it leads to fruit and seed set. One of the important advantages of using unilateral sexual polyploidization is that the resulting progenies possess intergenomic recombination. As a consequence of the presence of recombinant segments in the BC_1 progenies, there is scope for the expression of recessive loci that might become nulliplex in the BC_1 triploids (Barba-Gonzalez et al. 2005a, b). This means that selection can be effective in the BC_1 generation itself as it might have been the case in Dutch cultivars derived from LA hybrids (Zhou et al. 2008). A cardinal feature of 2*n* gametes in distant hybrids such as LA or OA genotypes is that they originate predominantly through first division restitution (FDR) and because of this the heterozygosity of the parental hybrids is largely preserved in the 2*n* gametes and transferred to the progenies. This is in contrast to the use of 2*x* gametes derived from the somatically doubled tetraploids, which can lead to "inbreeding depression" giving rise to weakly performing polyploid progenies.

The important advantages of using 2*n* gametes are that they help to overcome the F_1 hybrid sterility, transfer hybrid vigor, facilitate intergenomic recombination, and directly give rise to triploid progenies that are preferred for cultivar selection.

9.4.4 Bilateral Sexual Polyploidization

As was pointed out earlier, only very few genotypes of F_1 *Lilium* hybrids produce either 2*n* pollen or 2*n* eggs in reasonable frequencies but none of the genotypes that have been examined so far produce both types of 2*n* gametes in appreciable frequencies. Therefore, it has never been possible to obtain a tetraploid through the functioning of 2*n* pollen and 2*n* egg from the same diploid hybrid parent. However, by using two separate LA hybrids as parents, one donating 2*n* egg and the other 2*n* pollen, it has been possible to produce tetraploid progenies (Khan et al. 2010). Such bilateral sexual (tetraploid) progenies have certain advantages for using them as parents in breeding. In the first place, they are expected to be reasonably fertile because of their allotetraploid constitution (LLAA). Secondly, they will not have the drawback of being "permanent hybrids" in which no recombination can occur. On the contrary, because they have originated through 2*n* gametes, these allotetraploids do possess recombinant segments in some pairs of chromosomes. This means, such genotypes have the potential for segregation of genetic traits that might be present on the distal parts of the crossover segments. Thus, there is scope for selection of sexual tetraploids, which may be repeatedly used as parents in order to produce triploid progenies.

9.4.5 Somatic Chromosome Doubling

One of the widely used methods for overcoming the F_1 sterility of distant hybrids was to double the chromosome numbers of such hybrids and produce allopolyploids that are mostly fertile. Chemicals such as colchicine and oryzalin, among others (van Tuyl et al. 1992; Barba-Gonzalez et al. 2006a), have been successfully used for this purpose. One of the drawbacks of allopolyploids produced through somatic doubling is that there will not be any intergenomic recombination between the parental genomes due to autosyndetic pairing of chromosomes (Lim et al. 2000a, b). Nevertheless, this method has been successfully used for producing polyploid cultivars of lily. Apart from the use of colchicine or oryzalin, the use of nitrous oxide (N_2O) for chromosome doubling in the germinal cells has proven to be effective in producing 2*n* gametes with certain amount of intergenomic recombination (Barba-Gonzalez et al. 2006a, b). This method can be a substitute in order to induce 2*n* gametes in those genotypes that normally never produce such gametes (or only in very low quantities). The potential of this method must be further evaluated for large-scale application.

So far, lily breeding has been carried out through traditional methods. These approaches are time-consuming, especially in this crop, because its generation time is about 2–3 years from seed germination to maturity of fruits and seeds. In such situation, it is attractive to apply molecular methods of tagging desirable traits and practice the so-called marker-assisted selection, which might reduce time. In this context, linkage maps have been constructed using amplified fragment length polymorphism (AFLP) and diversity array technology (DArT) markers (Van Heusden et al. 2002; Khan 2009; Shahin et al. 2010). Like in other crops, these molecular methods might be potentially useful in lily as well.

9.5 Genomics Resources Developed

Besides morphological or phenotypic resources, few molecular aspects have also been investigated in *Lilium*. Zhang et al. (2008) characterized one of the protease inhibitors, a trypsin inhibitor (17 kDa), in the bulb of *Lilium brownii*. The amino acid sequence of this protease showed similarity to a short fragment of a known trypsin inhibitor from *Populus tremula* and a putative trypsin inhibitor from *Arabidopsis thaliana*. Trypsin (protease) inhibitors are quite important compounds due to their role as defense proteins against pests. Another compound "free mannose" has been characterized in *L. longiflorum* bulbs (Miller 1989). Besides the starch, which is known to be the main storage carbohydrate in *Lilium*, glucomannan has been recorded (Tomoda et al. 1978) to comprise

about 15% of the carbohydrate in the bulb (Matsuo and Mizuno 1974). This carbohydrate is a water-soluble polysaccharide that is considered as a dietary fiber and used in food as an emulsifier and thickener.

Because the sexual reproduction system is highly important in plants, many studies were carried out in order to understand the complex process of transportation of sperm to egg cell. Kim et al. (2003) identified a chemotropic molecule "chemocyanins" in lily stigma, which is a small basic protein that shows sequence similarity to plantacyanins, cell wall proteins of unknown function (Nersissian et al. 1998) and that belongs to the ancient phytocyanin family of blue copper proteins (Ryden and Hunt 1993). Genes that encode histone proteins have been reported in male gametic cells within the pollen grain of *L. longiflorum*. Histones are highly conserved throughout the evolution and are encoded by multigene families (Xu et al. 1999). H3 and H2B have been identified as potential tissue-specific histones in the generative cell of lily (Ueda and Tanaka 1995). Later on Xu et al. (1999) isolated and characterized *gcH2A* and *gcH3* histone genes from a cDNA library of *Lilium* generative cells. These two genes are expressed specifically in the generative cell but not in microspores undergoing pollen mitosis I or in other dividing cells of *Lilium* somatic tissue (Xu et al. 1999). Other three core histones were specifically expressed in the generative cell of lily: gH2A, gH2B and gH3, which had been proposed to be specific core histones that contribute to chromatin condensation of male gametes or to chromatin remodeling, and resulted in the repression of gene expression in male gametes (Ueda et al. 2000, 2005). Expressed sequence tags (ESTs) of the generative cells were constructed from *L. longiflorum* with an aim to detect gametophyte-specific genes. About 886 ESTs were generated and clustered into 637 unique ESTs of which 70% showed significant similarity to *Arabidopsis* genes with known function. Among these, 129 ESTs showed significant similarity to male gametophyte-specific transcripts, and 55 ESTs appeared to have significant hits in both maize sperm cell ESTs and *Arabidopsis* male gametophyte-specific genes, suggesting that these genes are common across different plant species (Okada et al. 2006). The expression of 83% of the generative-cell genes was up-regulated in generative cells, which suggests their specialized function (Okada et al. 2007).

Lily (*L. longiflorum*) pollen tube contains two exo-β-glucanases, i.e., *LP-ExoI* and *LP-ExoII*, secreted into its cell walls. These two exhibited over 80% similarity to each other. *LP-ExoI* transcripts were abundant in pollen grains. However, *LP-ExoII* transcripts found in all lily tissues were tested. In addition, it has been suggested that 1,3:1,4-β-glucan was present in lily pollen tubes. LP-ExoI and LP-ExoII may be involved in the regulation of pollen tube elongation by hydrolyzing callose and 1,3:1,4-β-glucan within pollen tube walls (Takeda et al. 2004).

The mature pollen of *L. longiflorum* has stable oil bodies that contain a protein of 18 kDa, which is accumulated massively in the late stages of pollen maturation. Immunological and sequence analysis suggest that it is a putative oleosin that represents a distinct class in comparison with oleosins found in seed oil bodies and tapetum (Jiang et al. 2007).

Many physiological processes in plant cells are highly correlated with actin cytoskeleton, such as the elongation of pollen tubes tips. The dynamic of actin cytoskeleton Rop1Ps and its importance for pollen tube elongation characteristics in *L. davidii* were investigated and have been shown by Zhao and Ren (2006). Many compounds were detected in the pollen including ABP29, LlLIM1, and LLA23. *Lilium* ACTIN BINDING PROTEIN29 (ABP29, 29 kDa) is the smallest identified member of the villin/gelsolin/fragmin superfamily, and it is a splicing variant of *Lilium* villin that plays important roles in remodeling of the actin cytoskeleton (Xiang et al. 2007). LlLIM1, also an actin-binding protein (ABPs), was identified in *L. longiflorum* pollen. It plays an important role in regulating the actin microfilaments, which is essential for polar cell tip growth (Wang et al. 2008). LLA23 is an abscisic acid-, stress-, and ripening-induced protein, which was isolated from *L. longiflorum* pollen. It was shown that LLA23 protein plays an important role against drought. This protein mediates stress-responsive ABA signaling under elevated salt concentration or dehydration (Yang et al. 2005).

MADS box genes consist of three major groups: A, B and C, and a number of functional genes have been investigated in lily. LMADS1 is homologous to the *Arabidopsis AP3* gene and was cloned and characterized from *L. longiflorum*. The messenger RNA of this gene was detected in the petals and stamens only, suggesting that this gene is possibly post-transcriptionally regulated in lily (Tzeng and Yang 2001).

Two other MADS box genes (*LMADS2* and *EgMADS1*) characterized in *L. longiflorum* showed expression in the carpel, mainly in ovules and partly in style tissues, whereas they were absent from other flower organs or vegetative leaves (Tzeng et al. 2002). *LMADS3* and *LMADS4* are two AGL2-like MADS-box genes also characterized in *L. longiflorum* and their expression was detected in the inflorescence meristem and floral buds at different developmental stages and in all four whorls of the flower organ. However, *LMADS4* was also expressed in vegetative leaf and in the inflorescence stem (Tzeng et al. 2003). *LMADS5*, *LMADS6*, and *LMADS7* have been isolated and characterized from *L. longiflorum* (Chen et al. 2008). The expression of these three genes was similar, and their RNAs were detected in vegetative stem and inflorescence meristem. *LMADS5* showed high-sequence similarity to oil palm (*Elaeis guineensis*) *EgSQUA3*, and *LMADS7* is more close to an orchid gene *DOMADS2* (Chen et al. 2008).

Very little sequence information is available for *Lilium*. Some regions of *Lilium*'s chloroplast DNA (*trn*T–*trn*L, *trn*L–*trn*F, and *atp*B–*rbc*L) have been sequenced. These sequences were used to study the phylogenic relationships among different cultivars of the *Lilium* and *Archelirion* sections (Nishikawa et al. 2002). Besides that, Sentry and Smyth (1989) characterized a 9.35 kb transposable element with 2.4 kb long terminal repeats (LTRs) from *L. henryi*).

In near future, a considerable amount of DNA sequences will be available as they are currently regenerated for genetic mapping purposes.

9.6 Domestication History of *Lilium*

Lilium has a long history of cultivation. Madonna lily (*L. candidum*) and Tiger lily (*L. tigrinum*) are believed to be the oldest domesticated floral species. The artistic and botanical evidences suggested that the wild Madonna lily (*L. candidum*) had become a garden plant during the Late Minoan period. However, little is known where and when a garden Madonna lily became sterile and vegetatively propagated by man and consequently a domesticated plant (Negbi and Negbi 2000). *L. candidum* most likely originated from the Middle Orient and the first record on cultivation of this species dates back to 3000 BC. Probably during the Iron Age, the Madonna lily was introduced into Egypt by Greek colonists. In southern Europe, the Madonna lily was introduced by the Romans and until the middle of the fifteenth century this was the only cultivated species in European gardens, but by the end of the sixteenth century other European species were introduced, e.g., Orange lily (*L. bulbiferum* L.) (Pelkonen et al. 2007) or in the Netherlands the so-called rye lily (Bos 1993). During the seventeenth and eighteenth centuries, *L. canadense* and *L. speciosum* were introduced into European gardens from North America and Asia (Woodcock and Stearn 1950). China is considered as a second center of origin of domesticated lilies. *L. tigrinum* is believed to be cultivated in China since ancient times (Haw 1986).

9.6.1 *Lilium* as Traditional Medicinal Plant Species

In the Middle Orient and eastern Asia, various lily species have been widely used as important medicinal plants for more than 2,000 years. In "The Canon of Medicine" (980–1037 AD), the bulbs, leaves and oil of the flowers of *L. candidum* have been suggested to be useful for curing injures, burns, and inflammation (Avicena 980–1037 AD after Farsam et al. 2003). In China, the history of cultivation of lily for medicinal purposes can be traced back to the Han dynasty (202 BC–220 AD). One of the oldest Chinese medical books, *jin kui yao lue*, described that lilies were prescribed for such diseases as chronic coughing, for certain blood disorders and against sleeplessness (Zhang Zhongjing and Xiwen 1995). According to Chinese herbal records, the bulbs of *L. dauricum*, *L. pumilum*, *L. longiflorum*, and *L. brownie* var. *colchesteri* have been used as a medical material (Mimaki et al. 1992) where the later has been prescribed as an antitussive and sedative (Lin et al. 2003).

The fresh and dried bulbs of *L. candidum* have been suggested to be useful in gynecological disorders, ulcer problems, burns, injuries, and may be used as a diuretic (Gruenwald 2000). Furthermore, the bulbs of several other *Lilium* species exhibited a wide spectrum of biological activities (Duke 2002).

9.6.2 Lily as Human Food

Some lily species have a long history of being used as a food ingredient. The bulbs of *L. brownie*, *L. pumilum*, and *L. tigrinum* were already collected as vegetables around 900–960 AD in China (Zhao et al. 1996).

Nowadays, there are two main cultivation centers for vegetable lilies in China: Yixing of Jiangsu Province and Lanzhou of Gansu Province. Beside China, the bulbs of *L. tigrinum*, *L. pumilum*, *L. candidum*, and *L. brownii* are grown for food purposes on a large scale in Japan, Korea, and Vietnam (Simoons 1991; Chau and Wu 2006). Lily bulbs may be dried, eaten fresh, baked, made into soup, or processed to extract starch (Simoons 1991). Similarly, native Americans used the bulbs of many species of *Lilium* for food. The Cherokee made flour from *L. canadense* during famine whereas in Saskatchewan, the Cree dried the bulbs scales of *L. philadelphicum* as snack.

9.6.3 Chemical Composition

Numerous investigations have been carried out on chemical constituents of lily species (Mimaki and Sashida 1990; Mimaki et al. 1992; Nakamura et al. 1993; Farsam et al. 2003; Eisenreichová et al. 2004). *Lilium* have turned out to be a rich source of steroidal compounds, such as steroidal saponins (Eisenreichová et al. 2004), steroidal glycosides (Mimaki et al. 1992), and steroidal alkaloids (Mimaki and Sashida 1990). Steroidal saponins have been extracted mainly from bulbs, e.g., of *L. candidum* (Haladová et al. 1998), *L. brownii* (Mimaki and Sashida 1990) and *L. speciosum* × *L. nobilissimum* "Star Gazer" (Nakamura et al. 1993), as well as from flowers of *L. candidum* (Haladová et al. 1998) and from flowers and corm of *L. ledebourii* (Farsam et al. 2003). Furthermore, phenolic constituents have been isolated, e.g., from fresh bulbs of *L. brownii* (Mimaki and Sashida 1990) and *L. pumilum* (syn. *L. tenuifolium*, Mimaki et al. 1989). In flowers and leaves of numerous *Lilium* species, the presence of flavonoids such as kaempferol, quercetin and isorhamnetin was confirmed (Skrzypczakova 1967). The dried bulbs of *L. tigrinum* are rich in calories, iron, and ascorbic acid (Simoons 1991).

The bulbs of some *Lilium* species exhibited different biological activities, e.g., the extract from *L. brownie* var. *colchesteri* showed the inhibitory effect on monoamine oxidases (MAOs) (Lin et al. 2003). Ethanolic extracts of *L. candidum* L. were found to express antifungal and antiyeast activity (Eisenreichová et al. 2004). Some of the saponins present in the bulbs of this species can inhibit epidermal carcinogenesis promoter activity (Vachlkov et al. 2000).

9.7 Recommendations for Future Actions

Normally, all crop plants have the threat of pests and diseases. This includes the commercially grown ornamental plants as well. But in recent years, there is a new threat. This is the implementation of laws against the use of protective chemicals that will be harmful for the environment. When such laws are rigidly implemented, it can seriously affect the cultivation of ornamental crops. This means, in addition to all other horticultural traits, breeders will have to make serious efforts to introduce disease resistances into new cultivars of lily if it has to survive as a crop. Fortunately, the genus *Lilium* includes about 100 species that are distributed widely in the northern hemisphere extending up to some tropical areas as well. This indicates that there might be scope to discover more useful genetic variation than hitherto has been done. Keeping this in mind, the following lines of work may be contemplated for the future: (1) more in-depth screening of wild germplasm for (partial) disease resistance; (2) interspecific hybridization; (3) screening for haploid and 2*n* gametes in distant hybrids; (4) perfecting methods for polyploidization; and (5) development of molecular linkage maps and tagging of useful traits.

1. The diversity within and between wild species and the current cultivated assortment is very large and for many (disease) traits not fully explored leaving significant perspective for crop improvement under current and new culture conditions.
2. The importance of creating new breeding material through interspecific hybridization cannot be overemphasized. Fortunately, methods for producing hybrids between distantly related species have been

well developed and fairly successful in lily (Van Tuyl et al. 1991, 2000; Van Tuyl and De Jeu 1997). Besides the three important hybrids of groups of lily, viz., Longiflorum, Asiatic and Oriental, it might be desirable to extend to other groups as well and pay attention to the use of wild species of other sections that might possess disease resistances and other desirable traits.

3. Based on the experience gained from LA hybrids, it is apparent that distant hybrids can produce normal haploid gametes in spite of their genome differentiation. This certainly depends on the genotypes of the parents. Once such genotypes are identified, the benefits can be highly rewarding because it can open up the way for breeding at the diploid level and by using them for producing polyploids of desired level, i.e., triploids. Besides haploid gametes, it might be worthwhile to screen for genotypes that produce 2*n* gametes. This is because, as in numerous other ornamental plants (Van Tuyl et al. 2002), polyploid cultivars in lily will be the most successful ones.
4. Not all distant F_1 hybrids might be able to produce genotypes with 2*n* gametes. In such cases, it might be desirable to use traditional methods or preferably, through the use of nitrous oxide treatment, to restore fertility. But this later method may have to be refined further so as the results of the treatment will become more predictable.
5. It is imperative that molecular genetic maps might be potentially useful in breeding. At present, however, none of the molecular markers are assigned to chromosomes as yet and the number of linkage groups exceed the basic chromosome number (i.e., $x = 12$). In this context, the cytological maps of the three genomes of lily cultivars (Khan et al. 2009a) might be useful for assigning linkage groups to the respective chromosomes. Regardless of the availability of general high-quality linkage maps, however, it might be useful to develop markers to tag useful quantitative traits and increase the efficiency of selection in breeding. The use of sequences of the lily genome will become important in the near future for the construction of genetic maps, which are needed for molecular marker-assisted breeding (P Arens and JM van Tuyl, unpublished results).

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