

A Molecular Cytogenetic Analysis of Introgression in Backcross Progenies of Intersectional *Lilium* Hybrids

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ABSTRACT

The genus *Lilium* is comprised of about 100 species and has been divided into seven taxonomic sections. The abundance and diversity of species within the genus *Lilium* offers numerous and rewarding possibilities to lily breeders. Species within the same section can be crossed by conventional hybridization and this has led to different hybrid groups of great commercial importance such as Longiflorum, Asiatic and Oriental lilies. On the other hand, the divergence of species between various taxonomic sections causes considerable difficulties for intersectional crosses. Such difficulties include crossing incompatibility barriers, embryo abortion, sterility and reduced fertility in intersectional hybrids. For these reasons, various pollination techniques followed by *in vitro* embryo (sac) rescue and ovary culture, chromosome doubling, and $2n$ gametes are used frequently to obtain progeny between parents from different sections. Being the largest genome in the plant kingdom, lily is used as a model plant for cytogenetic analysis. The genome composition of the hybrids and backcross progenies were monitored through genomic *in situ* hybridization (GISH). The progress in molecular cytogenetic studies has been associated with the analysis of introgression of chromosomal segments in backcross progenies of various interspecific hybrids, contribution of individual genome in the resultant progenies, and the mechanism of $2n$ gamete formation. Based on the cytological analysis of progenies derived from the use of both haploid and $2n$ gametes, cytological maps of three different genomes have been constructed and the relevance of these analyses for introgression in *Lilium* is demonstrated.

Keywords: chromosomal recombination, *in situ* hybridization, inter specific hybrids, $2n$ gametes, polyploidization

Abbreviations: BC, back cross; GISH, genomic *in situ* hybridization; FISH, fluorescence *in situ* hybridization; LA, Longiflorum × Asiatic; LMoV, Lily-Mottle-Virus; OA, Oriental × Asiatic; QTL, quantitative trait locus

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INTRODUCTION

Lilies originated in the Himalayan region and they have been extended over the mountain areas in the Northern hemisphere (De Jong 1974). They have been cultured since ancient times and with the passage of time became one of the most important ornamental crops (Woodcock and Stern 1950). The genus *Lilium* belongs to the Liliaceae family and it is comprised of about 100 species (Comber 1949), and thousands of cultivars (Leslie 1982-2005). The lily species are taxonomically classified into seven different sections based on various morphological and physiological characteristics. The seven sections are *Lilium*, *Martagon*, *Pseudolirium*, *Archelirion*, *Sinomartagon*, *Leucolirion* and *Oxypetalum* (Comber 1949; De Jong 1974).

The species from these sections and their hybrids are used as garden plants and attractive for gardeners in Europe and North America. The lilies were sold in the markets

which were propagated by seeds or clones from species until the attempts were made to make lily hybrids by Oregon Bulb Farm (OBF) after World War II (Lim *et al.* 2008). Initially, Asiatic hybrids were developed by OBF like 'Enchantment', 'Tabasco', 'Showboat' etc. Only since 1970's lily became an important cut flower in the commercial market and Oriental hybrids were introduced such as 'Sans Souci', 'Black Beauty', 'Le Rêve', etc. Since early 1980's Dutch lily breeding companies played a prominent role in the growth and commercialization of lily cultivars. More than 300 cultivars are registered per year and approximately 9,500 lily cultivars have been registered (<http://www.lilyregister.com>). The new cultivars are mostly intersectional (interspecific) hybrids and their demand is increasing year by year. It was estimated that the total bulbs production acreage of lily in the Netherlands was 4,161 ha in 2006 (Lim *et al.* 2008). Altogether more than 24% of total lily bulb production occupies interspecific hybrids. The lily



Fig. 1 Figure from different lily hybrids. (A) Longiflorum hybrids, (B) Asiatic hybrids (C) Oriental hybrids, and (D) Trumpet hybrids.

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. Most of the cultivars, however, are interspecific hybrids within the sections (especially the Longiflorum, Oriental, Asiatic and Trumpet groups) and the most important cultivated groups are:

The **Longiflorum** hybrids (L-genome) originate from intra- or interspecific hybridization within the Leucolirion section. Most of these hybrids have trumpet-shaped, pure white flowers such as *L. longiflorum*, while *L. formosanum* have purple backed tepals. The hybrids in this section have a distinctive fragrance, year-round forcing ability and mostly outward-facing flowers (McRae 1990). Longiflorum hybrids involve the species *L. formosanum* and *L. longiflorum*.

The **Asiatic** hybrids (A-genome) are derived from interspecific crosses among at least 13 species of the Sinomartagon section such as *L. amabile*, *L. bulbiferum*, *L. cernuum*, *L. concolor*, *L. dauricum*, *L. davidii*, *L. duchartrei*, *L. langkense*, *L. leichtlinii*, *L. maculatum*, *L. nepalense*, *L. pumilum*, and *L. tigrinum* (Leslie 1982-2005). Cultivars of Asiatic hybrid lilies have a wide colour variation in their flower tepals (orange, white, yellow, pink, red, purple and salmon) and early to late flowering (Woodcock and Stern 1950). Some species in this section show resistance to *Fusarium* and viruses (McRae 1998).

The **Oriental** hybrids (O-genome) are nowadays the most important lily hybrid group. They result from hybridization among six species of the Archelirion section. Generally, Oriental hybrids are late-flowering, with big and showy flowers with a pleasant fragrance (McRae 1998). Most Oriental hybrids show a fair degree of resistance to *Botrytis elliptica* (Barba-Gonzalez *et al.* 2005b). *L. alexandrae*, *L. auratum*, *L. japonicum*, *L. nobilissimum*, *L. rubellum*, *L. speciosum*, together make the Oriental lily hybrids.

The **Trumpet** hybrids (T-genome) are mostly selected for their flower type. The flowers are mostly yellow, sometimes pink and upright in position with distinctive fragrance. *L. henryi* and *L. regale* are included in this section. Longiflorum, Asiatic, Oriental and Trumpet hybrids have been shown in **Fig. 1**.

The species mentioned within each section are closely related. Since their genomes have close affinity, they are relatively easy to cross, and most of the F₁ hybrids are fertile (McRae 1990; Van Tuyl *et al.* 2002). These intra specific or intra sectional hybrids will not be referred to as 'hybrids' hence forth in this manuscript. On the contrary, the species that belong to different sections are difficult to hybridize, their genomes are clearly differentiated and the F₁ hybrids are sterile (with a few exceptions) and they will be indicated as hybrids. The species of the mentioned four main groups of lilies possess valuable horticultural characters such as flower size, shape, colour, direction of the flowers, stem length, different forcing periods, and the resistance to various fungal and viral diseases caused by *Fusarium oxysporum*, *Botrytis elliptica* and LMoV (Straathof and Van Tuyl 1994; Straathof and Löffler 1994a, 1994b; McRae 1998). The main aim of lily breeding is to combine desirable traits from different sections into new cultivars.

The present review focuses on cytogenetic of lily species with relation to introgression in the back cross pro-

genies of intersectional lily hybrids. The attention was given to the species which are used in breeding of improved cultivars, including interspecific hybrids. Another important topic which is discussed in this article is meiotic analysis in *Lilium* species and 2n gametes formation in interspecific lily hybrids. Some recent work on chromosomal recombination and their rearrangements in interspecific lily hybrids using molecular cytogenetic studies is also reviewed.

INTERSPECIFIC HYBRIDIZATION IN LILY

As mentioned above lilies possess a wide variety of horticultural characteristics of major importance, like distinctive small and large flowers, various shapes and direction, wide variation in colours, different forcing time, variability in stem length and strength. However, resistance to certain pathogens restricted only to some species within different section. To combine these characteristics into a new hybrid, crosses are made despite of having pre- and post-fertilization barriers; however, this is the most important tool to combine traits from different sections to generate a completely new cultivar. **Fig. 2** represents the crossing polygon of successful crosses of species between different sections of the genus *Lilium*. It was found that some of the hybrids in Oriental and Asiatic lilies are resistant to *F. oxysporum*, and *B. elliptica* (Barba-Gonzalez *et al.* 2005b). The F₁ hybrids of these interspecific crosses are obtained by overcoming the pre- fertilization and post fertilization barriers by employing different strategies such as cut style, inter stylar or *in vitro* pollination techniques followed by ovule, ovary and embryo rescue techniques (Van Tuyl *et al.* 1989a, 1989b; Van Tuyl *et al.* 1991). However, the diploid interspecific hybrids of lily are sterile. The sterility of diploid F₁ hybrids can be overcome e.g., *via* induction of polyploidization by treating diploid hybrids with colchicine or oryzalin (mitotic polyploidization) (Van Tuyl *et al.* 1992). Resulting tetraploid plants can be used for backcrossing with one of the putative parents to get triploid progenies in 4x - 2x cross combination. Although in such case, F₁ hybrid fertility is recovered, there are very little chances to get genetic variation in the subsequent progenies due to autosyndetic chromosome pairing between the parental genomes. However, it was found that some F₁ LA and OA hybrids produced 2n gametes. These hybrids were successfully used for back crossing to Asiatic parents and as the result of these crosses triploid progenies were obtained which exhibited intergenomic recombination (Lim *et al.* 2003; Barba-Gonzalez *et al.* 2004; Zhou *et al.* 2008; Khan *et al.* 2010). An important feature of sexual polyploidization through the use of 2n gametes is that it facilitates genetic variation in the BC₁ progenies which is absent in the case of BC₁ progenies generated from somatically doubled F₁ hybrids.

LILY: A MODEL PLANT IN CYTOGENETICS

Most of the species in the genus *Lilium* are diploid (2n = 2x = 24), with few exceptions of triploids (2n = 3x = 36) lilies such as *L. tigrinum* (syn. *L. lancifolium*) (Noda 1978; Kim *et al.* 2006). However, in several species aneuploids were found with additional chromosomes or chromosome fragments (Stewart 1943). The species of the genus *Lilium* have

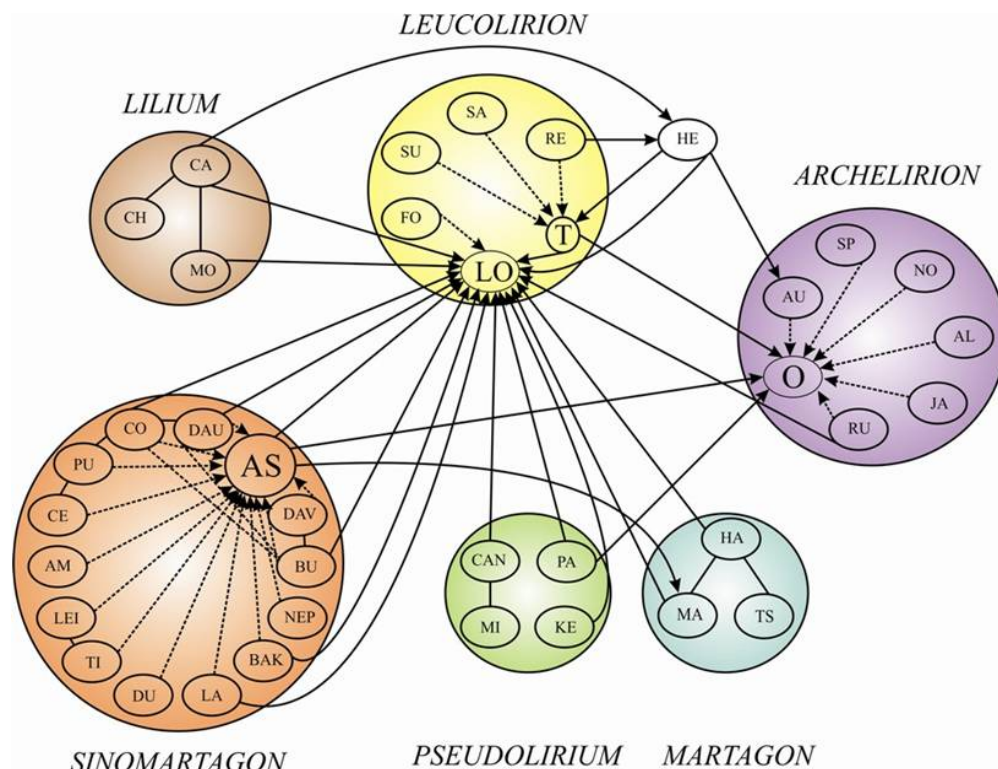


Fig. 2 Crossing polygon of the genus *Lilium* including all the successful crosses of species between different sections of the genus *Lilium* developed at Plant Research International, Wageningen University and Research Centre, The Netherlands. In this figure, the connection between the Asiatic, Trumpet, and Oriental hybrid groups (large circles) are shown by solid lines. In successful intra-sectional crosses between species (ellipses) of different sections (large circles) the arrows point towards the female parent. The dotted lines within each section pointed (arrow heads) towards the main section (AS, O, LO and T). 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. formosum*; 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. sempervivoides*; SP: *L. speciosum*; SU: *L. sulphureum*; T: trumpet hybrids; TI: *L. tigrinum*; TS: *L. tsingtauense* (Khan 2009).

probably the largest genomes among plants, almost a 250-fold larger amount of DNA than the *Arabidopsis* genome (Bennett and Smith 1976, 1991; Bennett *et al.* 2003; Leitch *et al.* 2007). *Lilium* chromosomes are exceptionally large and have proved to be outstanding material for cytogenetic research for more than a century. Since Strasburger's paper on the chromosomes of *Lilium* (Strasburger 1880), many studies have been published on the morphology of lily chromosomes. For example, basic studies on chromosome identification and karyotype analysis (Stewart 1947; Noda 1991), chiasma formation and crossing-over (Mather 1940; Brown and Zohary 1955; Fogwill 1958), and time and duration of female meiosis (Bennett and Stern 1975) were conducted. Later on, different techniques for longitudinal differentiation of chromosomes such as C- and Q banding were used to study the chromosome structure of different *Lilium* species (Holm 1976; Von Kalm and Smyth 1984; Smyth *et al.* 1989). Silver staining has been used for detecting nucleolar organizing regions (Ag-NORs) (Von Kalm and Smyth 1980, 1984; Smyth *et al.* 1989, 1991). Detailed karyotypes have been constructed for many lily species and cultivars on the basis of chromosome arm lengths, C-banding, AgNO₃ staining, fluorescence banding (PI-DAPI, CMA₃), together with fluorescence *in situ* hybridization (FISH) with the 5S and 45S rDNA sequences as probes (Lim *et al.* 2001b; Siljak-Yakovlev *et al.* 2003; Marasek *et al.* 2005).

The karyotype is the phenotypic aspect of the chromosome complement as seen at mitotic metaphase. Comparative karyotype analysis of related species has traditionally been used to describe patterns and directions of chromosomal evolution within a group and to infer the evolutionary role that such karyotype changes may have played (Zhang

et al. 2008). A description of the karyotype typically includes the chromosome number, the absolute and/or relative length of chromosomes (reflecting genome size), the position of primary and secondary constrictions (Levan *et al.* 1964), the distribution of material with different staining properties (Stebbins 1971; Stace 2000; Levin 2002; Lysak and Lexer 2006) and the degree of symmetry. A symmetrical karyotype is characterized by mainly metacentric and sub-metacentric chromosomes of approximately equal size. Changes leading to an asymmetric karyotype can arise by shifts in centromere position towards the telomere (intra-chromosomal) and/or by the addition or deletion of chromatin from some but not all chromosomes, leading to differences in size between the largest and smallest chromosomes (interchromosomal). Different cytogenetic studies have been made in *Lilium* species; for example, Siljak-Yakovlev *et al.* (2003) studied three related and taxonomically close diploid species of the genus *Lilium* (*L. pyrenaicum*, *L. pomponium* and *L. carnolicum*), for chromosomal differentiation, using fluorochrome banding and fluorescence *in situ* hybridization (FISH), and for genome size and GC (guanine and cytosine) percentage using flow cytometry. Muratović *et al.* (2005) used different cytogenetic techniques for interspecific differentiation between *L. bosniacum* and *L. carnolicum*. Recently a large-scale analysis of karyotype was done to determine the patterns of chromosome evolution in the family Liliaceae (Peruzzi *et al.* 2009). Sultana *et al.* (2010) employed FISH and GISH approaches to elucidate inter-specific relationships among wild *Lilium* species distributed in Korea. It was found that 5S rDNA site was located adjacent to 45S rDNA in *L. distichum*, *L. hansonii*, and *L. tsingtauense*. Furthermore, GISH analysis using genomic DNA probes detected strong hybridization of

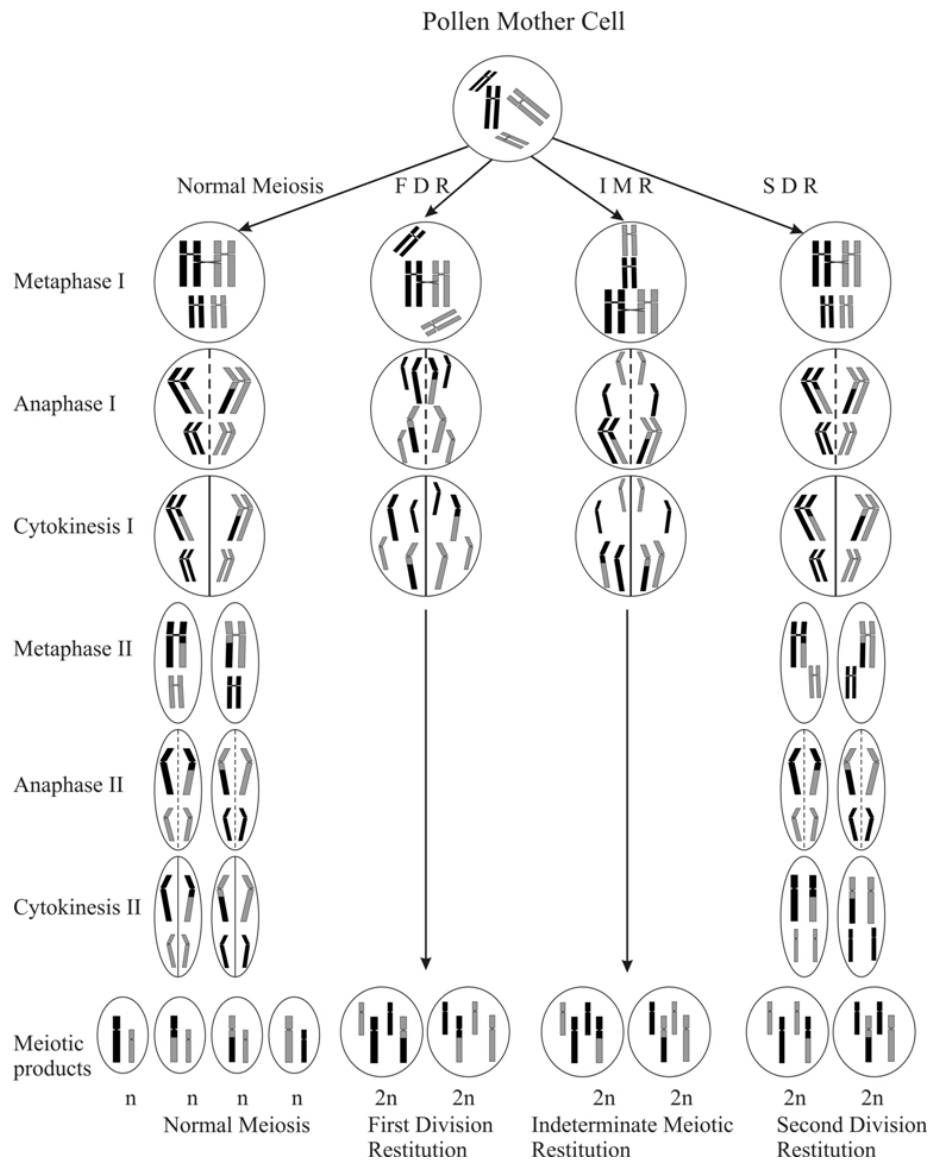


Fig. 3 An illustration of the meiotic process and three restitution mechanisms of $2n$ gametes formation in interspecific lily hybrids. The black and gray chromosomes represent Longiflorum and Asiatic genomes respectively.

genomes between diploid and triploid *L. lancifolium* (syn. *L. tigrinum*) species, demonstrating that triploid plants were derived from diploid parents.

MEIOSIS AND CYTOGENETIC EVALUATION OF $2N$ GAMETE FORMATION IN INTERSPECIFIC LILY HYBRIDS

Chromosome pairing, crossing-over and the distribution of chromosomes are the most important events during meiosis (Sybenga 1975). The outcome of these events leads to the formation of different types of gametes in interspecific lily hybrids. Despite the obvious genetic differentiation of chromosomes of these lily hybrids, homoeologous chromosomes occasionally pair and recombine in meiosis (Lim *et al.* 2001a; Barba-Gonzalez *et al.* 2004; Zhou 2007). Recently it was found that in diploid F_1 interspecific LA hybrids the meiosis seems to be regular, with a high frequency of bivalent formation (Zhou 2007). This normal meiosis resulted in the formation of diploid BC_1 progenies with homoeologous recombination (Khan *et al.* 2009b). Despite regular meiosis their fertility is still to be investigated further.

The products of meiosis are dependent on chromosome pairing at metaphase I and chromosome distribution at anaphase I. If all of the homoeologous chromosomes pair then normal meiosis occurs and four haploid gametes are produced. When homoeologous chromosomes fail to pair,

first division restitution (FDR) can occur resulting in two $2n$ FDR gametes formation. In case of second division restitution (SDR) first meiotic division occurs normally i.e., chromosomes divide reductionally and cytokinesis occurs giving rise to two SDR, $2n$ gametes. However, if some of the bivalents disjoin reductionally and some of the univalents divide equationally at anaphase I, such meiosis leads to indeterminate meiotic restitution (IMR) and produces two $2n$ IMR gametes. These three types of gametes have been reported in lily interspecific hybrids (Lim *et al.* 2001a, 2004). The mechanism of $2n$ gamete formation with a single pair of chromosome assortment is illustrated in the Fig. 3.

$2n$ gametes are useful for different reasons. They are used to overcome F_1 sterility. But more importantly, the use of $2n$ gametes can facilitate intergenomic recombination in the subsequent progenies. Because in lily interspecific hybrids FDR, SDR and IMR occur, the resulting genetic consequences of using $2n$ gametes will be different. One of the important pre-requisites for genetic variation is the occurrence of intergenomic recombination. Thus, in the absence of intergenomic recombination, an allotetraploid gives rise to identical $2n$ gametes without any potential for genetic variation. On the contrary, intergenomic recombination is most likely to occur in the diploid hybrid during restitutional meiosis and has the potential to produce considerable genetic variation as illustrated in Fig 3. In the case of FDR

and IMR derived triploids it is possible to achieve homozygosity for the recessive loci (nulliplex condition). In case of FDR when there is a single crossover, then heterozygosity is attained to the proximal segment and homozygosity retained in the distal segment. In case of SDR, the homozygosity for the proximal segment while heterozygosity retained in the distal segment (**Fig. 3**).

It was found that most of the LA and OA hybrids have possibilities to produce a high frequency of aneuploid gametes and a relatively small amount of balanced $2n$ gametes. However few LA hybrids were shown to produce n gametes as well (Khan *et al.* 2009a). This high percentage of aneuploid gametes is probably responsible for the sterility of the F_1 hybrids. Based on the homoeologous distribution at anaphase I, the F_1 LA hybrids have more potential to produce IMR $2n$ gametes than FDR $2n$ gametes (Zhou 2007). However, it was found that most of the BC_1 progenies result from FDR $2n$ gametes and less from IMR $2n$ gametes (Lim *et al.* 2001a; Barba-Gonzalez *et al.* 2005a; Zhou *et al.* 2008). It might be argued that FDR $2n$ gametes have better viability than IMR $2n$ gametes due to the balanced chromosomal composition.

Production of $2n$ gametes is influenced by environmental factors like temperature (Bretagnolle and Thompson 1995; Ramsey and Schemske 1998) and their production can be enhanced by using certain chemicals like colchicine (Wu *et al.* 2007) or treating flower buds with N_2O (Barba-Gonzalez *et al.* 2006; Akutsu *et al.* 2007). More recently, Kitamura *et al.* (2009) found that N_2O mediates polyploidization by inhibiting microtubule polymerization, but not actin filament formation, during microsporocyte meiosis in *Lilium*.

MOLECULAR CYTOGENETIC APPROACH IN LILIUM BREEDING

The F_1 hybrids that display attractive combinations of parental characteristics are used for selection and successive stabilization of hybrid genomes by employing intercross or backcross strategies. Some of the resulting genotypes, which are of interspecific origin, have already been registered as cultivars. The ability to produce interspecific hybrids between *L. longiflorum* × Asiatic (LA), Oriental × Asiatic (OA), Oriental × Trumpet (OT), and *L. longiflorum* × Oriental (LO) and the commercial success of these interspecific hybrids stimulated research on the structure and behavior of their genomes. The genetic material of *Lilium* can be investigated in detail by using molecular cytogenetic techniques such as fluorescence *in situ* hybridization (FISH) and genomic *in situ* hybridization (GISH) by applying specific DNA sequences as probes (Schwarzacher 2003) or total genomic DNA as probes (Schwarzacher *et al.* 1989), respectively. Due to the fact that the genomes of interspecific lily hybrids are distant enough at the DNA level, GISH technique enables the discrimination of parental genomes in hybrids and identification of the sites of genomic recombination (Marasek *et al.* 2004; Barba-Gonzalez *et al.* 2005a, 2005b; Zhou *et al.* 2008). Chromosome banding and FISH have been applied for identification of particular chromosomes and karyotype construction of *L. longiflorum* and *L. rubellum* (Lim *et al.* 2001b). GISH has been used extensively in lily to recognize the three most common genomes of *Lilium* viz. Longiflorum (L), Asiatic (A) and Oriental (O) and to study the recombinant chromosomes in the BC_1 and BC_2 progenies of LA and OA hybrids (Karlov *et al.* 1999; Lim and Van Tuyl 2002; Lim *et al.* 2003; Barba-Gonzalez *et al.* 2004, 2005b; Zhou *et al.* 2008). Furthermore, this technique is also employed to study the mechanisms of $2n$ gamete formation in interspecific hybrids of LA and OA (Lim *et al.* 2001a; Barba-Gonzalez *et al.* 2004, 2005a). GISH has been successfully employed to determine the potential of analytic breeding in interspecific lily LA hybrids (Khan *et al.* 2009a). Recently, Khan *et al.* (2009a) constructed a chromosomal recombination map of three genomes of lilies (Longiflorum, Asiatic and Oriental)

based on GISH analysis. So the characterization of genome composition by GISH may play an important role in selecting potentially useful hybrids during early stages of breeding. Moreover, the high frequency of intergenomic recombination can be detected by GISH in interspecific lily hybrids. This method is an attractive tool for introgression breeding and cytogenetic mapping of traits of interest.

In crosses involving a diploid F_1 hybrid that produces $2n$ gametes with a diploid cultivar that produces normal n gametes, the progeny is expected to be triploid. In crosses involving both parents producing $2n$ gametes, tetraploid progeny is possible. Analyses of the progeny of LA hybrids producing functional $2n$ gametes, obtained by crosses with diploid Asiatic (A) cultivars, showed that most of the progeny obtained was triploid as expected and considerable amounts of intergenomic recombination had occurred (Lim *et al.* 2003; Zhou *et al.* 2008). Based on GISH analysis it was found that BC_1 progenies in LA hybrids obtained from functional $2n$ gametes exhibited more homoeologous recombination as compared to the progenies obtained by somatic chromosome doubling to overcome F_1 sterility (Lim *et al.* 2001a). Similarly, when OA hybrids producing functional $2n$ gametes were backcrossed with diploid Asiatic cultivars they also produced triploid progenies with a certain amount of intergenomic recombination (Barba-Gonzalez *et al.* 2005a). Generally such allotriploid hybrids cannot be used in breeding because of their sterility. However, it has been demonstrated that some triploid hybrids can produce aneuploid and euploid (x , $2x$ and $3x$) gametes and have been successfully used to produce progenies in many different interspecific lily hybrids (Lim *et al.* 2003; Barba-Gonzalez *et al.* 2006; Prosevičius *et al.* 2007; Khan *et al.* 2009a). Furthermore, triploid hybrids might have contributed to the origin of a majority of new polyploids in nature (Husband 2004). In the case of *Lilium*, triploid ALA hybrids derived from $2n$ gametes, were successfully used in crosses and it has been shown that the recombinant chromosomes can be transmitted to the progeny. Crosses of these triploids with diploids and tetraploids produced aneuploid or near diploid and pentaploid progenies, respectively (Lim *et al.* 2003). Khan *et al.* (2009b) also used triploid LA hybrids obtained from functional $2n$ gametes and crossed with a diploid Asiatic cultivar. Here in case of $3x - 2x$ and in the reciprocal cross ($2x - 3x$), diploid progenies were obtained indicating the production of balanced n gametes by triploid cultivars. Obviously, in diploids obtained from $2x - 3x$ crosses one should expect the occurrence of genotypes with alien chromosome additions as well as substitutions which are valuable for creating novel genetic variation. Thus, the creation of polyploids from the progenies of such hybrids obtained from crosses between the species from different sections with the use of $2n$ gametes might have been rewarding with a higher degree of genetic variation.

AN ILLUSTRATIVE EXAMPLE OF INTROGRESSION BREEDING

An attractive aspect that has emerged in recent years is the prospect of breeding lily at the diploid level in spite of the hybrids being of interspecific origin (Khan *et al.* 2009b). It was found that some F_1 hybrids not only produced $2n$ gametes but also normal n gametes. This provides unique opportunities for generating allotriploid ($2n = 3x = 36$) as well as diploid ($2n = 2x = 24$) BC_1 progenies from backcrossing LA hybrids to Asiatic parents. Some diploid and triploid BC LA hybrids also had normal meiosis and gave rise to n gametes. The initial attempts indicated that it might be possible to generate fairly large numbers of diploid BC_1 and BC_2 progenies from the LA hybrids. **Fig. 4** shows the diploid, triploid LA and triploid OA hybrids obtained from the functional n and $2n$ gametes.

Intergenomic recombination and introgression was also estimated in the progeny of sib-mated LA hybrids (Khan *et al.* 2010). In this case both parents had contributed gametes with the somatic number of chromosomes (i.e., $2n - 2n$).

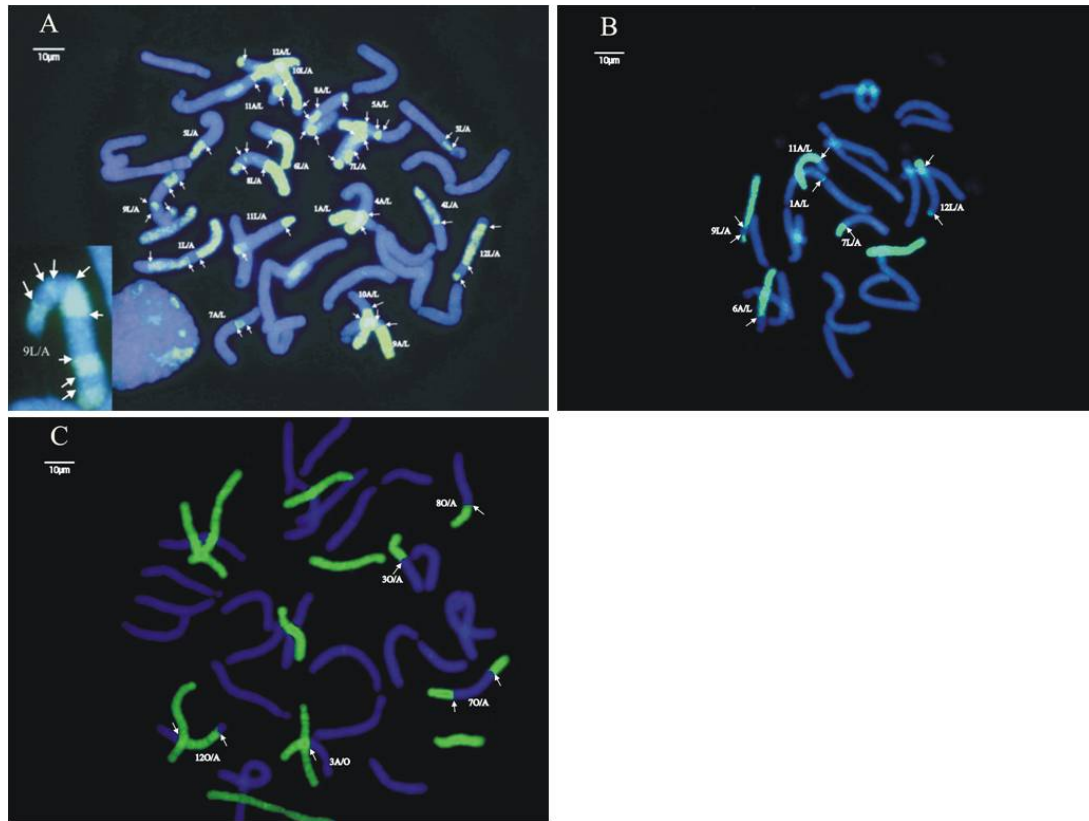


Fig. 4 Somatic metaphase chromosomes of BC₁ progenies of LA and OA hybrids showing recombination sites on different chromosomes after GISH (arrows). (A) A triploid ($2n = 3x = 36$) BC₁ progeny of LA hybrid (LAA, 066994-3) with 49 recombination sites (arrows). Inset: a recombinant chromosome showing 8 recombination sites in BC₁ LA hybrid (062071-2). (B) A diploid ($2n = 2x = 24$) BC₁ progeny of LA hybrid (LAA, 066828-5) with 8 recombination sites (arrows). (C) A triploid ($2n = 3x = 36$) BC₁ progeny of OA hybrid (AOA, 022605-24) with 7 recombination sites (arrows). Blue colour represents the Asiatic genome while green colour indicates the Longiflorum genomes. Reproduced from Khan N, Barba-Gonzalez R, Ramanna MS, Visser RGF, Van Tuyl JM (2009a) Construction of chromosomal recombination maps of three genomes of lilies (*Lilium*) based on GISH analysis. *Genome* 52, 238-251, ©2009, with kind permission of Canadian Science Publishing (NRC Research Press), Ottawa.

GISH was applied to access the intergenomic recombination in the resulted progenies. Based on these results it is revealed that bilateral sexual polyploidization through $2n$ gametes could successfully be carried out in interspecific lily (*Lilium*) hybrids to produce allotetraploid genotypes. Such tetraploids were obtained for the first time in interspecific lily hybrids. Being allotetraploids, they are expected to be fertile and be used repeatedly in crossing to get tetraploid or triploid progenies by overcoming the disadvantage of restoring fertility using various spindle inhibitors like oryzalin and colchicine (Van Tuyl *et al.* 1992) which promotes autosyndetic chromosome pairing amongst the parental genomes.

CYTOGENETIC MAPPING OF DIFFERENT LILY GENOMES

In *Lilium*, unfortunately there are no complete single or double chromosome substitution lines available (Lim *et al.* 2003; Barba-Gonzalez *et al.* 2006; Xie *et al.* 2010) which would permit comprehensive studies of the genetic control of desirable horticultural characters except in few cases. Furthermore, the molecular mapping in *Lilium* is hindered by its large genome which is mostly comprised of highly repetitive DNA sequences (Bennett *et al.* 2003; Leitch *et al.* 2007). So no high density genetic and physical maps are available to localize the genes or markers linked to such genes for specific traits. However, Shahin *et al.* (2011) have developed a molecular map including map positions of quantitative trait loci (QTL) for various ornamental traits and diseases resistances. Khan *et al.* (2009a) investigated that manipulation of genomes in lily could be facilitated by molecular cytogenetic techniques of individual chromosomes. The potential of $2n$ gametes in interspecific lily hybrids,

considerable frequency of homoeologous recombination and easy discrimination of parental genomes in hybrids by GISH created a new strategy for mapping, called 'the cytomolecular mapping'. Based on GISH, recombination sites were identified in BC progenies LA and OA hybrids and were mapped on 12 different chromosomes in the A, L and O genomes (Fig. 5). The availability of crossing over points, which are also called recombination sites, permits comprehensive studies of the chromosomal recombination and the localization of the introgressed segments in different backgrounds of interspecific lily hybrids. In this study mostly triploid progenies derived from functional $2n$ gametes with recombinant chromosomes were mapped. It was found that recombination sites were asymmetrically distributed on different chromosomes of three genomes. Furthermore, Hwang *et al.* (2010) reported the construction of a chromosome 1 specific library of *L. tigrinum*. As such, the essential components needed to integrate the physical and genetic map become available.

CONCLUSION AND FUTURE PERSPECTIVES

An extensive molecular cytogenetic approach has been done to elucidate the genome composition of lily hybrids as well as the nature of the production of $2n$ gametes and ability of the homoeologous chromosomes to pair and recombine. As the hybrids consist of distantly related species, the parental genomes can be easily discriminated by GISH. This means that every single recombination event can be visualized and as such, easily perpetuated for detailed analyses (Khan *et al.* 2009a). This system is not so commonly available in any other plant species. Therefore, it may be expected that cytogenetics of lily hybrids will remain in the center of attention of lily breeders as well as scientists for

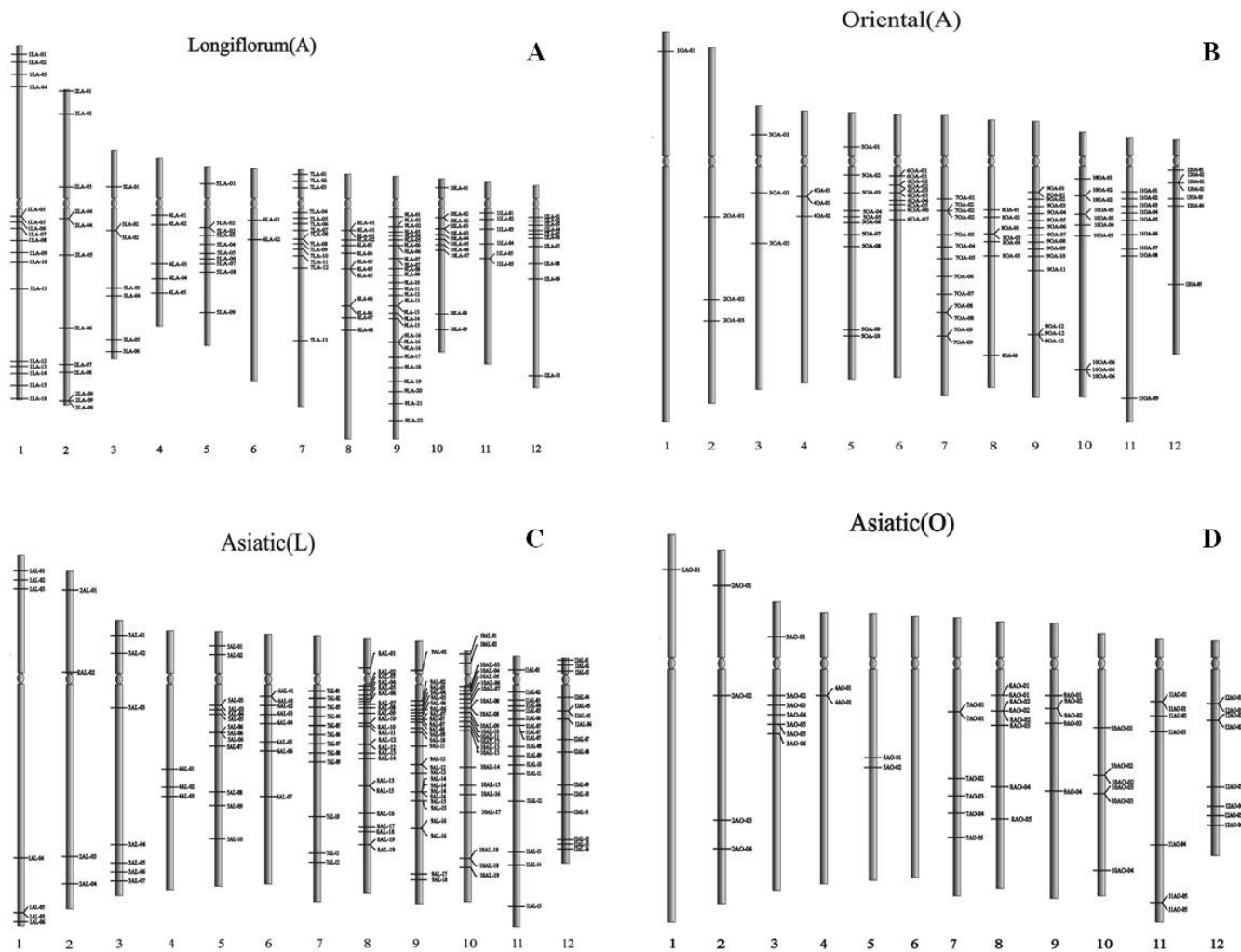


Fig. 5 Four chromosomal recombination maps resulting from the analysis of BC progenies of LA and OA hybrids. (A) Longiflorum (A); (B) Oriental (A) (C) Asiatic (L) and (D) Asiatic (O)- the recombination partner in each is given in parenthesis. Reproduced from Khan N, Barba-Gonzalez R, Ramanna MS, Visser RGF, Van Tuyl JM (2009a) Construction of chromosomal recombination maps of three genomes of lilies (*Lilium*) based on GISH analysis. *Genome* 52, 238-251, ©2009, with kind permission of Canadian Science Publishing (NRC Research Press), Ottawa.

fundamental research. As some of the F_1 inter-sectional hybrids facilitates intergenomic recombination leading to genetic variation and generate polyploids with introgressed chromosome segments. There is a need to explore more F_1 hybrids producing $2n$ gametes. Furthermore, the diploid and triploid BC_1 progenies could be readily used for further crosses and the recombinant chromosomes will be transmitted to the progeny assuring introgression of the alien chromosomes or chromosome segments. However, the impact of lily hybrids and progress in their breeding contrasts with a rather poor knowledge of their genome structure and behavior on a molecular basis. Lily may require advanced tools of genomics and biotechnology for its genomic structure analysis.

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