Introgression studies using GISH in interspecific *Lilium* hybrids of *L. longiflorum x Asiatic, L. longiflorum x L. rubellum and L. auratum x L. henryi*

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1. Interspecific hybridization

Interspecific hybridization proved to be the most important tool for developing complete new hybrid groups. After the Asiatic and the Oriental hybrid groups that could be developed with relative simple crossing methods also the **LA** and **OT**-groups were bred. And for the near future also the **OA**-hybrids, as the most advanced and up till now the most difficult crossing combination to realize, will give new possibilities for the lily market.

The development of methods for overcoming fertilization barriers was essential in the successes in lily breeding. A range of techniques were investigated and applied, besides pollination methods (cut-style, grafted style) to overcome pre-fertilization barriers and a number of techniques for post-fertilization barriers are needed. Embryo-rescue methods (ovary-slice, ovule and embryo culture) are needed to circumvent the problems with the embryo-endosperm development, polyploidization (chromosome doubling using oryzalin or colchicine or by 2n-gametes) could overcome the F_1-sterility which most frequently occurs. The last method to prove and speed up introgression of characters in interspecific hybrids is the so-called GISH (Genomic in situ hybridization), which can distinguish the parental genomes of interspecific hybrids

We succeeded in making numerous new combinations between many sections of the genus *Lilium* by the use of various pollination and embryo rescue methods. Examples include *L. longiflorum* (Leucolirion section) × *L. monadelphum* (Lilium section), *L. longiflorum* × *L. lankongense* (Sinomartagon section), *L. longiflorum* × *L. martagon* (Martagon section), *L. longiflorum* × *L. candidum* (Lilium section), *L. henryi* (Leucolirion section) × *L. candidum*, *L. longiflorum* × *L. rubellum*

(Archelirion section), *L. longiflorum* × Oriental hybrid, Oriental × Asiatic hybrid, *L. longiflorum* × *L. canadense* (Pseudolirium section) and Oriental hybrid × *L. pardalinum* (Pseudolirium section). The crossing polygon (Figure 1) shows the crossing compatibility within and between the sections achieved by our research group so far (Van Tuyl et al., 2002).

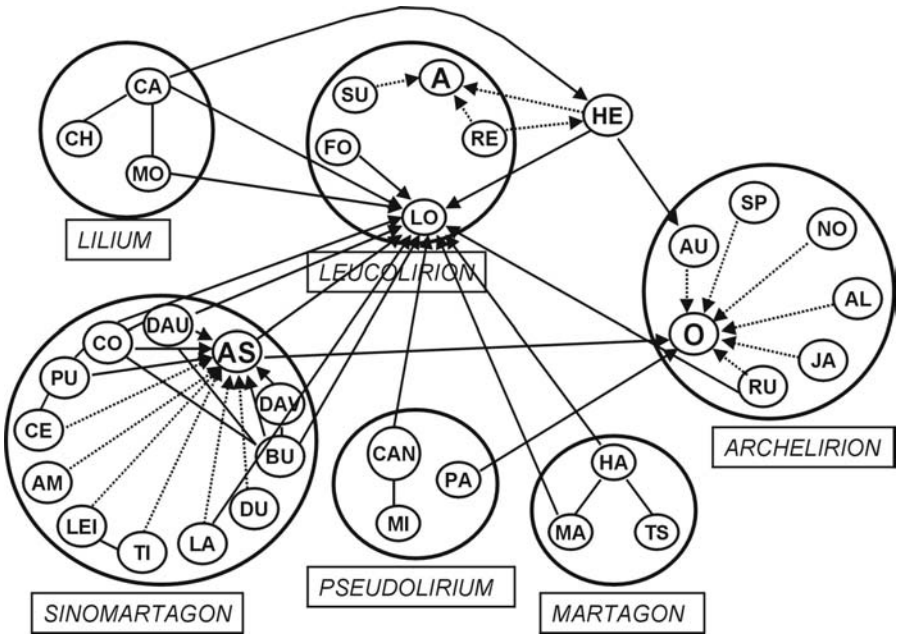

Figure 1. A crossing polygon of the genus *Lilium* including all successful crosses of species between different sections of the genus *Lilium* developed at Plant Research International, The Netherlands. In this figure, the connection between the Asiatic, Aurelian, and Oriental hybrid groups (large ellipses) are shown by dotted lines. In successful crosses between species (small circles) of different sections (large circles) the arrows point towards the female parent. Abbreviations: A: Aurelian hybrids; AL: *L. alexandrae*; AM: *L. amabile*; AS: Asiatic hybrids; AU: *L. auratum*; 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*; LA: *L. lankongense*; LEI: *L. leichtlinii*; LO: *L. longiflorum*; MA: *L. martagon*; MI: *L. michiganense*; MO: *L. monadelphum*; NO: *L. nobilissimum*; O: Oriental hybrids; PA: *L. pardalinum*; PU: *L. pumilum*; RE: *L. regale*; RU: *L. rubellum*; SP: *L. speciosum*; SU: *L. sulphurenum*; TI: *L. tigrinum*; TS: *L. tsingtauense.*

2. Chromosome analysis by genomic *in situ* hybridization (GISH)

Genomic *in situ* hybridization (GISH) can distinguish the parental genome chromosomes of interspecific hybrids. This technique, which utilizes total genomic DNA from one of the parental species as a probe and from the other counterpart species as a block, provides a new technique for effective parental genome analysis in both sexual and somatic hybrids. This technique also detects translocations involving chromosomes from different genomes and to monitoring chromosomes behaviour during meiosis. Therefore, the level of introgression in back-crossed progenies between different species can be measured by GISH analysis. Due to its large chromosome size lily has advantages analyzing the number of parental chromosome composition and homoeologous recombination breakpoints that are important to know whether or not there are recombination had been taken place between parental chromosomes. In this article three examples of groups of interspecific hybrids are mentioned in which this technique is utilized.

3.1 Longiflorum-rubellum hybrid 'Elegant lady' derived from mitotic polyploidization

One of the successful examples of interspecific hybridization is 'Elegant Lady' (triploid, **LLR).** This hybrid was derived from crossing of *L. longiflorum* and amphidiploid (**LLRR) of** F_1 interspecific hybrid. *L. longiflorum* possesses long-white-tubular shaped flowers with a pleasing fragrance, and *L. rubellum* has very early flowering habit (ca. 35 days) and a pink flower with a pleasing fragrance. **LR** the hybrid showed pink flower and early forcing habit. Since **LR** F_1 hybrid is sterile, it was necessary to make tetraploid by chromosome doubling to recover pollen fertility. An amphidiploid (**LLRR**) successfully recovered its fertility and was crossed with *L. longiflorum* to make a tubular shape of pink *longiflorum* flower. Genomic *in situ* hybridization (GISH) confirmed that **LLR** triploid was composted of two sets of *L. longiflorum* chromosomes and one set of *L. rubellum* chromosomes without any homoeologous recombination between parental chromosomes (Lim et al, 2000). The characters were intermediate between *L. longiflorum* and *L. rubellum* (Table 1). This plant exhibits elegant tubular-pink flower with very early flowering and a pleasing fragrance. The hybrid was named 'Elegant lady' and was commercially released in 2000.

Table 1. Characteristics of *L. longiflorum* and its F_1 and BC_1 hybrids.

Genotype	Plant height (cm)	Flower length (cm)	Forcing time (days)
'Gelria'	94.0	15.9	95.0
'Snow Queen'	116.3	18.2	96.7
LR (F_1)	47.9	11.2	51.7
LLR 'Elegant lady'	79.4	15.9	75.2

3.2 Longiflorum-Asiatic hybrids derived from meiotic polyploidization

LA interspecific hybrids commercially available for a decade and currently about 30 cultivars are marketed. Most of them are triploids derived from backcrossing by mitotic or meiotic polyploidization. There are two ways, so called, mitotic and meiotic polyploidization for further crossing. A study of **ALA**-hybrids derived from 2n-gametes of several **LA**-hybrids was performed and showed with GISH that homoeologous recombination can be found quite often. In the next generation the degree of recombination will be increased (Lim et al. 2002). Only using GISH it was possible to discover a new mechanism of 2n-gametes-production (Lim et al 2001). Further we analyzed one (**A**)**LA** hybrid 'Fangio' (2n=3x=36, triploid, **A** genome=24, **L** genome=12) and confirmed by GISH that there are many recombinant chromosomes between **L** and **A** genomes. Based on GISH analysis data, it was assumed that this hybrid was derived from spontaneous meiotic polyploidization.

3.3 Auratum-henryi hybrids derived from meiotic polyploidization

Using the *Lilium auratum* × *L. henryi* hybrid obtained by Yoshito Asano (1977), we produced a range of triploid Oriental-henryihybrids in the early eighties. Now, 20 years later, we could, using the GISH-technique, demonstrate that in many of these hybrids genetic recombination between the *L. henryi* and *L. auratum* chromosomes took place during meiosis of F_1 hybrid. In Table 1, GISH-results are presented of a number of hybrids we obtained in 1982, 1983 and 1985 using Journey's End, Stargazer, Dominique and Darling as female in crosses with F_1 hybrid of *L. auratum* × *L. henryi.*

Table 2. Chromosome composition of progeny derived from Oriental × (Auratum-henryi)hybrids

Cross no	Female	Male	Ploidy level	Chromosome no	Originated from	
					Female	Male [z]
82111	*L. auratum (Au)*	*L. henryi(H)*	2x	24	12Au	12H
82396-1	Journey's End	82111	3x	36	12O	12(1Au/H)+12(2H/Au)
82396-2	J E	82111	3x	36	12O	12(1Au/H)+12(1H/Au)
82396-3	J E	82111	3x	36	12O	12(1Au/H)+12(1H/Au)
82396-4	J E	82111	3x	36	12O	12(1Au/H)+12H
82396-5	J E	82111	3x	36	12O	12(1Au/H)+12(1H/Au)
82342-3	Star Gazer	82111	3x	36	12O	12Au+12H
82342-6	SG	82111	3x	36	12O	12Au+12(2H/Au)
83275-1	SG	82111	3x	36	12O	12Au+12H
83275-3	SG	82111	3x	36	12O	12Au+12(1H/Au)
83275-5	SG	82111	3x	36	12O	12(2Au/H)+12(3H/Au)
83275-7	SG	82111	3x	36	12O	12(2Au/H)+12(1H/Au)
83275-8	SG	82111	3x	36	12O	12(2Au/H)+12(1H/Au)
83275-12	SG	82111	3x	36	12O	12(1Au/H)+12(2H/Au)
83275-15	SG	82111	3x	36	12O	12(2Au/H)+12(1H/Au)
85863-1	Dominique	82111	3x	36	12O	12(1Au/H)+12(1H/Au)
85863-2	Dominique	82111	3x	36	12O	12Au+12(1H/Au)
85864-1	Darling	82111	3x	36	12O	12(2Au/H)+12(2H/Au)
85864-2	Darling	82111	3x	36	12O	12Au+12H
85864-5	Darling	82111	3x	36	12O	12(1Au/H)+12(1H/Au)
85864-6	Darling	82111	3x	36	12O	12Au+12H

[z] Numbers in parenthesis indicate the breakpoint of *L. auratum* (Au) chromosome with *L. henryi* (H) chromosome segment(s) and *L. henryi* chromosomes with *L. auratum* chromosome segment(s).

3.4 Concluding remarks

Using Genomic In Situ Hybridization (GISH) techniques, it is possible to visualize genetic recombination in interspecific hybrids between different parents. It has been shown that using mitotic doubled material no homologeous recombination take place, while with tetraploids from meiotic origin (2n-gametes) this is shown many homoeologous recombinations in ALA, AOA and OAuH-hybrids. This recombination is essential for introgression of characters, like disease resistance for *Fusarium*, Virus and *Botrytis*, in the next generations of the breeding process.

Acknowledgements

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Photo's

1. 82111 *L. auratum x L. henryi*
2. 82342-4 Stargazer x *L. auratum x L. henryi*
3. 85863-1 Dominique x *L. auratum x L. henryi*
4. 83275-7 Stargazer x *L. auratum x L. henryi*
5. 82396-5 Journeys End x *L. auratum x L. henryi*
6. 85364-5 Darling x *L. auratum x L. henryi*
7. Photo 7-12 GISH on somatic metaphase chromosomes of AuH hybrid, 82111 and BC_1 progenies in cross between Oriental hybrid and spontaneous chromosome doubled AuH hybrid with total genomic DNA of *L. henryi* as a probe.. Photo 7. AuH hybrid, 82111; yellow green fluorescence: 12 chromosomes of *L. henryi* ; red fluorescence: 12 chromosomes of *L. auratum*
8. Oriental hybrid 'Stargazer'×AuH hybrid (82342-6); yellow green fluorescence: 12 chromosomes of *L. henryi* ; red fluorescence: 24 chromosomes of *L. auratum* and Oriental hybrid. Arrows indicate the recombination sites between *L. auratum* and *L. henryi.*
9. Oriental hybrid 'Journeys End'×AuH hybrid (82396-5); yellow green fluorescence: 12 chromosomes of *L. henryi* ; red fluorescence: 24 chromosomes of *L. auratum* and Oriental hybrid. Arrows indicate the recombination sites between *L. auratum* and *L. henryi.*
10. Oriental hybrid 'Stargazer'×AuH hybrid (83275-7); yellow green fluorescence: 12 chromosomes of *L. henryi* ; red fluorescence: 24 chromosomes of *L. auratum* and Oriental hybrid. Arrows indicate the recombination sites between *L. auratum* and *L. henryi*
11. Oriental hybrid 'Stargazer'×AuH hybrid (83275-15); yellow green fluorescence: 12 chromosomes of *L. henryi* ; red fluorescence: 24 chromosomes of *L. auratum* and Oriental hybrid. Arrows indicate the recombination sites between *L. auratum* and *L. henryi.*
12. Oriental hybrid 'Darling'×AuH hybrid (85864-5); yellow green fluorescence: 12 chromosomes of *L. henryi* ; red fluorescence: 24 chromosomes of *L. auratum* and Oriental hybrid. Arrows indicate the recombination sites between *L. auratum* and *L. henryi.* Arrows indicate the recombination sites between *L. auratum* and *L. henryi.*
13. LRLR Tetraploid *L. longiflorum x L. rubellum*
14. LLR Elegant Lady *L. longiflorum* "Gelria'x *Longiflorum* x *L. rubellum*
15. GISH on somatic metaphase chromosomes of LLR Elegant Lady *L. longiflorum* "Gelria'x *Longiflorum* x *L. rubellum;* yellow fluorescence: 24 chromosomes of *L.* longiflorum ; red fluorescence: 12 chromosomes of *L. rubellum;* no recombination.
16. AOA Asiatic hybrid 'Gran Sasso' x (Oriental hybrid 'Bel Paso' x Asiatic hybrid 'Connecticut King')


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AURXHENRYI
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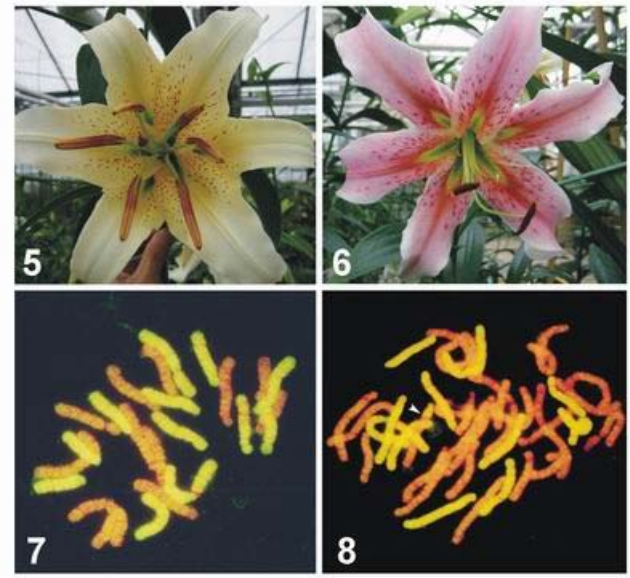
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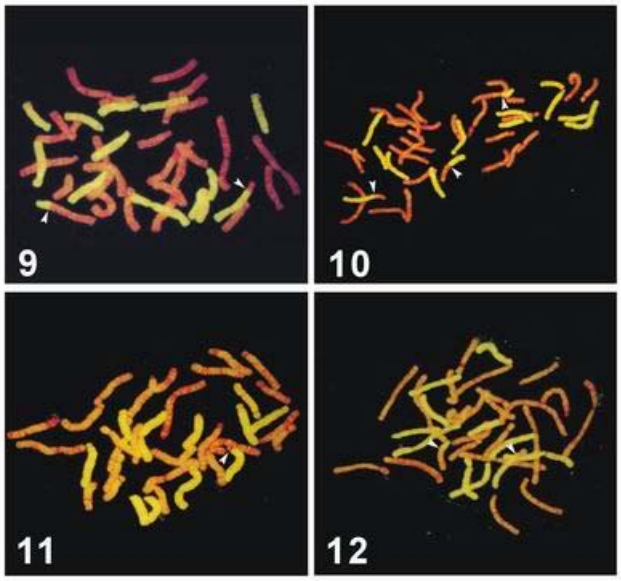
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