Molecular Cytogenetics in *Lilium* Breeding

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Abstract

In horticulture, interspecific hybridization is one of the most important tools to achieve genetic variation; this is especially true when it comes to ornamental crops, where it is always necessary to introduce new traits, such as flower colour, petal shapes, stem size and strength, longevity, disease resistances and more. However, to maintain such traits in the progeny is necessary to introgress the genes of the alien species into the gene pool of the receptor species. To accomplish this, first sterility must be overcome, because, as a general rule, interspecific hybrids tend to be sterile. Mitotic polyploidization is a useful technique to come to such end, but the use of unreduced gametes is preferred because recombination occurs between the parental genomes and introgression might be achieved. Here it is described i) the production of interspecific, intersectional lily hybrids, obtained through the use special pollination techniques combined with ovary- and embryo-rescue techniques, in crosses of distantly related lily hybrids, cultivars and species from different taxonomical sections (*L. longiflorum* × Asiatic hybrids (LA), *L. longiflorum* × Oriental hybrids (LO), *L. longiflorum* × *L. rubellum* (LR), *L. longiflorum* × *L. henryi* (LH), *L. henryi* × *L. candidum* (HC), *L. auratum* × *L. henryi* (AuH), *L. martagon* × Asiatic hybrids (MA), Oriental hybrids × Asiatic hybrids (OA), Oriental hybrids × Trumpet hybrids (OT) and Oriental hybrids × *L. pardalinum* (OP)); ii) the use of molecular Genomic *In Situ* Hybridization (GISH) to depict the mechanisms of *2n* gamete formation and their use for the production of sexual polyploids; iii) the

utilization of allotriploid BC_1 progenies in introgression breeding and iv) the application of N_2O to induce the formation of unreduced gametes in sterile lily hybrids.

INTRODUCTION

The horticultural importance of the lily places it as the forth ornamental crop. The genus *Lilium* L. (2n=2*x*=24) belongs to the monocotyledonous family Liliaceae. The genus is comprised by over 80 species distributed throughout the mountainous areas of the Northern Hemisphere (Lim et al., 2000), which were classified by Comber (1949) and later revised by Lighty (1968) and De Jong (1974) into seven sections: Lilium (Liriotypus), Martagon, Pseudolirium, Archelirion, Sinomartagon, Leucolirion and Oxypetala. Breeding history of lily can be traced back to more than 200 years ago; however, there are three groups of hybrids that have dominated the market over the last 50 years: the Asiatic, Oriental and Longiflorum hybrids, interspecific hybrids within species from the Sinomartagon, Archelirion and Leucolirion sections, respectively (McRae, 1998; Lim et al., 2008); in the last decades, cultivars of interspecific, intersectional hybrids has been added to a collection of about 15,000 lily cultivars (Matthews, 2007). The introduction of novel cultivars has followed the necessity of growers and consumers of new combinations of flower colours, shapes and orientations, forcing times, disease resistances, stem lengths and more. Breeding of the Asiatic, Oriental and Longiflorum hybrids has been done with relatively ease, however, the obtention of the intersectional, interspecific hybrids has required the combination of biotechnological tools and furthermore, chromosome analyses and the study of reproduction mechanisms in order to understand and achieve introgression in the novel cultivars. Molecular cytogenetics, a combination of cytogenetics and molecular biology, has been a crucial technique in the depiction of mechanisms of gamete formation in interspecific hybrids, the identification of recombinant chromosomes and their segregation in further progeny. Here, introgression in intersectional, interspecific lily hybrids and the importance of molecular cytogenetics in the process is described.

INTERSPECIFIC HYBRIDIZATION

The demanding market in ornamentals is always requesting for products with new colours, shapes, forcing times and disease resistances among other important traits. To accomplish this, it is necessary to introduce the desired genes from different species into existing cultivars to finally introgress the coveted traits into novel cultivars. Interspecific hybridization and repetitive backcrossing is the obligated path to attain introgression. Lilies are not the exception and interspecific hybridization has been the rule to create novel cultivars. There are three main groups of lily hybrids obtained by interspecific hybridization within species belonging to the same taxonomic section with relatively ease viz. Asiatic (A), hybrids within species of the Sinomartagon section; Orientals (O), interspecific hybrids within the Archelirion section and Longiflorum (L), hybrids within species of the Leucolirion section. These intrasectional, interspecific hybrids dominated the lily market in the last decades (McRae, 1998). However, intersectional, interspecific hybridization was not possible until the late 70s, due to incompatibility and incongruity barriers between the distantly related lily species (Van Tuyl et al., 1982). A great breakthrough in lily hybridization was the introduction of special pollination techniques as well as ovule and embryo rescue techniques (Asano and Myodo, 1977a; 1977b; Van Tuyl et al., 1982; 1991), with such techniques it has been possible to create completely

new lily hybrids. Actually, some of these intersectional, interspecific hybrids have been released as new cultivars in the market, gaining importance rapidly (Lim et al., 2008). At Wageningen University and Research Centre (Wageningen, The Netherlands) a considerable number of interspecific hybrids have been obtained. These new hybrids involve different species in complex combinations and have been classified into new groups: Oriental hybrids x Asiatic hybrids (OA); *L. longiflorum x L. rubellum* (LR); *L. longiflorum x* Asiatic hybrids (LA); *L. Longiflorum x L. henryi* (LH): *L. henryi* x *L. candidum* (HC); *L. auratum x L. henryi* (AuH); *L. Longiflorum x* Oriental hybrids (LO); Oriental hybrids x *L. pardalinum* (OP); Oriental hybrids x Trumpet hybrids (OT) and *L. martagon x* Asiatic hybrids (MA) (Van Tuyl et al., 1982; 1991; 2000; Van Tuyl, 1990; Van Tuyl and Van Holsteijn, 1996; Lim et al., 2001; Lim and Van Tuyl, 2002; Barba-Gonzalez et al., 2004; Lim et al., 2005; Luo et al., 2012). The parentage of some of these hybrids is described to detail in Table 1.

There is a major drawback in these hybrids that hampers further hybridization; this is the sterility in the F_1 hybrids. Most of these hybrids are sterile due to lack of chromosome pairing and irregular chromosome segregation during meiosis (Asano, 1982). The traditional method to restore fertility has been the utilization of chemicals as colchicine and oryzalin (Van Tuyl et al., 1992) to produce polyploids. Even though, fertility is restored, these polyploids are not so useful in further breeding because these hybrids show fixed heterozygosity in their gametes (Soltis and Soltis, 2000) due to autosyndetic chromosome pairing during meiosis (Lim et al., 2000; Wendel, 2000; Van Tuyl et al., 2002b; Ramanna and Jacobsen, 2003). An alternative to the use of these artificial polyploids is the use of "unreduced gametes".

MECHANISMS OF *2n* GAMETE FROMATION REVEALED BY GISH

"Unreduced gametes", also known as "*2n* gametes" are gametes with somatic chromosome numbers. They occur in most of the angiosperm species and they might be responsible for the origin of polyploid species (Harlan and De Wet, 1975). In lilies, several intersectional, interspecific hybrids have been detected to produce this kind of gametes (Table 1). The development of genomic *in situ* hybridization (GISH), a molecular cytogenetic technique that can distinguish between the parental genome chromosomes in an interspecific hybrid (Van Tuyl et al., 2002), allowed the description of mechanisms of *2n* gamete formation in lilies (Lim et al., 2001; Barba-Gonzalez et al., 2005a). There are several abnormal cytogenetic events that led to the formation of the *2n* gametes in these hybrids, however, despite of them, there are three main genetic consequences regarding chromosome segregation and nuclear restitution during meiosis. These are: i) First Division Restitution (FDR), where the whole chromosome complement divides "equationally" before telophase I, segregating each chromatid of each chromosome to the opposite poles in the cell (Fig. 2a), followed by cytokinesis, forming a dyad. ii) Second Division Restitution (SDR), where the chromosomes divide "reductionally" at anaphase I, without further segregation of the sister chromatids, as a consequence, both chromatids of a chromosome remain in the same cell. iii) Indeterminate Meiotic Restitution (IMR), in this case, some chromosomes might segregate "equationally" (each chromatid of a chromosome to a different pole of the cell), while other chromosomes might be segregated "reductionally" (complete chromosomes with both chromatids to a pole in the cell) during anaphase I, without further division forming a dyad. In all three cases, the meiosis is not complete and the stages of meiosis cannot be strictly defined (Fig. 1). The detection of these mechanisms, and more

important, the description of them, was possible with the use of GISH, because it was possible to discriminate between the parental genomes in the interspecific hybrid during meiosis and literally "observe" the segregation of each parental chromosome and its chromatids in the forming dyad.

One of the most important features of the unreduced gametes in contrast to mitotically doubled hybrids, is that recombination is present. Through the use of GISH techniques it was possible to identify recombination sites between the parental genomes during meiosis, predicting the presence of recombinant chromosomes in the progeny.

INDUCTION OF UNREDUCED GAMETE FORMATION

Despite the number of intersectional, interspecific lily hybrids created, only a few of them produced functional *2n* gametes. The unreduced gametes are both, genetically and environmentally controlled (Xu and Joppa, 2000; Kynast et al., 2001; Barba-Gonzalez et al., 2005a). Selection of male *2n* gamete producers is an arduous job, because it consists in measurements and monitoring of pollen grain germination in all the hybrids; the selection of female unreduced gametes producers is even more difficult or time consuming, because to detect the genotypes that might produce them, crosses must be performed in each genotype and analyses in the progeny must be done, at least, chromosome counting or flow cytometry tests to determine the DNA content; however, successful attempts to induce unreduced gametes in lily hybrids have been performed. Heat shock treatments resulted in generation of unreduced gametes (Lokker et al., 2005), nonetheless, different frequencies of *2n* gametes were obtained, even among clones. Another successful attempt was the injection of caffeine into young flower buds; after anthesis, the flowers were treated as both, male and female parents, producing progeny in both cases, when the progeny was analysed by GISH it showed to contain recombinant chromosomes when the flowers were utilized as female parents (Lim et al., 2005). N_2O has also been utilized; it is used by exposing the plants with young flower buds to the gas under pressure in a chamber. These treatments resulted in an increment in the frequency of unreduced gamete production in already known *2n* gamete producers and restored fertility by producing unreduced gametes in completely sterile lily hybrids. These treatments also functioned to induce unreduced female gametes (Barba-Gonzalez et al., 2006a). When the progeny plants were analysed through GISH, recombinant chromosomes were found.

PRODUCTION OF SEXUAL POLYPLOIDS

As mentioned before, the main goal in a breeding program is to introgress genes of important traits from one species to another, this might be accomplished by several backcrosses only if recombination between the parental genomes is present, otherwise, the genes from one of the parental genomes will be lost after few generations. In the case of lilies, thousands of crosses were performed at Plant Research International in different combinations to introgress the desired traits into the existing cultivars and moreover, to create completely new hybrids.

Several different F_1 intersectional, interspecific lily hybrids (AuH, HC, LA, LH, LO, LR, MA, OA, OP, and OT) were utilized to produce progeny. In some cases, fertility of the F_1 hybrids was obtained through mitotic chromosome doubling, in other cases unreduced gametes were induced by heat shock, caffeine or N_2O treatments, and in most of the cases the natural occurring *2n* gametes were utilized (Tables 1 and 2). Even though an important number of BC_1 and BC_2 hybrids were produced by the use of autopolyploid

F_1 hybrids (tetraploids obtained by mitotic chromosome doubling), particular focus to the progeny obtained through the use of *2n* gametes (induced and naturally occurring) will be given here. BC_1 progeny was obtained by utilizing the *2n* gamete producers as both, male and female parents in different backcross combinations to diploid cultivars (A x OA; O x OA; OA x A; A x LA; La x A; O x LA; O x AuH; OT x O and MA x A) and to tetraploid cultivars in the combination OAOA x OA (Table 2) (Lim et al., 2003; Barba-Gonzalez et al., 2006b; Khan et al., 2009a; Luo et al., 2012; Chung et al., 2013).

INTROGRESSION BREEDING

A representative sample of the BC_1 hybrids was analysed by flow cytometry and GISH, in most of the cases the ploidy of the progeny was triploid (2n=3*x*=36) as expected, because the unreduced gamete provides two sets of chromosomes (24) and the diploid cultivar provides one set of chromosomes (12). The BC_1 progeny of the OLA and OAuH hybrids was triploid, while the progeny of the OA hybrids was mainly triploid, only one aneuploid (2n=3*x*+1=37) and a few tetraploids were detected (Table 2); the tetraploids originated by the functioning *2n* eggs of an Asiatic cultivar used as female progenitor in the A x OA crosses and thus contributed with two sets of the A genome. Contrasting results were found in the BC_1 of the LA, OT and MA hybrids; in the MA hybrids three of them were analysed through GISH, only one of them was triploid, while the other two were aneuploid, nearly triploid (Luo et al., 2012). The BC_1 progeny of the LA and OT hybrids was unlike the progeny of the other hybrids; their progeny was constituted by diploid and triploid hybrids (also a couple of tetraploids in the case of the progeny of the LA hybrids, originated in the same way as the tetraploid OA hybrids), as well as for aneuploid nearly diploid and triploid (Table 2). The GISH analyses in the progeny of the LA and MA hybrids showed the availability of these hybrids to produce aneuploid, *n* and *2n* gametes; of relevant importance are the *n* gametes, because they allows breeding superior lily cultivars at the diploid level and introgression can be achieved with a minimum of linkage drag and just in a few generations (Khan et al., 2009a).

In all cases, the chromosome analyses by GISH detected the presence of recombination of the parental genomes in the chromosomes of the BC_1 hybrids (Fig. 2b); at some extent, the number of recombinant chromosomes and the number of recombination sites per chromosome is higher in the LA hybrids than in any other hybrids (Khan et al., 2009b; 2010). Despite of this, the GISH analyses of the BC_1 progeny showed a complex chromosome/centromere constitution, this organisation in some progeny plants was balanced, while others were unbalanced, as an example, some triploid AOA hybrids showed 12 O + 24 A chromosomes; thus, 12 A and 12 O chromosomes were contributed by the *2n* gamete of the F_1 OA hybrid, while the other 12 chromosomes were contributed by the Asiatic progenitor, showing a balanced chromosome/centromere constitution; however, there were several cases were the chromosome conformation of the triploid hybrids was 13 O +23 A chromosomes or 11 O + 25 A chromosomes, this indicated that those unbalanced chromosome constitutions were originated through IMR, while the balanced chromosome constitution were originated by FDR (Barba-Gonzalez et al., 2005b). The same was observed in the progeny of the LA and MA hybrids (Lim et al., 2001; Luo et al., 2012). The importance of the presence of IMR in the progeny is that chromosome assortment is achieved. The presence of recombinant chromosomes is also important, because chromosome substitutions can be achieved and the FDR and IMR gametes serve different purposes regarding the chromosome segment were recombination

occurs and the genetic recessive loci in the substituted regions can attain a nulliplex condition (aaa); being this, the basis of genetic variation (Barba-Gonzalez et al., 2005b).

Further crosses in different directions were performed in three main lily hybrid groups viz., OA, LA and OT hybrids, in order to obtain BC_2 progeny utilizing the BC_1 triploid hybrids obtained from unreduced gametes and diploid and tetraploid cultivars. The crosses performed include: AOA x AA; AOA x OA; AOA x OAOA; ALA x AA; LAA x AA; ALA x LALA and OO x OTO (Table 3). Regarding the ploidy of the parents utilized two groups could be identified in the BC_2 hybrids; when the crosses involved the BC_1 triploid and diploid hybrids ($3x - 2x$ or $2x - 3x$) most of the progeny was aneuploid, ranging from diploid to triploid, just a few diploid cultivars were produced (Table 3). In the case of the LA hybrids some of these crosses involved diploid BC_1 hybrids, thus, these hybrids contributed with 12 chromosomes as well as the other diploid cultivar, obviously resulting in diploid BC_2 hybrids. However, these BC_2 hybrids contained recombinant chromosomes, achieving introgression (Khan et al., 2009a). Regarding the aneuploid BC_2 progenies, the gametes of the triploid BC_1 hybrids contributed with an aneuploid chromosome number; GISH analysis on meiotic cells of triploid AOA showed chromosome segregation of A chromosomes, while the O chromosomes laggard (Fig. 2c), thus, in the triploid hybrids, chromosome segregation is expected to be random, resulting in aneuploid gametes.

The BC_2 progeny from the crosses involving triploid BC_1 hybrids to mitotically doubled tetraploids showed contrasting ploidy levels regarding the hybrid groups, in the OA hybrids the ploidy of the progeny ranged from nearly triploid to nearly tetraploids (38 – 40 chromosomes), while the ploidy of the LA hybrids was closer to the pentaploid level (54–63) (Table 3); when the crosses involved the triploid BC_1 hybrids and other F_1 *2n* gamete producers the ploidy of the progeny was pentaploid or nearly pentaploid in both groups of hybrids (LA and OA) with one exception in a nearly triploid OA hybrid with 38 chromosomes (Table 3). In most cases, the BC_2 progeny contained recombinant chromosomes, achieving chromosome assortment and introgression in the lily hybrids.

CONCLUSIONS

Introgression can be achieved in lily breeding by the use of *2n* gametes. These studies show that intersectional, interspecific F_1 lily hybrids are not always sterile and are able to produce both, *n* and *2n* gametes, which can be utilized to breed at the diploid and triploid breeding; even though, screening for *2n* gamete producers might be an arduous job it is worthwhile to identify them. In addition, *2n* gametes can be induced by different treatments. Triploid lily hybrids might be fertile and they can be used in further breeding.

The use of molecular cytogenetics in lily breeding has been a useful tool to understand and identify mechanisms of unreduced gamete formation; furthermore, it has been possible to distinguish between the parental genomes in interspecific hybrids and to monitor chromosome segregation through several generations, detecting introgression and chromosome assortment.

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Tables

Table 1. Parentage and origin of the gametes of a selection of F_1 intersectional, interspecific lily hybrids utilized in breeding programs at Wageningen UR.

Crossing Code	Progenitors		Group	Gametes' origin	Reference
	Female	Male			
OA Hybrids	Oriental	Asiatic			
951462-1	"Romero Star"	"Connecticut King"	OA	2n	Barba-Gonzalez et al., 2004
951502-1	"Pesaro"	"Connecticut King"	OA	2n	Barba-Gonzalez et al., 2004
951584-1	"Acapulco"	"Sancerre"	OA	2n	Barba-Gonzalez et al., 2004
952088-1	"Expression"	"Au Revoir"	OA	2n	Barba-Gonzalez et al., 2004
952400-1	"Mero Star"	"Gran Sasso"	OA	2n	Barba-Gonzalez et al., 2004
952462-1	"San Marco"	"Connecticut King"	OA	2n	Barba-Gonzalez et al., 2004
962119-1	"Acapulco"	"Connecticut King"	OA	2n	Barba-Gonzalez et al., 2004
962120-1	"Bernini"	"Connecticut King"	OA	2n	Barba-Gonzalez et al., 2004
962433-1	"Sissi"	"Mirella"	OA	2n	Barba-Gonzalez et al., 2004
951301-5	"Mero Star"	"Connecticut King"	OA	i2n	Lim et al., 2005
951301-5	"Mero Star"	"Connecticut King"	OA	i2n	Barba-Gonzalez et al., 2006a
952059-9	"Touch"	"Connecticut King"	OA	i2n	Barba-Gonzalez et al., 2006a
969023-2	"Casa Blanca"	"Connecticut King"	OA	i2n	Barba-Gonzalez et al., 2006a
951469	"Bel Paso"	"Connecticut King"	OA	Mitotic	Van Tuyl et al., 2002
LR Hybrids	*L. longiflorum*	*L. rubellum*			
940303	"Gelria"	*L. rubellum*	LR	Mitotic	Lim et al., 2000
950039	"Gelria"	*L. rubellum*	LR	Mitotic	Lim et al., 2004
LA Hybrids	*L. longiflorum*	Asiatic			
"Loblanca"	"White Europe"	"Mont Blanc"	LA	f2n, Mitotic	Van Tuyl & Van Holsteijn 1996
88542-24	"Gelria"	"Whilito"	LA	2n	Lim et al., 2001
88542-52	"Gelria"	"Whilito"	LA	2n	Lim et al., 2001
88542-69	"Gelria"	"Whilito"	LA	2n	Lim et al., 2001
024004-5	"White Heaven"	"Connecticut King"	LA	2n	Khan et al., 2009a
LH Hybrids	*L. longiflorum*	*L. henryi*			
89352-1	*L. longiflorum*	*L. henryi*	LH	2n, Mitotic	Van Tuyl, 1990;
89356-1					Van Tuyl and Van Holsteijn, 1996
HC Hybrids	*L. henryi*	*L. candidum*			
87506-1	*L. henryi*	*L. candidum*	HC	Mitotic	Van Tuyl and Van Holsteijn, 1996
AuH hybrids	*L. auratum*	*L. henryi*			
82111	*L. auratum*	*L. henryi*	AuH	2n	Van Tuyl and Van Holsteijn, 1996
LO Hybrids	*L. longiflorum*	Oriental			
940277	"Snow Queen"	"Acapulco"	LO	Mitotic	Lim & Van Tuyl, 2002
OP Hybrids	Oriental	Pseudolirium			
962753-3	"Anton Geesink"	*L. pardalinum*	OP	Mitotic	Van Tuyl et al., 2000
OT Hybrids	Oriental	Trumpet			
082171-1	"Cherbourg"	062433	OT	f2n	Luo et al., 2012
MA Hybrids	Martagon	Asiatic			
064045-3	72296	061079	MA	2n; f2n	Luo et al., 2012

2n = *2n* Gametes (pollen)
i2n = induced *2n* gamete
f2n = female *2n* gamete
Mitotic = Mitotic polyploidization

Table 2. Number and ploidy level of BC_1 progeny of intersectional, interspecific lily hybrids analysed by GISH.

Progenitors		Number of progenies analysed	Chromosome number of the progenies											Reference
Female	Male		23	**24**	25	26	27	32	35	**36**	37	**48**	**72**	
OA Hybrids														
AA	OA	56								51	1	4		Barba-Gonzalez et al., 2005b; Lim et al., 2005; Khan et al., 2009b.
OO	OA	14*								14				Barba-Gonzalez et al., 2005b; Lim et al., 2005.
OA	AA	6								6				Barba-Gonzalez et al., 2005b; Lim et al., 2005.
4x-OA	OA	6								1		4	1	Barba-Gonzalez et al., 2004; 2005b.
LA Hybrids														
AA	LA	24		1					3	17	1	2		Lim et al., 2001; Khan et al., 2009a;b; 2010.
LA	AA	73	1	21		1	2			46	2			Zhou et al., 2008; Khan et al., 2009a;b; 2010
OLA Hybrids														
OO	LA	1								1				Lim and Van Tuyl, 2002
LR Hybrids														
LL	4x-LR	9								9				Lim et al., 2000
OAuH Hybrids														
OO	AuH	20								20				Van Tuyl, 1990; Chung et al., 2013
OT Hybrids														
OT	OO	21		1	2	1			3	14				Luo et al., 2012
MA Hybrids														
MA	AA	3						1	1	1				Luo et al., 2012

The prefix 4x- denotes for tetraploid F_1 hybrids produced by mitotic chromosome doubling.
The Bold-faced chromosome number denotes euploid ploidy levels.
*Analysed through flow cytometry.

Table 3. Number and ploidy level of BC_2 progeny of intersectional, interspecific lily hybrids analysed by GISH

Progenitors		Number of progenies analysed	Chromosome number of the progeny																						
Female	Male		**24**	25	26	27	28	29	30	31	32	35	**36**	38	42	43	44	46	**48**	49	54	56	**60**	61	63
OA Hybrids																									
AOA	AA	10 [c,e]		1				3	1	1	2	2													
AOA	OA	2 [c]												1									1		
AOA	4x-OA	7 [c]												1	1	1	1	1	1	1					
LA Hybrids																									
ALA	AA	12 [b,d]	4		1	1	1		1	2			1											1	
LAA	AA	1 [d]		1																					
AA	LLA	2 [e]	2																						
ALA	LA	2 [e]																				1	1		
ALA	4x-LA	4 [b]																			2		1		1
LR Hybrids																									
4x-LL	LLR	3 [a]													1	1	1								
OT Hybrids																									
OO	OTO	9 [f]		3	1	2	2	1																	

Superscript refers to the bibliographic reference as follows: a Lim et al., 2000; b Lim et al., 2003; c Barba-Gonzalez et al., 2006b; d Khan et al., 2009a; e Khan et al., 2009b; Luo et al., 2012.

The Bold-faced chromosome number denotes euploid ploidy levels.

Figures

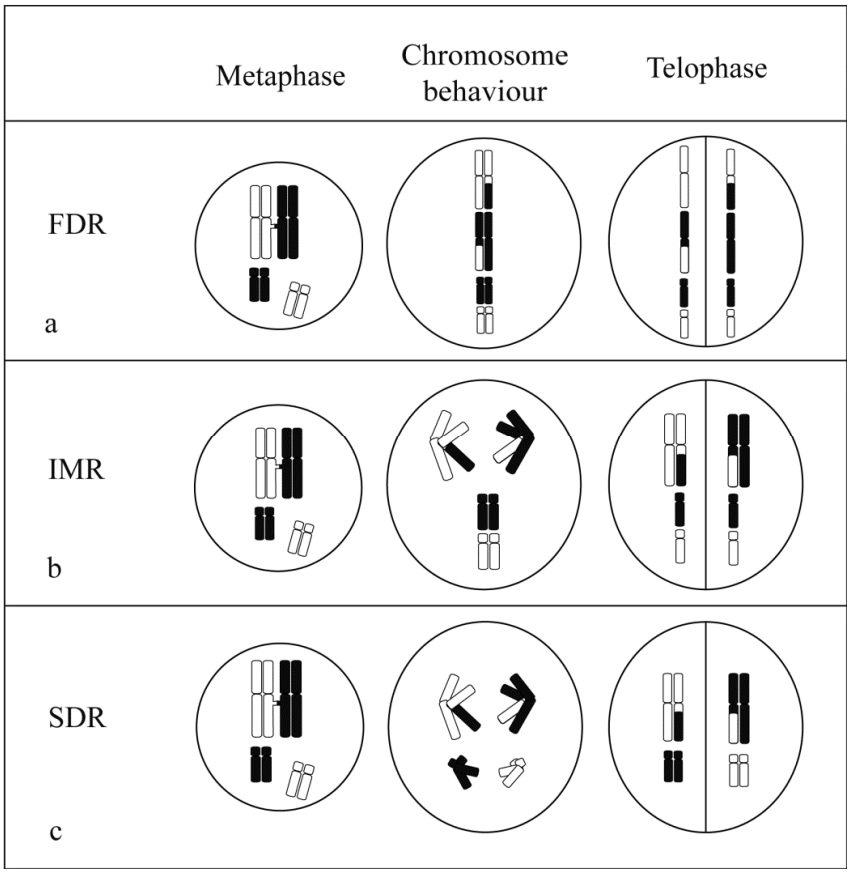

Fig. 1. Schematic representation of meiotic nuclear restitution in interspecific hybrids. The homeologous chromosomes are represented as black and white chromosomes. a) First Division Restitution (FDR), the whole chromosome complement divides equationally, segregating each chromatid of each chromosome to the opposite poles in the cell. b). Indeterminate Meiotic Restitution (IMR), some chromosomes segregate “equationally” (each chromatid of a chromosome to a different pole of the cell), while other chromosomes segregate reductionally (complete chromosomes with both chromatids to a pole in the cell). c) Second Division Restitution (SDR), the chromosomes divide reductionally, without further segregation of the sister chromatids, as a consequence, both chromatids of a chromosome remain in the same cell. In all three cases, the meiosis is not complete and the stages of meiosis cannot be strictly defined. Modified from Lim et al., 2001.

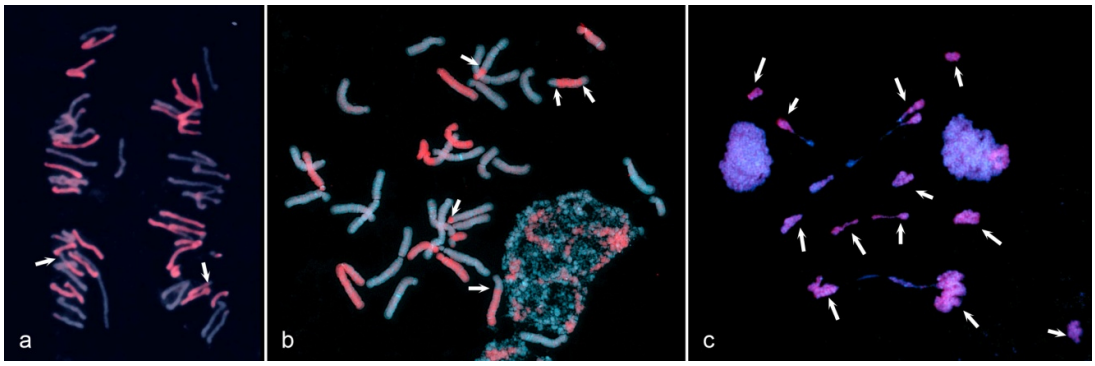

Fig. 2. GISH analysis in OA hybrids. The chromosomes are hybridized with total genomic DNA of an Oriental lily hybrid, detected with streptavidin-Cy3 (red-pinkish), Asiatic chromosomes are stained in blue. a) Meiotic anaphase in the intersectional, interspecific lily hybrid 951502-1, a *2n* gamete producer; chromosomes are segregated equationally as in First Division Restitution; arrows shows recombinant chromosomes. b) Triploid chromosome complement of the AOA hybrid 022538-15 with 11 O and 25 A chromosomes, arrows shows the recombination sites. c) Late telophase in the triploid hybrid 002531-12, laggard chromosomes are mostly Oriental (arrows) while the Asiatic chromosomes are forming two nucleuses (modified from Barba-Gonzalez, *Forthcoming* 2014).