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ORIGINAL PAPER

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Use of *2n* gametes for the production of sexual polyploids from sterile Oriental × Asiatic hybrids of lilies (*Lilium*)

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Abstract Sixteen Oriental and 12 Asiatic cultivars were crossed in 158 different combinations. A total of 708 F_1 hybrids were obtained from 86 of the different combinations of 15 Oriental and 11 Asiatic cultivars. Because the *Lilium* cultivars ($2n=2x=24$) used for the production of these hybrids belong to two different taxonomic sections —Archelirion (O) and Sinomartagon (A), respectively— the F_1 hybrids (OA) could be obtained only through embryo, embryo sac rescue, ovary slice or ovule culture. Most of the F_1 hybrids were highly sterile (did not produce viable *n* gametes) due to the failure of chromosome pairing. However, in a few cases F_1 plants were found that produced viable 2*n* pollen at variable frequencies. These 2*n* pollen grains were successfully used for the production of backcross progenies. Using genomic in situ hybridization we found intergenomic recombinant chromosomes in the sexual polyploid progenies. These results indicate that there are effective prospects for combining important horticultural traits from the two main groups of cultivars of lilies through sexual polyploidization.

Introduction

Lilium L. is a genus of the monocotyledonous family Liliaceae, which comprises over 80 species (Comber 1947; De Jong 1974), all of which are distributed throughout the mountainous areas of the Northern Hemisphere, mainly in Asia, North America and Europe (Lim et al. 2000a). Comber (1947) classified the genus into six sections: *Lilium* (Liriotypus), Pseudolirium, Martagon, Sinomartagon (Asiatic hybrids), Archelirion (Oriental hybrids) and Leucolirion.

The most important hybrid groups cultivated for cut flower production are the Longiflorum, Asiatic and Oriental hybrids. Crosses within a section can be made with relative ease, and while complete new hybrids can be developed—interspecific hybridization being the most important tool—pre- and post-fertilization barriers have to be overcome. There is a special interest in breeding Oriental × Asiatic hybrids. On the one hand there is a need to transfer characteristics such as the resistance to *Fusarium* and viral diseases from Asiatic hybrids to Oriental hybrids; on the other hand, some Orientals are resistant to *Botrytis*, a trait that would be valuable in breeding Asiatic lilies (Schenk 1990; Lim et al. 2000a).

As in other plant taxa, the F_1 hybrids between distantly related *Lilium* species are mostly sterile and as such they are useless in breeding. The traditional method of restoring fertility in such cases is to double the chromosome numbers of the F_1 hybrid and produce allopolyploids that might be fertile (Darlington 1967; Grant 1981; Van Tuyl et al. 1992). Such allopolyploids are appropriately called "permanent hybrids" because their progenies never segregate for parental characters due to the strictly autosyndetic pairing of different genomes in an allopolyploid. Therefore, from the point of view of creating genetic variation for breeding purposes, allopolyploids produced through somatic chromosome doubling have limited possibilities. In an attempt to combine desirable characteristics of two diploid species of *Lilium* ($2n=2x=24$), Lim et al. (2000b) successfully hybridized *L. longiflorum* Thunb. and *L. rubellum* Baker and doubled the chromosome number of the F_1 (LR) hybrid through oryzalin treatment. In the BC_1 and BC_2 progenies derived from LLRR allotetraploid, however, not even a single crossover between the L and R genomes was ever found (Lim et al. 2000b).

On the contrary, intergenomic recombination can occur readily in sexual polyploids induced through 2*n* gametes

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originating from the F_1 hybrids of distant species. This has been clearly demonstrated in the case of distant hybrids of *Gasteria* × *Aloe* (Takahashi et al. 1997), *Alstroemeria aurea* × *A. inodora* (Kamstra et al. 1999), *A. pelegrina* × *A. inodora* (Ramanna et al. 2003) and *L. longiflorum* × Asiatic lily hybrids (Karlov et al. 1999; Lim et al. 2001, 2003). A cardinal feature of intergenomic recombination in allopolyploids is that they can lead to the genetic segregation of parental characters in the progenies so that such polyploids do not behave as permanent hybrids (Ramanna and Jacobsen 2003). Thus, sexual polyploidization offers attractive possibilities for breeding allopolyploids of lilies.

With the aim of identifying F_1 hybrids that produce 2*n* gametes, we produced a large number of hybrids by crossing cultivars of Oriental and Asiatic lilies. The F_1 hybrids were screened for the production of 2*n* gametes, and those genotypes that had 2*n* gametes were used for producing sexual polyploid progenies. We present here our results with respect to the frequencies of 2*n* gametes found in the progenies of 12 different F_1 hybrids as well as their use in producing BC_1 progenies. In addition, we report and discuss intergenomic recombination in the progenies that was detected through genomic in situ hybridization (GISH).

Material and methods

Plant material Cultivars from two groups of lilies (*Lilium*) were used for hybridization: the so-called Oriental and Asiatic hybrids belonging to two different taxonomic sections, Archelirion and Sinomartagon, respectively. Because all of the cultivars in both sections are derived from hybridization of closely related, intra-sectional diploid (2*n*=2*x*=24) *Lilium* species (Van Tuyl et al. 2000), the accessions used in the crossing program are mentioned by their cultivar names, but not as botanical species. The Oriental and Asiatic cultivar (Table 1) genomes will be referred to as O and A, respectively. Using these symbols, we denote the F_1 hybrid as OA and the BC_1 plants as either OOA and AOA depending on the parent used in the backcross. The oryzalin-induced tetraploid OA hybrids will be indicated as 4*x*-OA (Van Tuyl et al. 1992).

Pollen germination Pollen was cultured for 24 h at 25°C in artificial agar medium containing 100 g sucrose, 5 g bacteriological agar, 20 mg boric acid and 200 mg calcium nitrate per litre. The pollen was classified as large (2*n*) and small (*n*), and the germination percentage was scored by counting only large germinated pollen grains.

Embryo, embryo sac ovary slice and ovule culture Embryo, embryo sac ovary slice and ovule culture methods were used to overcome post-fertilization barriers (Asano 1980; Van Tuyl et al. 1991; Van Creij et al. 1993; Okazaki et al. 1994).

Flow cytometry Leaves from BC_1 plants were collected to determine the ploidy level as described by Van Tuyl and Boon (1997).

Chromosome preparation To prepare somatic metaphase chromosomes, we collected root tips early in the morning from in vitro plantlets and pre-treated them in a 0.7 m*M* cyclohexamide solution at room temperature for 4 h. For the analysis of meiotic chromosomes, young anthers were collected. Both the anthers and pre-treated root tips were fixed in an ethanol:acetic acid solution (3:1) for at least 12 h and stored at −20°C until use. The root tips were incubated in a pectolytic enzyme mixture containing 0.2% (w/v) pectolyase Y23, 0.2% (w/v) cellulase RS and 0.2% (w/v) cytohelicase in 10 m*M* citrate buffer (pH 4.5) at 37°C for about 1–1.5 h. Squash preparations were made in a drop of 45% acetic acid and frozen in liquid nitrogen; the cover slips were removed using a razor blade. Slides were dehydrated in absolute ethanol and air-dried. The pollen

Table 1 Twelve selected OA hybrids of *Lilium* that produced well-filled *2n* pollen: their germination percentages and ability to produce viable embryos after being used as pollen parents (*NA* not available)

Crossing code	Parents		Pollen germination (%) (range)	Occurrence of embryos[a]
	Oriental	Asiatic		
951462-1	Romero Star	Connecticut King	31.4 (0–75)	+
951447-1	Bel Paso	Gran Sasso	2.0 (0–2)	-
951502-1	Pesaro	Connecticut King	16.6 (0–100)	+
951584-1	Acapulco	Sancerre	23.3 (5–60)	+
952088-1	Expression	Au Revoir	25.0 (NA)	+
952381-5	Mero Star	Connecticut King	2.6 (0–10)	-
952400-1	Mero Star	Gran Sasso	0.5 (0–20)	+
952462-1	San Marco	Connecticut King	37.5 (20–50)	+
962119-1	Acapulco	Connecticut King	6.0 (0–40)	+
962120-1	Bernini	Connecticut King	1.9 (0–25)	+
962254-2	Tenerife	Lanzarote	2.1 (0–30)	-
962433-1	Sissi	Mirella	24.4 (0–75)	+

[a]+, Embryos were produced; -, no embryos were produced

mother cells (PMC) were dissected from the anther and squashed in a drop of 1% aceto-orceine.

DNA probe preparation Sonicated genomic DNA (1–10 kb) from the Oriental cultivar 'Sorbonne' was used as a probe after labeling with Biotin-16-dUTP (Biotin-16–2′-deoxyuridine-5′-triphosphate) by nick translation according to the manufacturer's instructions (Roche). Autoclaved DNA (100–500 bp) from the Asiatic cultivar 'Connecticut King' was utilized for blocking the non-hybridized sequences.

Genomic in situ hybridization The in situ hybridization protocol was carried out according to Lim et al. (2000b) with minor modifications. In brief, slides were pretreated with RNase A (100 μg/ml) for 1 h and pepsin (5 μg/ml) for 10 min, both at 37°C, then by formaldehyde (4%) for 10 min at room temperature, dehydrated sequentially with 70%, 90% and absolute ethanol for 3 min at each concentration and air-dried. Hybridization followed using a mixture of 20× SSC, 50% formamide, 10% sodium dextran sulphate, 10% sodium dodecyl sulfate, 25–50 ng/ml probe DNA and 5–10 μg/ml blocking DNA. The DNA was denatured by heating the hybridization mixture at 70°C for 10 min and then placed on ice for at least 10 min. For each slide, 40 μl hybridization mixture was used. The preparations were denatured at 80°C for 10 min. After an overnight hybridization at 37°C in a humid chamber, the slides were washed at room temperature in 2× SSC for 15 min and 0.1× SSC at 42°C for 30 min. Biotin-labelled DNA was detected with Cy3-labelled streptavidin (Amersham Biosciences, UK), and amplified with biotinylated goat-antistreptavidin (Vector laboratories, Burlingame, Calif.). Chromosomes were counterstained with 1 μg/ml DAPI (4,6-diamidino-2-phenylindole) and examined under a Zeiss Axiophot microscope equipped with a triple filter. Images were photographed on 400 ISO color negative film and scanned at 1,200 dpi for digital processing in Photoshop (Adobe).

Results

Production of F_1 hybrids

Because hybridization between cultivars of two different sections could not be achieved through normal crossing and seed production, pollination was carried out by the

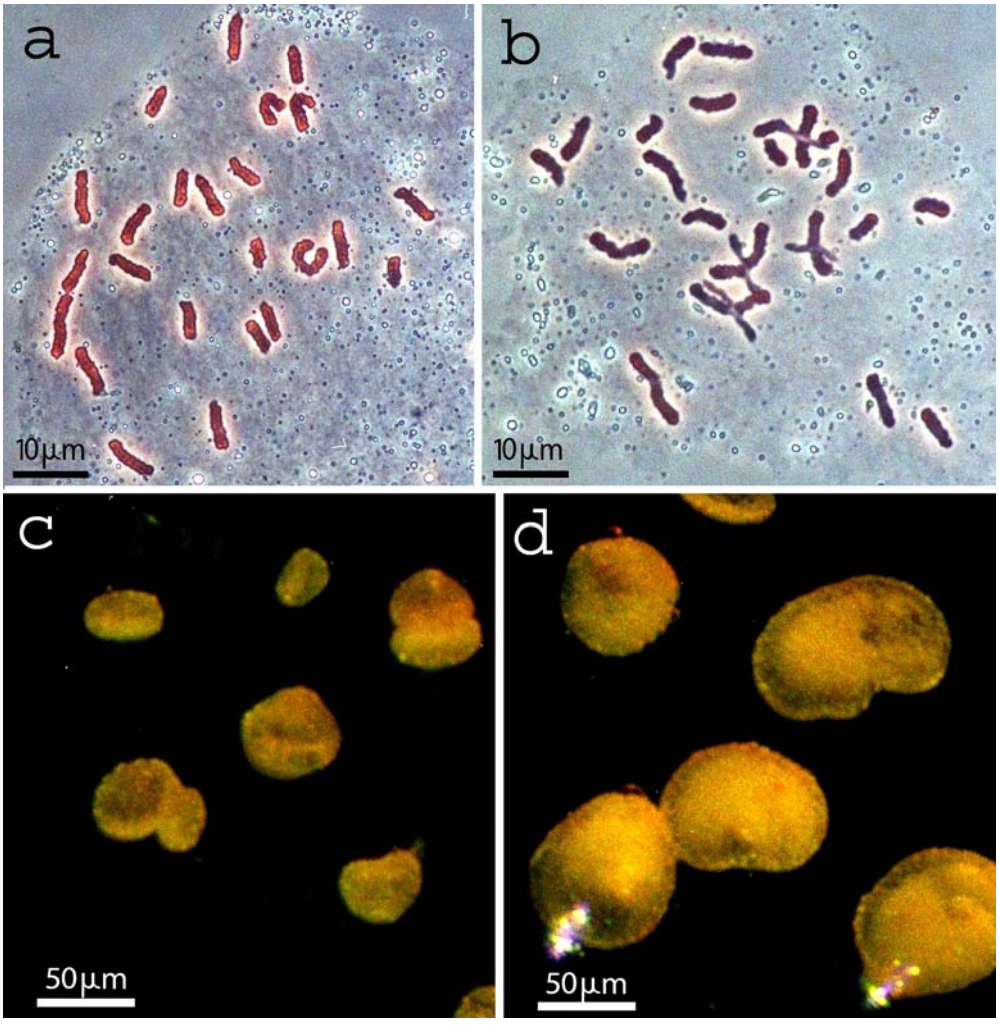

Fig. 1a–d Metaphase I stages during microsporogenesis in a OA hybrid (951502-1). **a**, **b** Two pollen mother cells, one with 24 univalents (**a**) and one with four bivalents + 16 univalents (**b**). **c**, **d** Pollen grains in the same OA hybrid showing sterile (**c**) and fertile (**d**) 2*n* pollen grains

Table 2 Results of crossing of 2*n* pollen producing OA hybrids with the parental diploid, 2*x*-OA and 4*x*-OA genotypes

Parents		Number of flowers pollinated	Number of germinations	Based on theoretical estimate of 100 crosses
Female	Male			
Asiatic				
Amarone	951462-1	9	5	56
Gironde	951502-1	24	54	225
Amarone	951502-1	23	160	696
Mont Blanc	951502-1	16	44	275
Amarone	951584-1	2	10	500
Gironde	952400-1	16	27	169
Amarone	952400-1	5	25	500
Mont Blanc	952400-1	5	15	300
Gran Sasso	952400-1	5	2	40
Lanzarote	952400-1	18	36	200
Gironde	952462-1	6	1	17
Amarone	952462-1	6	26	433
Mont Blanc	952462-1	3	20	667
Mont Blanc	962119-1	2	3	150
Amarone	962120-1	2	1	50
Gironde	962433-1	12	10	83
Amarone	962433-1	13	26	200
Mont Blanc	962433-1	14	59	421
Total		181	524	2.9 e/p[a]
Oriental				
Sorbonne	951502-1	28	45	161
Lombardia	951502-1	31	27	87
Lombardia	951584-1	11	14	127
Sorbonne	952462-1	5	8	160
Lombardia	952462-1	4	6	150
Tiber	952462-1	6	3	50
Lombardia	962119-1	2	4	200
Lombardia	962120-1	2	2	100
Sorbonne	952400-1	8	2	25
Time out	952400-1	5	2	40
Bramante	952400-1	4	1	25
Total		106	114	1 e/p
OA Hybrid				
952400-1	Gironde	7	12	171
952400-1	Connecticut King	2	4	200
952400-1	Amarone	11	6	55
952400-1	Mont Blanc	4	11	275
952400-1	Mero Star × Connecticut King	12	5	42
952400-1	Expression × Lady Rosa	14	2	14
Total		50	40	0.8 e/p
4*x*-OA				
Romero Star × Lady Rosa	951502-1	10	1	10
Expression × Lady Rosa	951502-1	19	8	42
Expression × Lady Rosa	952088-2	2	1	50
Romero Star × Connecticut King	952400-1	12	2	17
Expression × Lady Rosa	952400-1	11	3	27
Expression × Lady Rosa	952462-1	2	7	350
Total		56	22	0.4 e/p

[a]e/p, Embryos/pollination

cut style method and either embryo, embryo sac rescue, ovary slice or ovule culture was required in all cases (Van Tuyl and De Jeu 1997). In order to rescue embryos, we used ovules from capsules that had developed any time between 20 days and 70 days post-pollination. This means that the embryo and endosperm were allowed to develop in vivo for a considerable amount of time just as if they were following the normal course of seed development. There were cases in which the embryo, endosperm, or both failed to develop in the ovules in vivo. Only those ovules that developed embryos were dissected and used for in vitro culture. Although embryo rescue is a laborious technique, we were able to obtain 708 F_1 plants from the 86 crossing combinations. In addition to confirming the F_1 hybrids on the basis of plant morphology, we used chromosome pairing (Fig. 1a, b) and a high degree of male sterility as criteria.

Pollen of F_1 hybrids and their viability

With the exception of a small fraction of the F_1 hybrids, all possessed aborted small pollen grains and were completely sterile (Fig. 1c). However, in a small number of OA hybrid combinations, well-filled large pollen grains (Fig. 1d) were present in some genotypes, and these were considered to be 2*n* pollen grain producers. Of the 708 OA hybrids that were produced, only 12 (1.7%) were found to produce 2*n* pollen in notable frequencies. We investigated these further for pollen viability by means of germination tests and their ability to produce embryos when used as male and female parents (Tables 1, 2). The frequencies of 2*n* pollen grains in all the genotypes were highly variable, probably due to environment (data not included). In order to establish whether these 2*n* pollen grains were viable and functional, they were germinated in vitro as well as used as male parents in crossing with 2*x* parents and 2*x*-OA and 4*x*-OA hybrids. Average percentage and range of pollen germination in the different genotypes and the outcome of the crossing experiments, as evidenced from viable embryo formation in vitro of 12 OA hybrids, are presented in Table 1. For calculating average germination percentages we took into account only those of the large pollen grains that had germinated. Although 2*n* pollen germinated in all 12 hybrids, nearly a 40-fold variation was observed for germination percentages among different genotypes of the same hybrid as well as among the 12 different hybrids that were investigated (Table 1). There was, however, no strict relationship between germination percentage and the formation of viable embryos in vitro. For example, of the nine cases where embryo formation was observed, seven had relatively high percentages of germination, whereas two (952400-1, 962120-1) had low percentages (2% or less). Embryos were formed when higher pollen germination percentages were found (Table 1). The possible influence of the environment on the formation and the viability of 2*n* pollen could explain this absence of a strict relationship between pollen germination and viable embryo formation. This implies that those genotypes that showed a lower percentage of germination had formed a higher percentage of viable pollen at the time of making the crosses. In an estimate of germination, percentages of 2*n* pollen in eight clones of one genotype (962433-1) showed nearly a 30- to 40-fold variation between the different stems as well as among the individual flowers of the same plant (results not included). This implies that 2*n* pollen may have to be repeatedly tested before any conclusion can be drawn on whether a pollen parent can be used successfully.

Use of 2*n* pollen for backcrossing

In order to investigate whether the selected 2*n* pollen producers were suitable for sexual polyploidization, we used all of the selected genotypes of the 12 OA hybrids as male and some as female parents for crossing with the diploid parental cultivars as well as with the 2*x*-OA and 4*x*-OA hybrids. The successful results obtained in four different sets of hybridizations are presented in Table 2. Of all the 2*n* pollen-producing genotypes tested as male parents, nine gave rise to germinating embryos. In the backcrosses, a relatively larger number of germinating embryos was obtained in the case of Asiatic × OA hybrids (2.9 embryos/pollination) as compared to Oriental × OA hybrids (1 embryo/pollination) (Table 2).

In addition to the male fertility of the 2*n* gamete-producing genotypes, one genotype (952400-1) showed positive results for female fertility (Table 2). In this case, four Asiatic cultivars as well as two 4*x*-OA hybrids were used as male parents. The occurrence of female fertility in the OA hybrids indicates the potential for using 2*n* eggs for (bilateral) sexual polyploidization. The crosses with 4*x*-OA hybrids with four 2*n* pollen-producing genotypes were successful. On the basis of these crossing data it was evident that most of the selected genotypes (Table 1) were potentially useful for producing a large number of backcross and other progenies through unilateral as well as bilateral sexual polyploidization.

Analysis of the sexual polyploid progenies (BC_1, + 4*x*-OA × OA crosses)

In 263 BC_1seedlings derived from three different combinations— AOA, OOA and OAA and six 4*x*-OA × OA— seedlings were analysed for their ploidy levels by determining DNA values through flow cytometry according to Van Tuyl and Boon (1997). Assuming that 2*n* gametes from OA hybrids contributed 24 chromosomes, we expected that the BC_1 progenies would possess triploid (2*n*=3*x*=36) chromosome numbers and the 4*x*-OA × OA progenies would have tetraploid (2*n*=4*x*=48) chromosome numbers. Of the 263 BC_1 progenies that were analysed, 246 (93.5%) were triploid and 14 (5.3%) were tetraploid (Table 3). In the 6 4*x*-OA × OA progenies, one was a triploid, four were tetraploid and one was a hexaploid (not shown). The observation that the BC_1 progenies were

mostly triploids proved that the F_1 OA hybrids had contributed balanced diploid chromosome complements. And, as expected, the 4*x*-OA × OA progeny were mostly tetraploid. The few tetraploids that occurred in the BC_1 progenies had obviously originated through the functioning of 2*n* gametes from both the diploid Asiatic and the OA backcross parent. A notable result was the presence of one hexaploid among the 4*x*-OA × OA progeny which was probably the result of the production of a 2*n* egg in the mitotically doubled OA hybrid. The results of flow cytometric analysis were confirmed through cytological analysis of the somatic chromosomes in several triploids and tetraploids (Fig. 2a,b).

Of particular interest is that in the BC_1 progenies the presence of intergenomic recombinant segments could be demonstrated by means of GISH analysis (Fig. 2a). Such recombination was anticipated because the cytological analysis of metaphase I stages of the F_1 OA hybrids revealed bivalent formation between O and A genomes (Fig. 1b).

Discussion

Failure to obtain intergenomic recombination in the intersectional species hybrids of *L. rubellum* × *L. longiflorum* (Lim et al. 2000b) is a clear illustration of the limitation of using somatically doubled allotetraploids in breeding lilies. In contrast, the results of the present investigation clearly demonstrate the value of sexual polyploidization in obtaining crossovers between O and A genomes. There are indeed BC_1 progenies that are predominantly triploid as well as 4*x* progenies from $BC_1$and 4*x*-OA × OA crosses that possess more than one recombinant chromosome (results not included). Allotriploid BC_1 plants, although difficult to hybridize, can be crossed either with diploid or tetraploid parents, as shown by previous results from *L. longiflorum* × Asiatic crosses (Lim et al. 2003). In these cases the progenies that possess near-diploid or near-pentaploid chromosome numbers have to be selected for the presence of recombinant chromosomes. In the case of 4*x*-OA × OA crosses, most of the progeny plants are allotetraploids. Some of these possess recombinant chromosomes. The allotetraploids are expected to behave

Table 3 Number and types of progenies obtained from different types of backcrosses

Progeny code	Parents		Number of progenies analyzed	Ploidy levels of the progenies	
	Female	Male		3*x*	4*x*
A OA					
002433	Gran Sasso	952400-1	2	2	0
002526	Lanzarote	952400-1	20	20	0
002529	Lanzarote	952400-1	16	16	0
002531	Gironde	952400-1	21	21	0
022217	Gironde	952400-1	6	6	0
022218	Gironde	952400-1	9	6	3
022219	Mont Blanc	952400-1	4	3	1
022538	Amarone	951502-1	14	12	0
022542	Amarone	962433-1	3	3	0
022604	Gironde	951502-1	16	15	1
022605	Amarone	951502-1	75	71	4
022610	Mont Blanc	951502-1	18	17	1
022611	Gironde	951502-1	12	11	1
022612	Amarone	951502-1	25	23	2
022643	Amarone	951502-1	3	2	1
O OA					
992682	Sorbonne	952400-1	2	2	0
992738	Time Out	952400-1	2	2	0
022552	Lombardia	951584-1	1	1	0
022572	Lombardia	962119-1	1	1	0
022574	Lombardia	952462-1	1	0	0
022582	Sorbonne	952462-1	3	3	0
022609	Sorbonne	951502-1	2	2	0
022624	Lombardia	951502-1	1	1	0
022636	Lombardia	951502-1	1	1	0
OA A					
022215	952400-1	Mont Blanc	1	1	0
022204	952400-1	Connecticut King	4	4	0
Total			263	246	14

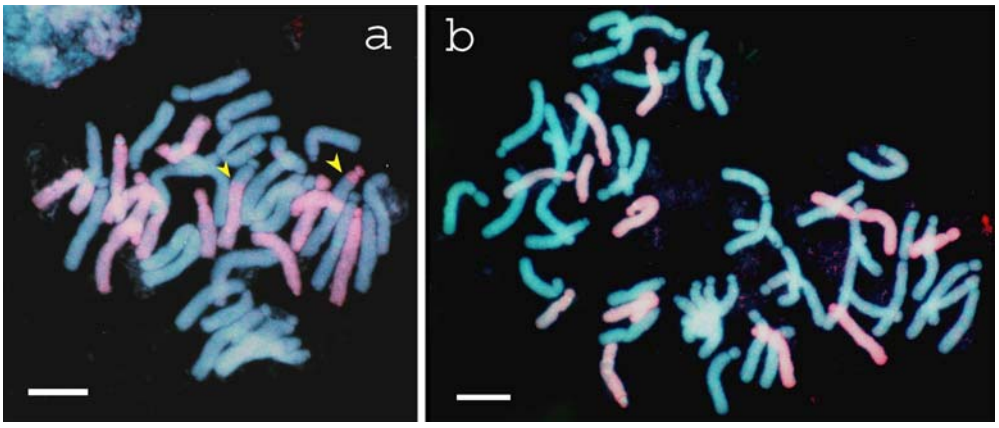

Fig. 2a,b Chromosome complements of the BC_1 progenies showing a triploid and a tetraploid chromosome number. **a** 36 chromosomes of the BC_1, 022217-3, plant with two recombinant chromosomes (*arrows*). The biotin-labelled Oriental DNA of 12 chromosomes was detected with the Cy3-streptavidin system (*pink fluorescence*) and 24 Asiatic chromosomes were counterstained with DAPI (*blue fluorescence*). **b** A tetraploid chromosome complement of the BC_1 plant, 022218-6, showing 12 Oriental and 36 Asiatic chromosomes. The probe DNA used and detection were the same as for **a**. *Bar*: 10 μm

almost like permanent hybrids except for those chromosomes that have recombinant segments that can form multivalents and assort independently. Thus, these tetraploids are much more directly accessible for breeding than the triploid BC_1 progenies.

The presence of 2*n* gametes in the present study is similar to results reported in distant F_1 hybrids in various other plant taxa (Ramanna and Jacobsen 2003). Most of these cases share certain common features: (1) they all have disturbed chromosome pairing; (2) they are highly sterile in the sense that they do not produce functional *n* gametes; (3) they all produce exclusively first-division restitution (FDR) gametes. Some of the other comparable instances are: wheat × *Aegilops squarrosa* (Fukuda and Sakamoto 1992), *Aegilops squarrosa* × *Triticum durum* (Sasakuma and Kihara 1981), rye × *A. squarrosa* (Xu and Dong 1992; Xu and Joppa 2000), *Alstroemeria* interspecific hybrids (Ramanna et al. 2003) and *L. longiflorum* × Asiatic hybrids (Lim et al. 2001). An important feature in many of these cases is that the frequencies of 2*n* gametes are greatly influenced by the environment. This also appears to be the case in the present *Lilium* hybrids (unpublished data).

There is a general view that 2*n* gametes may not be widely applicable in breeding because their occurrence is not always predictable. Although there are some instances in which 2*n* gamete formation has been claimed to be genetically controlled, this is not always the case (Ramanna and Jacobsen 2003). In the present study the cultivars of both Oriental and Asiatic hybrids were not found to produce FDR gametes at any noticeable frequencies. Yet there are a few genotypes among the F_1 hybrids (Table 1) that did produce considerable frequencies of 2*n* pollen through FDR. The success in the present study has probably been achieved for the following two reasons: (1) a fairly large number of F_1 hybrids using several genotypes were produced; (2) all of the F_1 hybrids were carefully screened for the occurrence of 2*n* gametes. Because of the relative ease with which the pollen could be screened, we have found several 2*n* pollen producers, and there is evidence of 2*n* eggs producing genotypes (952400-1). However, it will be important to identify more 2*n* egg producers because it would facilitate bilateral sexual polyploidization.

There appear to be certain trends in the hybrids of different plant species that can aid in the identification of the genotypes that might produce 2*n* gametes. For example, in wheat × *Aegilops* hybrids, the later emerging spikes and secondary culms produced a higher frequency of 2*n* gametes (Fukuda and Sakamoto 1992). In *Alstroemeria*, hybrids between Brazilian and Chilean species produced much higher frequencies of 2*n* gametes than those between the Chilean species (Ramanna et al. 2003). In the genus *Saccharum*, interspecific hybrids between *S. officinarum* × *S. spontaneum* produced 2*n* eggs on a more regular basis (Bremer 1961). In the present investigation, the hybrids with the Asiatic cultivar Connecticut King as the male parent predominantly produced 2*n* pollen. But it is important to note that we have screened a relatively larger number of hybrids from this combination for 2*n* pollen production. The genotype dependence of 2*n* gamete production has been observed in some of crop plants (Ramanna and Jacobsen 2003), but it remains to be established whether such genotype dependence is also present in lilies. In view of the variable tendencies found in the hybrids of different plant species, it might be helpful to identify the genotype dependence, if it exists, in each instance.

In *Lilium*, embryo rescue is required for producing the F_1 hybrids and the BC_1 plants and partly for producing the BC_2 and subsequent progenies. A comparable situation is observed in the interspecific hybrids of *Alstroemeria aurea* × *A. inodora* where embryo rescue is required for backcrossing (Kamstra et al. 1999). In other interspecific hybrids of *Alstroemeria* involving Brazilian and Chilean species, however, seed set occurs in vivo after selfing the F_1 hybrids as well as after backcrossing (Buitendijk et al. 1997; Ramanna et al. 2003). In many of the cereal

interspecific and intergeneric hybrids and polyhaploids, both 2*n* pollen and 2*n* eggs occur so frequently that selfing and backcrossing can be practised regularly (Ramanna and Jacobsen 2003). Despite embryo rescue being time-consuming and laborious in *Lilium* hybrids, as this investigation shows, success can be achieved in using 2*n* gametes for sexual polyploidization.

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