# Intergenomic recombination in $F_1$ lily hybrids (*Lilium*) and its significance for genetic variation in the $BC_1$ progenies as revealed by GISH and FISH

**R. Barba-Gonzalez, M.S. Ramanna, R.G.F. Visser, and J.M. Van Tuyl**

**Abstract:** Intergenomic recombination was assessed in a $BC_1$ population of Oriental (O) × Asiatic (A) lilies (*Lilium*) backcrossed to Asiatic parents. This population consisted of 38 plants generated from the $2n$ gametes from 2 genotypes (951502-1 and 952400-1) of the diploid $F_1$, Oriental × Asiatic lilies ($2n = 2x = 24$) as parents. In the majority of $BC_1$ plants, there was evidence that first division restitution, with and without crossovers, resulted in functional gametes. However, there were 5 $BC_1$ plants in which $2n$ gametes originated from indeterminate meiotic restitution (IMR). Based on the number of recombinant chromosomes for a particular homoeologous pair, 3 types of plants were identified: (*i*) those with both the reciprocal product of a crossover ($^{O}/_{A}$, $^{A}/_{O}$, where O represents the centromere of the O genome and A the recombinant segment of Asiatic chromosome, and vice versa); (*ii*) those with 1 normal chromosome of the O genome and a recombinant chromosome (O, $^{A}/_{O}$); and (*iii*) those with 1 normal chromosome of the A genome and a recombinant chromosome (A, $^{O}/_{A}$). An important feature of A × OA backcross progeny is the occurrence of substitutions for the segment distal in the crossover wherever the recombinant chromosome $^{O}/_{A}$ was present. In the case of IMR, the substitution occurred for both proximal and distal recombinant segments. The significance of these substitutions is that they offer the potential for the phenotypic expression of recessive genes in polyploids (i.e., nulliplex genotype).

*Key words:* genomic in situ hybridization (GISH), fluorescence in situ hybridization (FISH), unreduced gametes, allopolyploid.

**Résumé :** La recombinaison intergénomique a été mesurée chez une population $BC_1$ de lys orientaux × asiatiques rétrocroisés à des parents asiatiques. La population comptait 38 individus et résultait de gamètes $2n$ fonctionnels produits par 2 $F_1$ (951502-1 et 952400-1) diploïdes ($2n = 2x = 24$) issus du croisement de lys orientaux × asiatiques. Chez la plupart des plantes $BC_1$, des évidences ont été obtenues de restitution à la première division (FDR; « first division restitution »), avec ou sans enjambement, ce qui menait à la production de gamètes fonctionnels. Chez 5 plantes $BC_1$, cependant, les gamètes $2n$ auraient été produits par suite de restitution méiotique indéterminée (IMR ; « indeterminate meiotic restitution »). En fonction du nombre de chromosomes recombinants pour une paire d'homéologues, 3 types de plantes ont été identifiés : (*i*) porteuses des 2 produits réciproques de la recombinaison ($^{O}/_{A}$, $^{A}/_{O}$, où O représente le centromère du génome O et A le segment recombinant du chromosome asiatique et vice versa) ; (*ii*) porteuses d'un chromosome normal O et d'un chromosome recombinant (O, $^{A}/_{O}$) et (*iii*) porteuses d'un chromosome normal du génome A et d'un chromosome recombinant (A, $^{O}/_{A}$). Une caractéristique importante des progénitures A × OA était la présence de substitutions du segment distal à l'enjambement à chaque fois qu'un chromosome recombinant $^{O}/_{A}$ était présent. Dans le cas des gamètes IMR, la substitution se produisait tant chez les segments proximaux que distaux. L'importance de ces substitutions provient du fait qu'elles offrent la possibilité d'exprimer des gènes récessifs chez des polyploïdes (e.g., un génotype nulliplexe).

*Mots clés :* hybridation génomique in situ, hybridation in situ en fluorescence, gamètes non-réduits, allopolyploïde.

[Traduit par la Rédaction]

## Introduction

There are 3 main groups of lily hybrids (*Lilium*) important in commercial horticulture: Longiflorum, Asiatic (A), and Oriental (O) hybrids. These groups belong to different taxonomic sections: Leucolirion, Sinomartagon, and Archelirion,

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respectively. The cultivars in all these groups are mostly diploid ($2n = 2x = 24$) and they are easy to hybridize within the same section. However, it is difficult to hybridize cultivars or species that belong to different sections; these hybrids can be produced only through special techniques, such as the cut-style method (Asano and Myodo 1977*a*, 1977*b*), the grafted-style method followed by in vitro pollination (Van Tuyl et al. 1991), in vitro pollination, and rescue methods, such as embryo, ovary slice and ovule culture (Van Creij et al. 2000).

In an effort to combine desirable horticultural traits from species of different sections, we have made many intersectional hybrids. These include Longiflorum × Asiatic (LA hybrids) and Oriental × Asiatic (OA) hybrids (Lim et al. 2003; Van Tuyl et al. 2002). Because of the high degree of sterility of $F_1$ hybrids, they cannot be directly used for backcrossing. To restore fertility, the chromosome numbers of the $F_1$ hybrids are doubled with colchicine or oryzaline treatment (Van Tuyl et al. 1992; Lim et al. 2000*a*), and the allotetraploid is used for backcrossing. A serious drawback of this approach has been that, because of autosyndetic pairing in the allotetraploid, there has been no intergenomic recombination in $BC_1$ and subsequent progenies (Lim et al. 2000*a*). An alternative approach is to use numerically unreduced (*2n*) gametes from $F_1$ hybrids for backcrossing. Although it is generally difficult to find genotypes of $F_1$ hybrids that produce large numbers of *2n* gametes, it is possible to select such genotypes in both LA and OA hybrids and to backcross them extensively in both cases (Lim et al. 2000*b*; Barba-Gonzalez et al. 2004).

An important feature of the LA hybrids is that it is possible to obtain intergenomic recombination in the $BC_1$ progenies (Lim et al. 2003; van Tuyl and Lim 2003). This recombination includes not only crossing over between homoeologous chromosomes, but also chromosome assortment. The crucial factors for the occurrence of recombination in LA hybrids are the result of 2 types of nuclear restitution mechanisms: first division restitution (FDR), with crossing over; and indeterminate meiotic restitution (IMR) (Lim et al. 2001). As a consequence of intergenomic recombination, substitutions for recombinant segments, as well as for whole chromosomes, can occur in the $BC_1$ progenies of LA hybrids (Lim et al. 2001). First division restitution has also been found to occur in OA hybrids (Barba-Gonzalez et al., 2005).

The aims of this investigation on $BC_1$ progenies are to estimate the extent of intergenomic recombination, to determine the chromosome composition through genomic in situ hybridization (GISH) and fluorescent in situ hybridization (FISH), and to determine whether substitutions occur for recombinant segments in the $BC_1$ progenies of OA hybrids.

The implications of the results for the occurrence of genetic variation are discussed.

## Material and methods

### Plant material

A $BC_1$ population consisting of 38 plants generated through sexual polyploidization was used to estimate the extent of intergenomic recombination and to determine the chromosome constitution. Three diploid Asiatic cultivars (A genome) (‘Gironde’, ‘Mont Blanc’, and ‘Amarone’ ($2n = 2x = 24$)), 2 tetraploid OA hybrids (4*x*–OA) from the PRI collection (991108 and 991110), and a diploid OA genotype (952400-1) were used as female parents. With 3 exceptions, all these $BC_1$ progeny plants were derived from crossing a diploid cultivar with either of the *2n* pollen-producing OA hybrids: 952400-1 (13 plants) or 951502-1 (23 plants). Only 1 $BC_1$ plant originated from an OA × A cross in which *2n* eggs from the OA hybrid must have been functional (Table 1). Bulbs from all hybrids and cultivars were planted in a greenhouse and grown in standard conditions for lily growth and development (Van Tuyl and Van Holsteijn 1996).

### Chromosome preparation

For the study of somatic metaphase chromosomes, root tips were collected early in the morning, incubated in saturated α-bromonaphthalene solution in ice-water overnight, fixed in 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 mmol citrate buffer/L (pH 4.5), at 37 °C for approximately 2 h. Squash preparations were made in a drop of 50% acetic acid and frozen in liquid nitrogen; the cover slips were removed with a razor blade. Slides were dehydrated in absolute ethanol and air dried.

### DNA-probe preparation

Fluorescence in situ hybridization was performed using 2 different probes: clone pTa71, which contains the *Eco*RI fragment of 45S ribosomal DNA from wheat (9 kb) (Gerlach and Bedbrook 1979); and a synthetic telomeric probe that was generated using PCR, as described by Cox et al. (1993), with minor modifications. In brief, 2 oligomer primers, 1fw $(5'\text{-TTTAGGG-}3')_5$ and 1rev $(5'\text{-CCCTAAA-}3')_5$, were synthetized by Isogen Life Science (Maarssen, Netherlands). Reactions were amplified in the absence of template DNA. Each 100-µL reaction comprised 10 µL of 10× *Taq* buffer (Promega, Madison, Wis.), 1.5 mmol $MgCl_2$/L, 2 units of *Taq* polymerase (Promega), 2.5 mmol deoxynucleoside triphosphate (dNTP), and 10 pmol of each primer (1fw and 1rev). Temperature cycling was performed as described by Ijdo et al. (1991), with the final extension step of 10 min at 72 °C.

For GISH, sonicated genomic DNA (1–10 kb) from the Oriental cultivar ‘Sorbonne’ was used as a probe. All probes were labeled with either biotin-16-dUTP or digoxigenin-11-dUTP by nick translation, in accordance with the manufacturer’s instructions (Roche, Germany).

### In situ hybridization

Fluorescence in situ hybridization was performed with metaphase chromosomes of 10 different $BC_1$ progeny plants by incubating slides in RNase A (100 µg/mL) for 1 h and pepsin (5 µg/mL) for 10 min, both at 37 °C, and then in paraformaldehyde (4%) for 10 min at room temperature; they were then dehydrated with 70%, 90%, and absolute ethanol for 3 min each, and air dried. Hybridization followed using a mixture consisting of 20× SSC, 50% formamide, 10% sodium dextran sulphate, 10% SDS, and 25–50 ng/mL of each probe. The DNA was denatured by heating the hybridization mixture at 70 °C for 10 min and then placing it

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**Table 1.** Genotypic information on ploidy level and number of Oriental (O), Asiatic (A), and recombinant chromosomes of a $BC_1$ population generated from the crossing of *2n* gametes, producing OA hybrids with 3 parental diploid Asiatic cultivars and 2 4×–OA genotypes.

| | Parents | | | | Genome composition | | |
|---|---|---|---|---|---|---|---|
| Genotype | Female | Male | Cross | Ploidy | O ($^{O}/_{A}$) | A ($^{A}/_{O}$) | Number of recombinant chromosomes |
| 022215-1 | 952400-1 | 'Mont blanc' | OA × A | 3× | 12 | 24 | 0 |
| 022217-1 | 'Gironde' | 952400-1 | A × OA | 3× | 12 (1) | 24 (1) | 2 |
| 022217-2 | 'Gironde' | 952400-1 | A × OA | 3× | 12 | 24 | 0 |
| 022217-3 | 'Gironde' | 952400-1 | A × OA | 3× | 12 (1) | 24 (1) | 2 |
| 022217-4 | 'Gironde' | 952400-1 | A × OA | 3× | 12 | 24 | 0 |
| 022217-5 | 'Gironde' | 952400-1 | A × OA | 3× | 12 | 24 (2) | 2 |
| 022218-1 | 'Gironde' | 952400-1 | A × OA | 3× | 12 | 24 | 0 |
| 022218-3 | 'Gironde' | 952400-1 | A × OA | 3× | 12 | 24 | 0 |
| 022218-4 | 'Gironde' | 952400-1 | A × OA | 3× | 12 | 24 (1) | 1 |
| 022218-5 | 'Gironde' | 952400-1 | A × OA | 3× | 12 | 24 | 0 |
| 022218-7 | 'Gironde' | 952400-1 | A × OA | 3× | 12 | 24 | 0 |
| 022218-10 | 'Gironde' | 952400-1 | A × OA | 3× | 12 | 24 | 0 |
| 022218-11 | 'Gironde' | 952400-1 | A × OA | 3× | 12 | 24 | 0 |
| 022219-2 | 'Mont blanc' | 952400-1 | A × OA | 3× | 12 | 24 (1) | 1 |
| 022538-1[1,3] | 'Amarone' | 951502-1 | A × OA | 3× | 12 (4) | 24 (3) | 7 |
| 022538-3[3] | 'Amarone' | 951502-1 | A × OA | 3× | 12 (4) | 24 (2) | 6 |
| 022538-5 | 'Amarone' | 951502-1 | A × OA | 3× | 12 (3) | 24 (2) | 5 |
| 022538-7[1,3] | 'Amarone' | 951502-1 | A × OA | 3× | 13 (3) | 23 (3) | 6* |
| 022538-[8 3] | 'Amarone' | 951502-1 | A × OA | 3× | 12 (2) | 24 (2) | 4 |
| 022538-[9 3] | 'Amarone' | 951502-1 | A × OA | 3× | 13 (2) | 23(2) | 4* |
| 022538-14 | 'Amarone' | 951502-1 | A × OA | 3× | 12 (3) | 24 (2) | 5 |
| 022538-15[1,4] | 'Amarone' | 951502-1 | A × OA | 3× | 11 (1) | 25 (3) | 4* |
| 022544-2 | 991110 | 951502-1 | OA × OA | 4× | 24 | 24 | 0 |
| 022604-6 | 'Gironde' | 951502-1 | A × OA | 3× | 12 | 24 | 0 |
| 022604-9 | 'Gironde' | 951502-1 | A × OA | 3× | 12 (1) | 24 | 1 |
| 022604-10 | 'Gironde' | 951502-1 | A × OA | 3× | 12 | 24 | 0 |
| 022605-1 | 'Amarone' | 951502-1 | A × OA | 3× | 12 (1) | 24 (1) | 2 |
| 022605-3[2,4] | 'Amarone' | 951502-1 | A × OA | 4× | 12 (4) | 36 (3) | 7 |
| 022605-7 | 'Amarone' | 951502-1 | A × OA | 3× | 12 (3) | 24(3) | 6 |
| 022605-8[4] | 'Amarone' | 951502-1 | A × OA | 3× | 12 (2) | 24 (1) | 3 |
| 022605-9[2,4] | 'Amarone' | 951502-1 | A × OA | 3× | 12 (4) | 24 (3) | 7* |
| 022605-13 | 'Amarone' | 951502-1 | A × OA | 4× | 11 (1) | 37 (1) | 2* |
| 022605-15 | 'Amarone' | 951502-1 | A × OA | 4× | 12 | 36 | 0 |
| 022605-38 | 'Amarone' | 951502-1 | A × OA | 3× | 12 (1) | 24 (1) | 2 |
| 022605-40[4] | 'Amarone' | 951502-1 | A × OA | 3× | 12 (2) | 24 (2) | 4 |
| 022605-42 | 'Amarone' | 951502-1 | A × OA | 3× | 12 (1) | 24 (3) | 4 |
| 022611-4 | 'Gironde' | 951502-1 | A × OA | 3× | 12 (1) | 24 (3) | 4 |
| 22631 | 991108 | 951502-1 | OA × OA | 6× | 36 (1) | 36 (2) | 3 |

**Note:** Superscripts identify the Figs. in which more information is presented.
*Genomic composition generated from the indeterminate meiotic restitution (IMR) gametes.

on ice for at least 10 min. For each slide, 80 µL of the hybridization mixture was used. The preparations were denatured at 80 °C for 10 min. After overnight hybridization at 37 °C in a humid chamber, slides were washed at room temperature in 2× SSC for 15 min and 0.1× SSC at 42 °C for 30 min. Biotin-labeled probes were detected with Cy3-labeled streptavidin (Amersham Biosciences UK Limited, Buckingshire, UK) and amplified with biotinylated goat-antistreptavidin (Vector Laboratories, Burlingame, Calif.); digoxigenin-labeled probes were detected with antidigoxigenin-fluorescein (Roche, Germany) and amplified with fluorescein antisheep and fluorescein antirabbit (Vector Laboratories). Chromosomes were counterstained with 1 µg/mL DAPI (4′,6-diamidino-2-phenylindole), and a drop of Vectashield antifade (Vector Laboratories) was added before it was examined under a Zeiss Axioplan 2 Photomicroscope equipped with epifluorescent illumination and filter sets of DAPI, FITC, and Cy3.

The probes were removed, and the preparations were then reprobed, as described by Schwarzacher and Heslop-Harrison (2000), with genomic DNA from the Oriental cultivar 'Sorbonne'. Autoclaved DNA (100–500 bp) from the Asiatic

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cultivar 'Connecticut King' was used to block the non-hybridized sequences. The GISH protocol was carried out as described by Barba-Gonzalez et al. (2004). For the other $BC_1$ progeny plants, the same GISH protocol was used.

Selected images were captured by a Photometrics Sensys 1305 × 1024 pixel CCD camera and processed with Genus Image Analysis Workstation software (Applied Imaging Corporation). The DAPI images were sharpened with a 7 × 7 high-gauss spatial filter. DAPI fluorescence was displayed in grey for the FISH analysis and pseudocoloured for the GISH analysis. For both analyses, the probe fluorescence was pseudocoloured with either red or green. Optimal brightness and contrast were achieved with Adobe® Photoshop® image processing.

### Chromosome analysis and flow cytometry

Chromosome measurements were taken using the freeware computer application MicroMeasure (Reeves and Tear 1997) and arranged in decreasing order of short-arm length, as described by Stuart (1947), taking into account the position of the 45S hybridization signals. Leaves from $BC_1$ plants were collected to determine DNA values, which reflected ploidy levels, as described by Van Tuyl and Boon (1997).

## Results

### Ploidy levels of $BC_1$ progeny

Ploidy levels, the number of chromosomes from the O and A genomes, and the number of recombinant chromosomes in all 38 $BC_1$ progeny plants were monitored with GISH. Of the 38 $BC_1$ plants, there were 33 triploids ($2n = 3x = 36$), 4 tetraploids ($2n = 4x = 48$), and 1 hexaploid ($2n = 6x = 72$) (Table 1). All $BC_1$ plants, regardless of ploidy level, were euploid.

In GISH preparations, it was possible to clearly identify the chromosomes of parental genomes, including recombinant segments (Figs. 1 and 2). In a majority of the triploid $BC_1$ progeny plants, 12 chromosomes of the O genome and 24 of the A genome were clearly identified (Table 1). This was as expected because, in a backcross, the *2n* gamete from the $F_1$ hybrid contributes 1 set each of O and A genomes. However, there were 5 genotypes in which the number of chromosomes of the O and A genomes did not conform to the expected numbers (12 and 24, respectively) (Table 1, asterisks). As will be discussed later, the *2n* gametes in these cases failed to transmit 12 chromosomes each of the O and A genomes.

Of 3 tetraploids, 1 (022544-2) had 24 chromosomes of each O and A genome. On the other hand, 3 tetraploids (022605-3, 022605-13, and 022605-15) had originated from the *2n* eggs from the female parent, and thus contributed 2 sets of the A genome. Remarkably, the constitution of 1 of these plants was 11 O + 37 A instead of 12 O + 36 A (Table 1). The constitution of the only hexaploid that was found was 36 O and 36 A, indicating that the allotetraploid female parent had contributed the *2n* egg.

### Homoeologous recombination

Of the 38 $BC_1$ progenies derived from sexual polyploidization, 25 plants (65.8%) possessed recombinant chromosomes (Table 1). There was, however, a clear difference between the 2 groups of progenies within this population with regard to the number of plants with recombinant chromosomes. This difference was dependent on the genotype of the $F_1$ OA hybrid that was used as the parent in the cross. Thus, in the 14 plants derived from crossing 952400-1 as a parent, there were only 5 plants (35.7%) with recombinant chromosomes. On the other hand, among the 24 plants generated from crosses with the OA hybrid 951502-1 as a parent, there were 19 plants (79.1%) with recombinant chromosomes. Thus, there was an obvious difference between the 2 genotypes of OA hybrids with regard to the contribution of recombinant chromosomes to the $BC_1$ progeny plants. Furthermore, the range of recombinant chromosomes varied from 0 to 2 in crosses of 952400-1; in the case of 951502-1, it ranged from 0 to 7. And, whereas mostly single crossovers had occurred in the 952400-1 crosses, there was evidence of the occurrence of double or more crossovers per chromosome in the 951502-1 crosses. A notable feature of homoeologous recombination was that the crossovers were unevenly distributed within the genome. The larger chromosomes had fewer crossovers than the smaller chromosomes of the $F_1$ chromosome complement (data not shown).

### Chromosome constitution of $BC_1$ plants

As was mentioned earlier, the chromosome constitution of some of the $BC_1$ plants deviated from the expected 12 O + 24 A chromosomes. To investigate such plants more critically, and to determine the frequency and the types of recombinant chromosomes that were transmitted, 10 $BC_1$ plants were analyzed with a combination of GISH and FISH techniques; 5 of these are illustrated (Figs. 1 and 2). The FISH technique was used to identify individual chromosomes, using 45S RNA sites and a synthetic telomeric probe as markers (Figs. 1 and 2). All the genotypes that were investigated had a triploid chromosome constitution (Figs. 1–4), with 1 exception (022605-3), a tetraploid (Figs. 2*a*, 2*b*, and 4). In all the idiograms (Figs. 3 and 4), for each individual chromosome, the chromosomes derived from the backcross parent are on the left and the 2 chromosomes derived from the $F_1$, OA hybrid are on the right.

Among the 10 plants, 6 (022538-1, -3, -8; 022605-3, -8, and -40) received the full complement of 12 O + 12 A from the OA hybrid. However, in the case of recombinant chromosomes, only the centromeres were used to assess the chromosome complement. Hereafter, to avoid confusion, the recombinant chromosomes will be mentioned in the text only as centromeres. The 6 previously mentioned plants received 12 individual chromosomes/centromeres each of the O and A genomes from the *2n* gametes of OA hybrids. This indicates that in all 6 cases, FDR gametes were functional. In 4 cases (022538-7, -9, -15, and 022605-9), there was evidence that not all the individual chromosomes/centromeres of either the O or A genome were transmitted through *2n* gametes to the $BC_1$ progenies. For example, chromosome 5 in 022538-7 was represented by a pair of 1 each of the O genome centromere/chromosome, instead of 1 each of both A and O genomes (Fig. 3, marked as SC). Because of this anomalous situation, this plant possessed 13 O + 23 A instead of the expected 12 O + 24 A chromosome constitution. Similarly, in the case of 022538-9, chromosome 8 was repre-

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**Fig. 1.** Chromosome identification and detection of intergenomic recombination in 3 $BC_1$ progenies. In all cases, fluorescent in situ hybridization (FISH) images (left) show 45S rDNA sites, probed with either biotin or digoxigenin, and telomeric sites probed with digoxigenin. Biotin probes were detected with the Cy3-streptavidin system (orange–red fluorescence) and digoxigenin probes were detected with the antidigoxigenin detection system (green fluorescence). The centromeres are marked with arrowheads for only the relevant chromosomes. In all genomic in situ hybridization (GISH) images (right), the respective recombinant chromosomes are mentioned appropriately (e.g., O/A or A/O) and the arrows indicate the recombinant segments. The biotin-labeled Oriental (O) DNA was detected with the Cy3-streptavidin system (pink fluorescence) and the Asiatic (A) chromosomes were counterstained with DAPI (4′,6-diamidino-2-phenylindole) (blue fluorescence). In the case of recombinant chromosomes, the centromeres are taken into account when the number of chromosomes of each genome is estimated. (*a*) and (b): The triploid complement of 022538-1, showing 12 O + 24 A, with 7 recombinant chromosomes. (c) and (d): Late metaphase of the triploid complement of 022538-7, showing both chromatids of each 13 O + 23 A, with 6 recombinant chromosomes. (e) and (f): The triploid complement of 0022538-15, showing 11 O + 25 A, with 4 recombinant chromosomes. Bar represents 10 μm.

sented by a pair of O centromeres/chromosomes rather than 1 each of the O and A chromosomes. The consequence was a chromosome constitution of 13 O + 23 A, as in 022538-7 (Figs. 1*c* and 1*d*), just as the presence of a pair of O genome chromosomes/centromeres, instead of a single chromosome or centromere, led to 13 O + 23 A chromosome/centromere constitution. There were also instances in which 1 of the O genome chromosomes/centromeres was missing in the $BC_1$ plant. An example of this is 022538-15, in which chromosome 8 was represented by 2 centromeres of A rather than 1 each of O and A. This resulted in a chromosome constitution of 11 O + 25 A (Figs. 1*e* and 1*f*). Although the unbalanced chromosome/centromere constitution of 13 O and 23 A and 11 O + 25 A resulted from the transmission of duplicate chromosomes/centromeres from *2n* gametes to $BC_1$, it always produced the same result. For example, in the case of 022605-9, there were duplicate chromosomes/centromeres for chromosomes 4 and 6 (Figs. 2*c*, 2*d*, and 4). This resulted in a chromosome constitution of 12 O and 24 A. This gave the impression that 2 complete sets of O and A genomes were transmitted by the *2n* gamete. Closer inspection, however, revealed that the chromosomes/centromeres of 4 O and of 6 A genomes were not transmitted by the *2n* gamete to the progenies. These 4 anomalous chromosome constitutions are likely the result of the occurrence of *2n* gametes through IMR.

### Substitution of chromosome segments

One notable fact that emerged from the analysis of the $BC_1$ plants with recombinant chromosomes was the occurrence of substitutions for some of the segments of chromosomes. Assuming that a crossing over occurred between 2 nonsister chromatids in a bivalent in the OA hybrid, the 4 chromosomes that can result are O, A, $^{O}/_{A}$, and $^{A}/_{O}$; in the latter 2 recombinant chromosomes, O represents the centromere of the O genome and A the recombinant segment of the Asiatic chromosome, and vice versa. In the majority of $BC_1$ plants, the recombinant chromosomes had single crossovers, but there were also instances of 2-strand double, 3-strand double, and 4-strand double crossovers (discussed later). In the event of a single crossover, with FDR as the mechanism, the following 3 situations related to the recombinant chromosomes were observed: $^{O}/_{A}$, $^{A}/_{O}$; O, $^{A}/_{O}$; and A, $^{O}/_{A}$. The first of these 3 consisted of both the reciprocal products of a crossover (marked as RP in Figs. 3 and 4), and the latter 2 consisted of nonreciprocal products of a crossover (marked as NRP in Figs. 3 and 4). Among these, the A, $^{O}/_{A}$ combination of chromosomes produced substitutions for the distal recombinant segment of the $^{O}/_{A}$ chromosome. Such substitutions were observed in 8 of 10 plants that were analyzed (asterisks in Figs. 3 and 4). In 2 cases, despite the presence of recombinant chromosomes, either both reciprocal products were present or the recombinant chromosome was $^{A}/_{O}$ (e.g., chromosome 3 of 022538-7).

Other than single crossovers, there were cases in which the recombinant segments were interstitial, mostly resulting from a 2-strand double type of crossover (Figs. 3 and 4, chromosome 5 of 022538-3; chromosome 9 of 022538-9; chromosomes 7 and 11 of 022605-3; and chromosome 12 of 022605-9). Of these 5 cases, there were substitutions in 3 cases that possessed $^{O}/_{A}$ recombinant chromosomes but not the other ones. There were also instances of multiple crossovers (e.g., chromosomes 8 and 9 of 022538-8; chromosome 11 of 022538-9) in which there were substitutions in 2 cases (asterisks in Figs. 3 and 4).

In addition to the FDR mechanisms where all univalents or half-bivalents divide equationally, we observed 4 cases of IMR-derived $BC_1$ plants (022538-7, 022538-9, 022538-15, and 022605-9) in which *2n* gametes contributed a sister chromosome/centromere (marked as SC in Figs. 3 and 4). In some of these cases, substitutions occurred in proximal and distal regions of the recombinant chromosomes. Examples of proximal substitutions are chromosome 8 in 022538-15 and chromosome 4 in 022605-9; in the case of chromosome 6 of 022605-9, substitution of the recombinant segment was distal. The latter situation could be the result of a 4-strand double crossing over followed by IMR, where the sister chromatids of a half-bivalent were included in the same *2n* gamete.

## Discussion

In many respects, the OA hybrids used in backcrosses in this investigation conform to distant-species hybrids in numerous other plant taxa. It is difficult to produce $F_1$ hybrids; they are highly sterile, the chromosomes of alien genomes are highly differentiated, and when allotetraploids are produced they behave as “permanent hybrids.” In view of these obstacles, when the traditional approach of chromosome doubling of OA hybrids is used, progress can be slow and labourious. The use of *2n* gametes is advantageous for 3 reasons: (*i*) $F_1$ sterility can be readily overcome; (*ii*) intergenomic recombination can be accomplished; and, most

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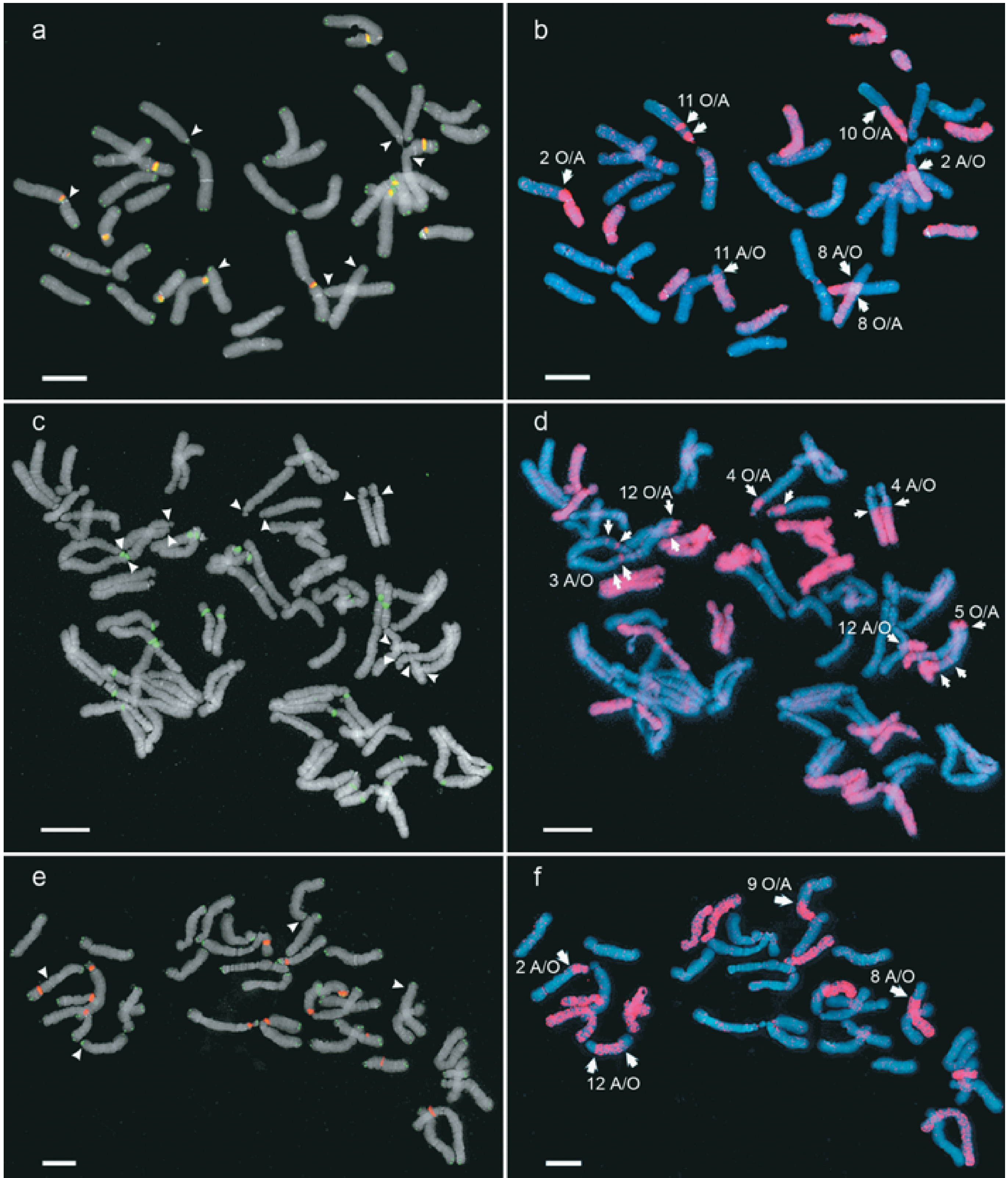


important, (*iii*) genetic variation can be obtained instantly in the $BC_1$ generation. All these aspects will be discussed later.

In OA hybrids, $F_1$ sterility is indeed a great impediment. However, with large-scale screening, it was possible to select some genotypes of OA hybrids with reasonably large amounts of *2n* pollen that are fertile (Barba-Gonzalez et al. 2004). This is similar to the success that has already been achieved in the LA hybrids used to produce a large number of backcross progenies (Lim et al. 2003) and numerous cultivars by breeders. The fertility of OA and LA hybrids

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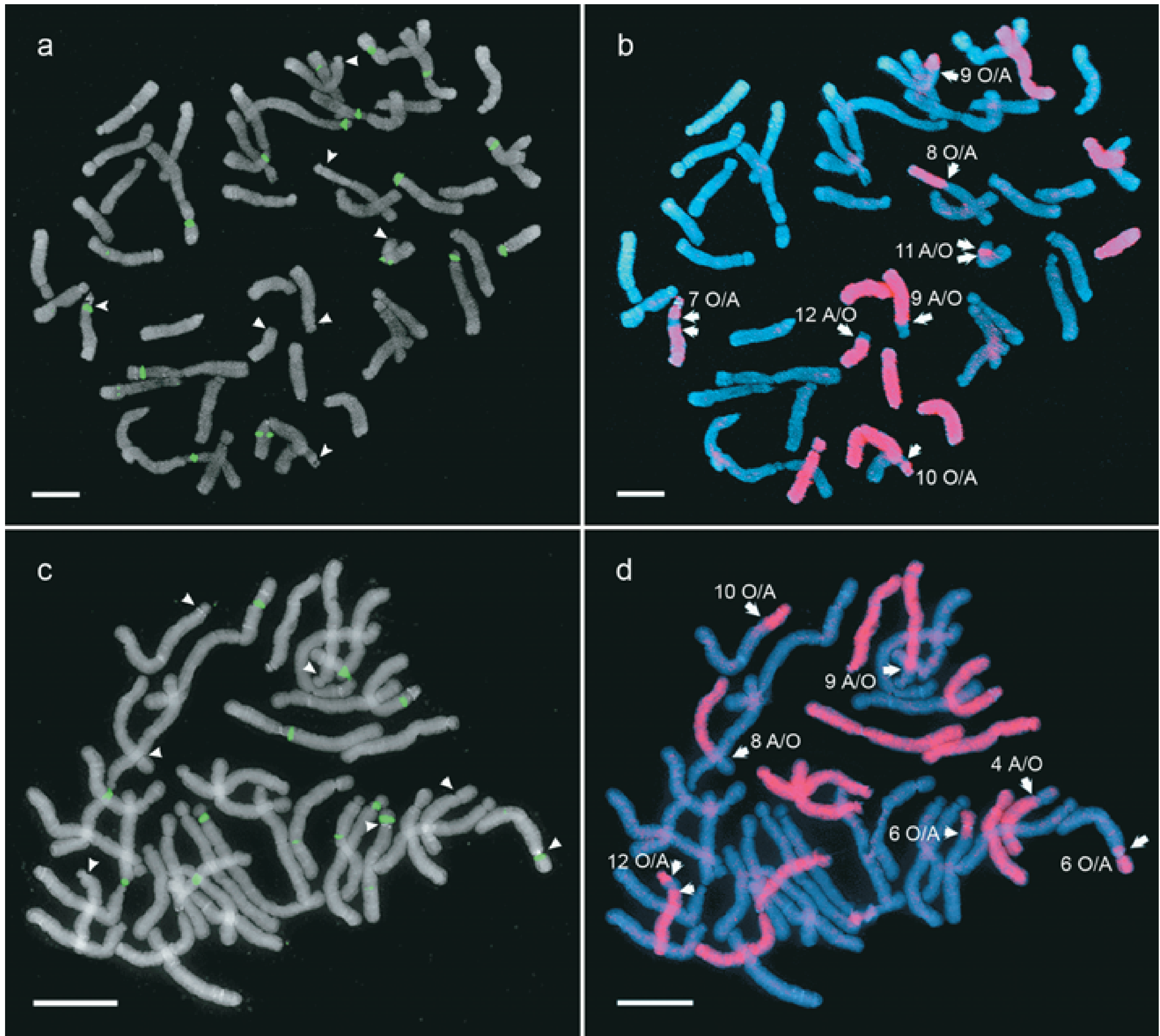
**Fig. 2.** Chromosome identification and detection of intergenomic recombination in 2 $BC_1$ progenies. In both cases, FISH images (left) show 45S rDNA probed with digoxigenin and detected with the antidigoxigenin detection system (green fluorescence). The centromeres are marked with arrowheads for only the relevant chromosomes. In all GISH images (right), the respective recombinant chromosomes are mentioned appropriately (e.g., O/A or A/O) and the arrows indicate the recombinant segments. The biotin-labeled Oriental DNA was detected with the Cy3-streptavidin system (pink fluorescence), and the Asiatic chromosomes were counterstained with DAPI (blue fluorescence). In the case of recombinant chromosomes, the centromeres are taken into account when the number of chromosomes of each genome is estimated. (*a*) and (b): The tetraploid complement of 022605-3, showing 12 O + 36 A, with 7 recombinant chromosomes. (c) and (d): The triploid complement of 022605-9, showing 12 O + 24 A, with 7 recombinant chromosomes. Bar represents 10 μm.




has been shown to result mainly from 2 main mechanisms of *2n* gamete formation: FDR and IMR (Lim et al. 2001; Barba-Gonzalez et al. 2004). Cytological analysis in previous studies on LA hybrids and in this investigation have clearly established that both types of *2n* gametes are functional and that $BC_1$ populations can be produced (Barba-Gonzalez et al. 2004).

With regard to intergenomic recombination, there is a difference between the 2 genotypes of OA hybrids (952400-1 and 951502-1). Whereas only 35.7% of $BC_1$ plants possessed recombinant chromosomes when 952400-1 was the *2n* gamete donor, 79.1% of the $BC_1$ progenies possessed recombinant chromosomes when 951502-1 was the parent. This clearly reflects the difference between the 2 genotypes in the extent of crossing over that occurs before restitution nucleus formation (Barba-Gonzalez et al., 2005). In a recent survey of diploid LA hybrids, we observed a clear quantitative difference among genotypes, showing complete failure

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**Fig. 3.** Idiograms of 5 $BC_1$ progeny plants, showing recombinant chromosomes and 45S rDNA sites. The reciprocal and nonreciprocal products of recombinant chromosomes are marked as RP and NRP, respectively. Sister centromeres are marked as SC in all cases. Substitutions for recombinant segments, when present, are marked with asterisks at the bottom of each group of chromosomes.

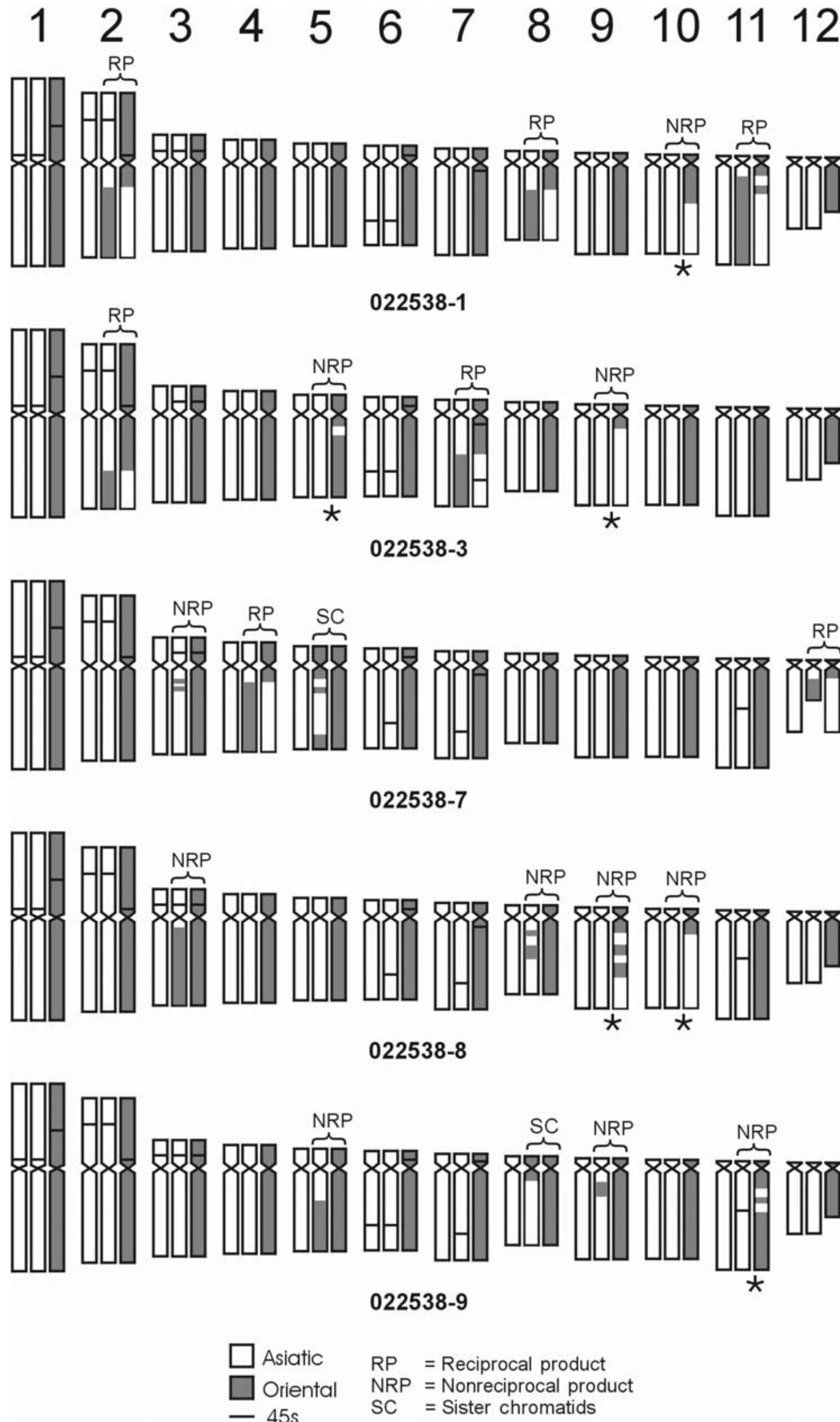


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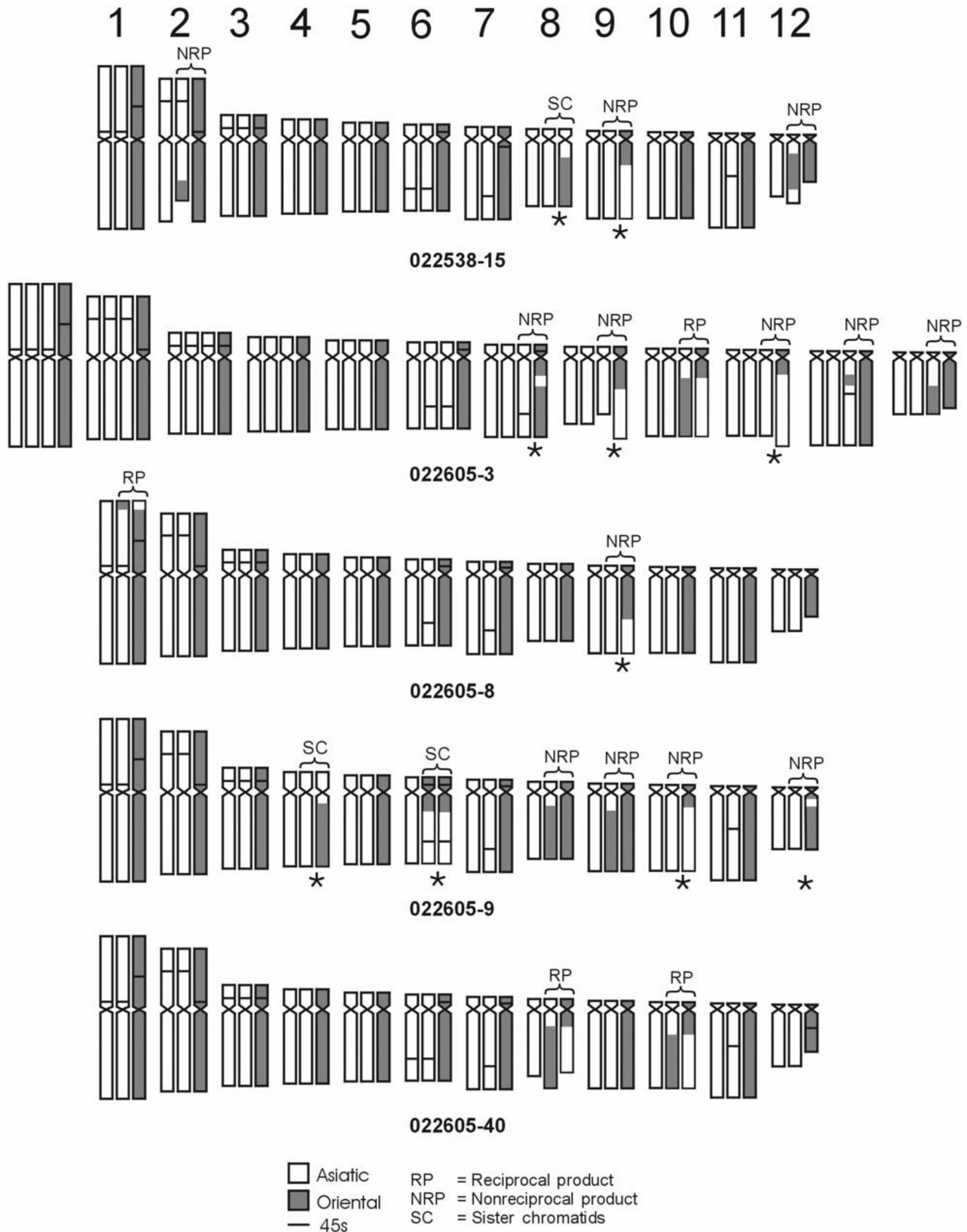
**Fig. 4.** Idiograms of 5 $BC_1$ progeny plants, showing recombinant chromosomes and 45S rDNA sites. The reciprocal and nonreciprocal products of recombinant chromosomes are marked as RP and NRP, respectively. Sister centromeres are marked as SC in all cases. Substitutions for recombinant segments, when present, are marked with asterisks at the bottom of each group of chromosomes.

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of chromosome pairing to almost normal pairing. Because intergenomic recombination can be crucial for generating genetic variation in $BC_1$ progenies (see later section), it might be desirable to screen diverse populations of OA hybrids for frequencies of chromosome pairing and chiasma formation.

An important feature of genetic recombination in OA and LA hybrids is that both homoeologous crossing over and chromosome assortment are accomplished. Chromosome assortment is not expected to occur in the case of FDR because the sister chromatids, as a rule, move to opposite poles during equational division of the nucleus. However, in the case of IMR, some of the half-bivalents disjoin and the 2 sister chromatids are included in 1 of the 2 restitution nuclei that result from a germ cell. This forms the basis of chromosome assortment (Lim et al. 2001). In this study, there were at least 5 $BC_1$ plants generated from the IMR gametes (Table 1, asterisks). To achieve substitutions for recombinant segments or whole chromosomes, FDR and IMR gametes serve different purposes.

In the case of FDR gametes with crossing over, nonsister chromatids may consist of reciprocal products ($^O/_A$; $^A/_O$) or nonreciprocal products (O, $^A/_O$ or A, $^O/_A$). When producing substitutions for crossover segments in a backcross involving A × OA parents, it is always the $^O/_A$ recombinant that is useful (asterisks in Figs. 3 and 4), not the $^A/_O$ recombinant. The substituted segment is invariably the distal one. If a backcross involves an O parent (i.e., O × OA cross), only the $^A/_O$ recombinant is useful. In the case of IMR, because both the sister chromosomes/centromeres are included in the *2n* gamete, most substitutions are for the proximal segment.

The importance of substitutions of recombinant segments cannot be overemphasized. This is because the recessive genetic loci in the substituted regions can attain a nulliplex condition (aaa). This forms the basis of genetic variation that can be observed in the $BC_1$ generation itself. With a traditional approach to producing an allotetraploid from a hybrid, such as OA, it would be a formidable task to achieve nulliplex genotypes through backcrossing.

Because intergenomic recombination is one of the important attributes of sexual polyploidization, this process is used extensively by horticultural breeders. There are numerous examples in which hybridization between distantly related species has been done and the fertile *2n* gametes are inadvertently used by the breeders to produce polyploid cultivars. Two examples are *Narcissus* (Brandham 1986) and *Alstroemeria* (Ramanna 1992) (for reviews, see Van Tuyl and Lim 2003; Ramanna and Jacobsen 2003). In this context, the phenomenon observed in lily OA hybrids and LA hybrids (Lim et al. 2003; Van Tuyl et al. 2002) is likely a repetition of what has occurred in some of the other horticultural crops.

Furthermore, some of these triploid hybrids have shown to be fertile and have been backcrossed in many directions. From such crosses in our laboratory, progeny have revealed the value of breeding these triploid hybrids and that the introgression of segments of the recombinant chromosomes can be transmitted to further generations.

Finally, the occurrence of small recombinant segments due to multiple crossover events in OA hybrids augers well for transferring specific horticultural traits to cultivars. If only large blocks of recombinant segments or whole chromosomes were transferred through introgression, many undesirable traits would be added to the cultivars along with the desirable ones (the so-called "linkage drag"). Fortunately, it appears possible to prevent the transfer of undesirable traits during the process of introgression.

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