Karyotype analysis of *Lilium longiflorum* and *Lilium rubellum* by chromosome banding and fluorescence in situ hybridisation

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Abstract: Detailed karyotypes of *Lilium longiflorum* and *L. rubellum* were constructed on the basis of chromosome arm lengths, C-banding, $AgNO_3$ staining, and PI–DAPI banding, together with fluorescence in situ hybridisation (FISH) with the 5S and 45S rDNA sequences as probes. The C-banding patterns that were obtained with the standard BSG technique revealed only few minor bands on heterologous positions of the *L. longiflorum* and *L. rubellum* chromosomes. FISH of the 5S and 45S rDNA probes on *L. longiflorum* metaphase complements showed overlapping signals at proximal positions of the short arms of chromosomes 4 and 7, a single 5S rDNA signal on the secondary constriction of chromosome 3, and one 45S rDNA signal adjacent to the 5S rDNA signal on the subdistal part of the long arm of chromosome 3. In *L. rubellum*, we observed co-localisation of the 5S and 45S rDNA sequences on the short arm of chromosomes 2 and 4 and on the long arms of chromosomes 2 and 3, and two adjacent bands on chromosome 12. Silver staining (Ag–NOR) of the nucleoli and NORs in *L. longiflorum* and *L. rubellum* yielded a highly variable number of signals in interphase nuclei and only a few faint silver deposits on the NORs of mitotic metaphase chromosomes. In preparations stained with PI and DAPI, we observed both red- and blue-fluorescing bands at different positions on the *L. longiflorum* and *L. rubellum* chromosomes. The red-fluorescing or so-called reverse PI–DAPI bands always coincided with rDNA sites, whereas the blue-fluorescing DAPI bands corresponded to C-bands. Based on these techniques, we could identify most of chromosomes of the *L. longiflorum* and *L. rubellum* karyotypes.

Key words: fluorescence in situ hybridisation, FISH, 5S rDNA, 45S rDNA, C-banding, reverse PI–DAPI banding.

Résumé : Des caryotypes détaillés du *Lilium longiflorum* et du *L. rubellum* ont été produits sur la base de la longueur des bras chromosomiques, des bandes C, de la coloration à l'$AgNO_3$ et la révélation des bandes PI–DAPI ainsi que l'hybridation in situ en fluorescence (FISH) à l'aide des séquences d'ADNr 5S et 45S comme sondes. La coloration des bandes C par la technique BSG standard n'a montré que quelques bandes mineures à des positions hétérologues sur les chromosomes du *L. longiflorum* et du *L. rubellum*. L'analyse FISH avec les sondes d'ADNr 5S et 45S sur les chromosomes du *L. longiflorum* en métaphase a permis d'observer : (*i*) des signaux se chevauchant dans les régions proximales des bras courts des chromosomes 4 et 7, (*ii*) un signal d'ADNr 5S au niveau de la constriction secondaire du chromosome 3 et (*iii*) un signal d'ADNr 45S adjacent au signal d'ADNr 5S dans la portion sub-distale de ce bras long. Chez le *L. rubellum*, les auteurs ont observé : (*i*) une co-localisation des séquences 5S et 45S sur le bras court des chromosomes 2 et 4 ainsi que sur les bras longs des chromosomes 2 et 3, et (*ii*) deux bandes adjacentes sur le chromosome 12. La coloration à l'argent (Ag–NOR) des nucléoles et des NOR chez le *L. longiflorum* et le *L. rubellum* a révélé un nombre très variable de signaux chez les noyaux en interphase et seulement quelques faibles dépôts d'argent sur les NOR de chromosomes en métaphase mitotique. Chez des préparations chromosomiques colorées avec le PI et le DAPI, les auteurs ont observé des bandes à fluorescence rouge ou bleue à différentes positions sur les chromosomes du *L. longiflorum* et du *L. rubellum*. Les bandes à fluorescence rouge, appelées bandes PI–DAPI inverses, coïncident toujours avec les sites d'ADNr tandis que les bandes DAPI à fluorescence bleue correspondent aux bandes C. Grâce à ces techniques, les auteurs ont pu identifier la plupart des chromosomes au sein des caryotypes du *L. longiflorum* et du *L. rubellum*.

Mots clés : hybridation in situ en fluorescence, FISH, ADNr 5S, ADNr 45S, révélation des bandes C, révélation des bandes PI–DAPI inverses.

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Introduction

The huge genomes of species of the genus *Lilium* are among the largest in the plant kingdom (Bennett and Smith 1976, 1991). The chromosomes of these species are also exceptionally large and have proved to be outstanding material for cytogenetic research for more than a century. Since Strasburger's paper on the chromosomes of *Lilium* (Strasburger 1880), many studies have been published, especially on chromosome morphology in *Lilium longiflorum* Thunb. (Stewart 1947), banding pattern (Holm 1976; Von Kalm and Smyth 1984), the detection of nucleolar organiser regions (NORs) (Von Kalm and Smyth 1980, 1984), and genome size (Van Tuyl and Boon 1997). Surprisingly, little attention was drawn to the chromosomes of *Lilium rubellum* Baker, which is an economically important species for interspecific hybridisation with *L. longiflorum*. Despite their phenotypic differences, most *Lilium* spp. have the same chromosome number ($2n = 2x = 24$) and almost identical chromosome portraits (Stewart 1947). *Lilium longiflorum* and *L. rubellum* (Noda 1991), which differ in nuclear DNA content by less than 2% (Van Tuyl and Boon 1997), were also found to have similar karyotypes. In addition, relatively high levels of meiotic recombination between the homoeologues of *L. longiflorum* × *L. rubellum* hybrids were observed (Lim et al. 2000), suggesting close synteny between the parental genomes (Fig. 3*a*).

The increased use of *L. longiflorum* and *L. rubellum* in lily breeding requires detailed knowledge of the parental chromosome portraits for tracing chromosome behaviour in interspecific hybrids and backcross plants. The ability to identify individual chromosomes is essential in localizing the breakpoints in translocation chromosomes and the sites of recombination between homoeologues. Detailed karyotypes could also be instrumental in assigning linkage groups to chromosomes and mapping genes on the chromosome arms.

Detailed karyotypes generally display chromosomes ordered in sequence of decreasing length, and include heterochromatin patterns based on C-, N-, and Q-banding and on other chromosome differentiation techniques. Satellite chromosomes can be recognised by their micro- or macro-satellites or by Ag–NOR staining, a technique that specifically visualises sites of metabolically active NORs (e.g., Linde-Laursen 1975). Fluorescence in situ hybridisation (FISH), which maps repetitive or single-copy sequences on the chromosomes, now complements banding technologies, along with the use of DNA-specific fluorochromes (Peterson et al. 1999), which elucidate local variation in DNA and (or) chromatin composition. The most common application of FISH is the localisation of rDNA families on the chromosomes. The 45S rDNA sequences were shown to be on the NOR of the satellite chromosomes and the 5S rDNA to be located, generally, on one of the other chromosomes (Gerlach and Dyer 1980; Leitch and Heslop-Harrison 1992). Other repeats in FISH mapping studies include microsatellites (Cuadrado and Schwarzacher 1998), satellite DNAs (Pedersen et al. 1996), and retrotransposon repeat families (Heslop-Harrison et al. 1997; Miller et al. 1998; Presting et al. 1998).

In *Lilium* spp., most chromosomes (4–6, 7–9, and 10–12) are morphologically too similar to be identified without additional diagnostic landmarks. C-bands were seen in several studies (Holm 1976; Son 1977; Kongsuwan and Smyth 1978; Smyth et al. 1989), revealing small bands in *L. longiflorum* chromosomes 1, 3, 4, 7, 8, 9, and 12, and NOR detection by isotopic in situ hybridisation and silver staining demonstrated nucleoli and active ribosomal sites on chromosomes 3, 4, and 7 (Von Kalm and Smyth 1980, 1984). However, so far no reports using FISH to detect ribosomal DNA sequences in *Lilium* have appeared.

Fluorescence microscopy of various plant chromosomes using a mixture of DAPI (4′,6-diamidino-2-phenylindole) and PI (propidium iodide) revealed small red-fluorescing bands (Peterson et al. 1999; Andras et al. 2000). Such reverse PI–DAPI bands were also detected in *L. longiflorum* × *L. rubellum* hybrid at the NOR regions of a few satellite chromosomes (Lim et al. 2000).

The aim of this study was a comparative analysis of the karyotypes of *L. longiflorum* and *L. rubellum*, using morphometric data, C-banding profiles, Ag–NOR staining, FISH detection of 45S rDNA and 5S rDNA sequences, and reverse PI–DAPI banding, to identify all the chromosomes in each complement.

Materials and methods

Plant materials and chromosome preparation

Lilium longiflorum 'Snow Queen' and *L. rubellum* (accession number 980085; originally from the mountainous area of the Fukushima prefecture, Japan) were obtained from the germplasm collection of Plant Research International, Wageningen, The Netherlands. Root tips were collected in the early morning, pretreated in a saturated α-bromonaphthalin solution for 2 h at 20°C, and kept in this solution at 4°C until the next morning. The material was then rinsed three times in tap water before being fixed in acetic acid – ethanol (1:3) for 2 h. Root tips were stored at –20°C until used. The material was rinsed thoroughly before incubation in a pectolytic enzyme mixture (0.3% pectolyase Y23, 0.3% cellulase RS, and 0.3% cytohelicase) for about 1 h at 37°C. Squash preparations were made in a drop of 60% acetic acid. The microscope slides were frozen in liquid nitrogen and the cover slips removed with a razor blade. Slides were then finally dehydrated in absolute ethanol, air-dried, and stored at –20°C in a freezer until used.

C-banding and silver staining

Prior to the $BaOH_2$ – saline – Giemsa (BSG) banding procedure, the preparations were baked overnight at 37°C. Denaturation was performed in a 6% $BaOH_2$ solution at 20°C for 8 min, followed by a 30 min wash with tap water. Slides were then re-annealed in 2× SSC (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0) at 60°C for 50 min and stained in 4% Giemsa in 10 mM Sörensen buffer (pH 6.8) for 45 min. After a brief wash, preparations were air-dried and mounted in Euparol.

For Ag–NOR staining, we used 200 mL of a freshly prepared 50% $AgNO_3$ solution per slide, which was covered with a 24 × 36 mm piece of Nybolt 300 nylon cloth. The slides were left in a humidified petri dish for 45 min at 60°C until the nylon patch turned yellowish. The slides were then washed briefly in running tap water, air-dried, and mounted with Entellan-M (Merck) for microscopic observation.

Table 1. Summary of the morphometric and karyotypic data for *Lilium longiflorum* and *L. rubellum*.

	L. longiflorum	*L. rubellum*
DNA content (pg/2C)	77.1±0.3	73.6±0.6
Chromosome length (μm)		
Longest	34.4 (chr. 1)	33.8 (chr. 1)
Shortest	18.1 (chr. 10)	16.3 (chr. 4)
Total length	≈286.1	≈269.9
Ag–NOR staining		
Signals/interphase	6	6–10
C-banded chromosomes	1, 3, 4, 7 (3*x*), 8(2*x*), 9, 11, 12	2 (2*x*), 3 (2*x*), 4 (2*x*), 6 (2*x*), 8 (2*x*), 12 (2*x*)
FISH		
5S rDNA alone	chr. 3	—
45S rDNA alone	chr. 3	chrs. 1, 6
5S + 45S rDNA	chrs. 4, 7	chrs. 2 (2*x*), 3, 4, 12
DAPI and PI bands		
DAPI alone	chrs. 1, 3, 9	chrs. 2, 4, 12
PI alone	chrs. 4, 7	chrs. 1, 2, 12
Reverse PI–DAPI bands	chrs. 4, 7	chrs. 2(2*x*), 3, 4, 12

Probe DNA

Clone pTa71 contains a 9-kb *Eco*RI fragment of the 45S rDNA from wheat (Gerlach and Bedbrook 1979), and pScT7 contains a 462-bp *Bam*HI fragment of the 5S rDNA from rye (Lawrence and Appels 1986). Isolated DNA of 45S and 5S rDNA sequences from pTa71 and pScT7 were labelled with biotin-16-dUTP or digoxigenin-11-dUTP by nick translation for in situ hybridisation, according to the manufacturer's manual (Boehringer Mannheim, Germany).

FISH

Slides were left overnight at 37°C before treatment with 1 μg RNase A/mL of 2× SSC at 37°C for 60 min. The slides were washed three times in 2× SSC at 20°C for 5 min, hydrolysed with 10 mM HCl at 37°C for 2 min, treated with 100 μL pepsin (5 μg/mL in 10 mM HCl) at 37°C for 10 min, washed two times in 2× SSC for 5 min, treated with 4% paraformaldehyde for 10 min, washed another three times in 2× SSC, dehydrated through a graded ethanol series (70, 90, and 98% for 3 min each), and finally air-dried. Samples (40 μL) of the hybridisation mixture (100 ng of the DNA isolated from pScT7 and pTa71, 2 mg of sheared herring sperm DNA (GibcoBRL), 50% deionised formamide, 10% (w/v) sodium dextran sulphate (Sigma), 2× SSC, and 0.25% (w/v) SDS) were denatured for 5 min at 70°C and then put directly on ice for at least 5 min. Each slide with 40 μL of the hybridisation mixture was covered with a slip of plastic sheet, denatured for 5 min at 80°C, and left overnight at 37°C in a tightly closed humidified container. Slides were washed in 2× SSC for 15 min, transferred to 0.1× SSC at 42°C for 30 min, and incubated for 60 min at 37°C in blocking buffer (0.1 M maleic acid, 0.15 M NaCl, 1% (w/v) blocking reagent from Boehringer Mannheim). Biotin- and digoxigenin-labelled probe DNA was detected by a Cy3™–avidin–streptavidin detection system (Vector Laboratories) and a fluorescein isothiocyanate (FITC) – anti-digoxigenin detection system (Boehringer Mannheim, Germany). Slides were counterstained with 10 mg/mL DAPI, 5 mg/mL PI, or a mixed solution of DAPI and PI. Images were photographed with a Zeiss Axiophot photomicroscope equipped with epifluorescence illumination and single-band filters for DAPI, FITC, and Cy3–PI, using 400 ISO colour negative film. Films were scanned at 1200 dpi for digital processing with Adobe Photoshop® (version 5.0; Adobe Inc. U.S.A.).

### Karyotype analysis and flow-cytometric analysis of nuclear DNA

Chromosomes were measured with a ruler and arranged in order of decreasing short arm length, according to Stewart (1947). Total nuclear DNA content of DAPI-stained leaf nuclei was measured with a Partec CA-II cell analyser. Relative DNA content was calculated using *Allium cepa* nuclei as internal standard (DNA content = 33.5 pg/2C).

Results

An overview of all the morphometric data, chromosome banding, and FISH results are given in Table 1. The positions of the bands and FISH signals are depicted in the ideograms of Fig. 1. Flow-cytometric analysis of DAPI-stained nuclei gave values of 77.1 ± 0.3 pg/2C for *L. longiflorum* and 73.6 ± 0.6 pg/2C for *L. rubellum*. In addition, the total length of the metaphase complement was ca. 286 μm for *L. longiflorum* and ca. 270 μm for *L. rubellum*. The (sub)metacentric chromosomes, 1 and 2, are far longer than all others, whereas chromosomes 3–12 are highly asymmetrical with centromere indexes ranging from 20 to 5%. Chromosomes 4 and 7 of *L. longiflorum* and chromosomes 2 and 4 of *L. rubellum* have satellites on their short arms. Further constrictions were observed on the long arms of chromosome 3 (subdistal) of *L. longiflorum* and chromosomes 1, 2, and 12 (proximal) of *L. rubellum*.

The C-banding technique revealed small bands at 11 positions on different chromosomes of *L. longiflorum*. We observed single proximal heterochromatin bands on chromosomes 1, 11, and 12, a single subdistal band on chromosome 3 at the secondary constriction, and several intercalary bands on chromosomes 7, 8, and 9. The *L. rubellum* karyotype showed a different pattern with a total of 12 small bands on chromosomes 2 (2*x*), 3 (2*x*), 4 (2*x*), 6 (2*x*), 8 (2*x*), and 12 (2*x*) (Figs. 2*b* and 2*d*). Ag–NOR staining of *L. longiflorum* chromosomes showed weakly stained spots on chromosome 4 only (Fig. 2*e*). Interphase nuclei, however, displayed six dark silver deposits or had completely stained nucleoli. In *L. rubellum*, the Ag–NOR dots on the mitotic metaphase

Fig. 1. Ideogram of *Lilium longiflorum* and *L. rubellum* indicating the positions of the C-bands and DAPI, PI, 5S rDNA, and 45S rDNA sites. The reversed PI–DAPI bands were obtained by staining the chromosome preparations with a mixture of DAPI and PI simultaneously.
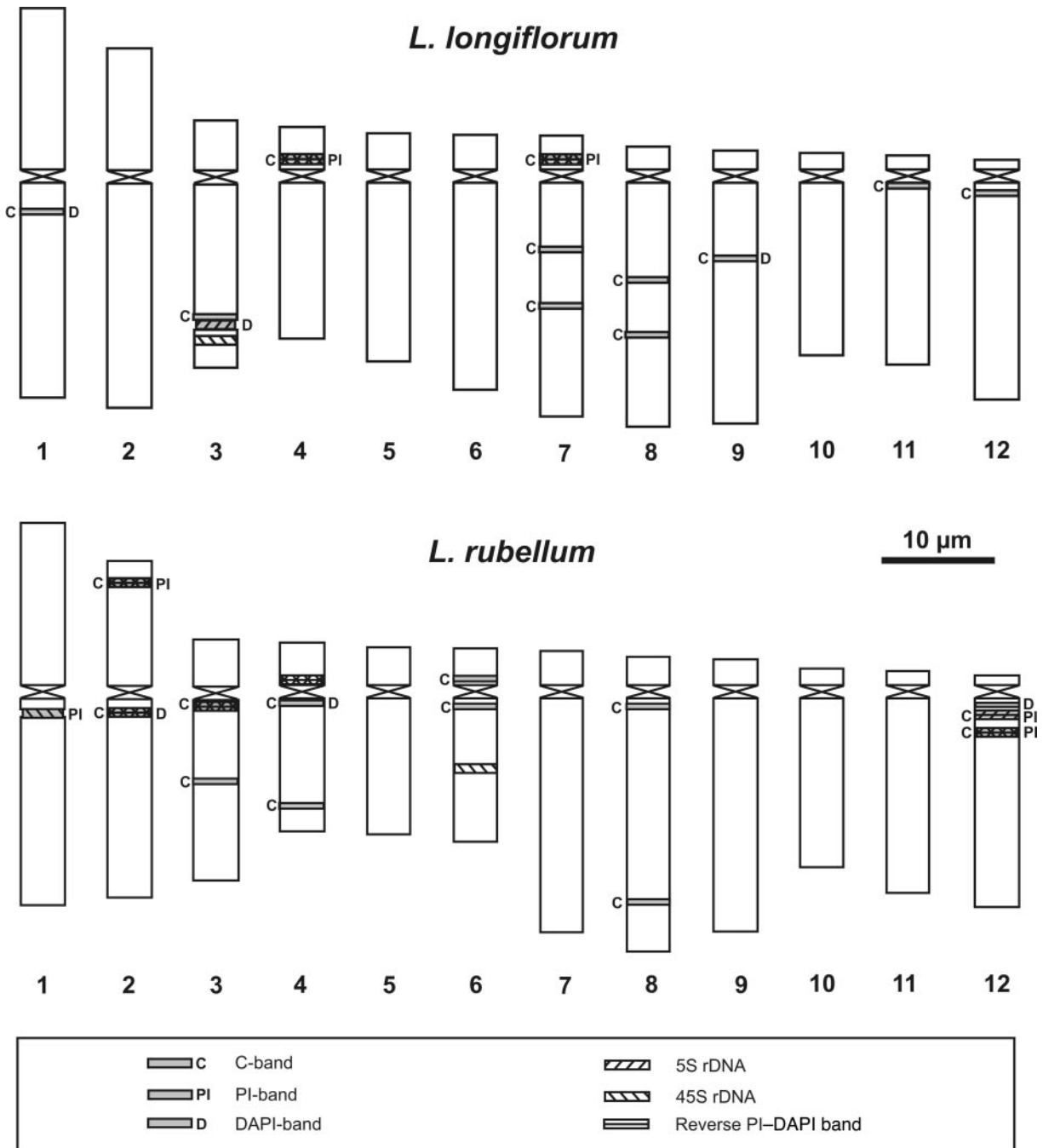

chromosome were even weaker than in *L. longiflorum* or not detectable, whereas interphase cells displayed 6–10 large spots.

The red-fluorescing reverse PI–DAPI bands were found on the secondary constrictions of chromosomes 4 and 7 in *L. longiflorum* and chromosomes 1, 2, 4, and 12 in *L. rubellum* (Fig. 3e). In *L. longiflorum*, DAPI bands were detected in chromosomes 1, 3, and 9, and PI bands were seen in chromosomes 3 and 4. In *L. rubellum*, DAPI bands appeared in proximal positions on chromosomes 2, 4, and 12, and PI bands appeared in a proximal position on chro-

mosome 1, in a subdistal position on chromosome 2, and at two adjacent sites close to the centromere of chromosome 12.

FISH of 45S rDNA (pTa71 probe) with *L. longiflorum* revealed signals on the secondary constrictions of chromosomes 4 and 7 and near the secondary constriction on chromosome 3. The 5S rDNA (pScT7 probe) hybridised to the long arm at the secondary constriction of chromosome 3 proximal from the 45S rDNA site. The probe also hybridised to the secondary constrictions of chromosomes 4 and 7 and co-localised with the 45S rDNA sites (Figs. 3b–3d).

Fig. 2. (*a* and *b*) Giemsa C-banding pattern of *Lilium longiflorum* (*a*) and *L. rubellum* (*b*) chromosomes. (*c* and *d*) Karyotypes of *L. longiflorum* (*c*) and *L. rubellum* (*d*). (*e*) Ag–NOR staining of *L. longiflorum* chromosomes.
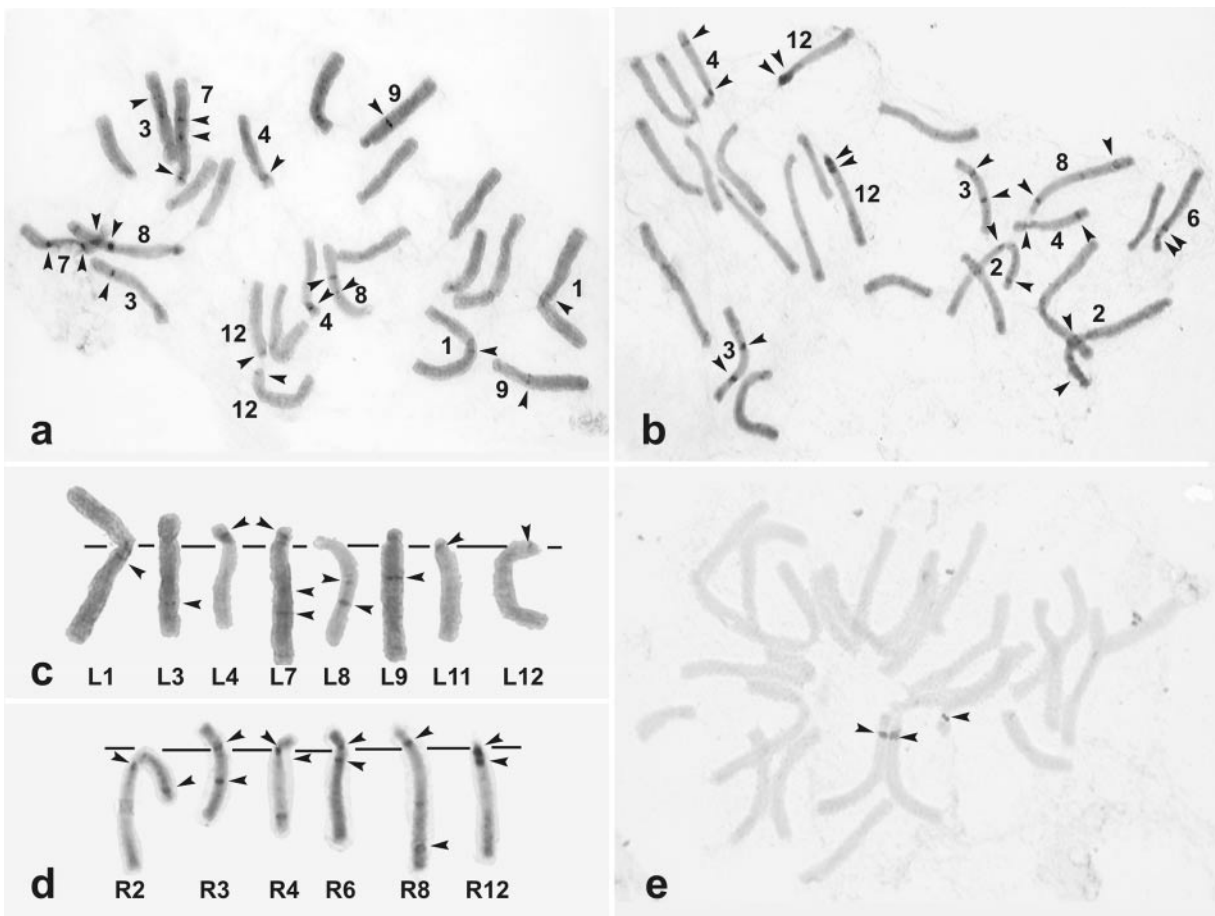
In *L. rubellum*, co-localisation of 45S rDNA and 5S rDNA was observed in the secondary constrictions of chromosomes 2, 3, 4, and 12, and a single spot of 45S rDNA was observed in the secondary constriction of chromosome 1 and in the middle of the long arm of chromosome 6 (Fig. 3*g*).

Discussion

This study elucidated various dissimilarities between the chromosomes of *L. longiflorum* and *L. rubellum* that are not obvious from comparison of their unbanded karyotypes. Firstly, the distribution of secondary constrictions differs between the species: three sites on chromosomes 3, 4, and 7 in *L. longiflorum* and five sites on chromosomes 1, 2, 3, 4 and 12 in *L. rubellum*. These differences between the two species are in agreement with the studies of Stewart (1947) and Ogihara (1968). Our observation made clear that secondary constrictions were associated with the nucleolus and that they contain 45S rDNA repeats. Other characteristics, such as the C-bands of *L. longiflorum*, were almost identical to those of *Lilium formosanum* (section *Leucolirion*) (cf. Stewart 1947; Smyth et al. 1989). The karyotype of *L. rubellum*, as far as the constrictions in chromosomes 1 and 2 are concerned, is a combination of the chromosome morphology of its putative parental species, *Lilium japonicum* and *L. auratum*. Most obvious is the constant number of single NORs on chromosomes 1, 2, 3, and 4

found in many species of *Lilium* studied so far (Stewart 1947). Sites of constitutive heterochromatin (C-bands) often coincided with the secondary constrictions, but did not always do so. In *L. longiflorum*, only the C-bands of chromosomes 3, 4, and 7 co-localise with these constrictions and contained 45S rDNA repeats (Fig. 1). These observations are in agreement with the previous studies of Stewart (1947), Von Kalm and Smyth (1984), Kongsuwan and Smyth (1978), and Smyth et al. (1989), and are similar to observations of *L. formosanum* of the same section (Smyth et al. 1989). In *L. rubellum*, the C-banding pattern, which strongly resembles the patterns of *L. auratum* and other species of section *Archelirion* (cf. Smyth et al. 1989), corresponds to the sites of the secondary constrictions except for three interstitial bands on the long arms of chromosomes 3, 4, and 8 (Fig. 2*d*). Unfortunately, our Ag–NOR staining produced insufficient evidence to determine the number of active NORs on metaphase chromosomes. It is questionable whether our in vitro root tip material grown at 25°C is suitable for the detection of nucleolar activity.

Further evidence for molecular differences in chromosomal organisation between *L. longiflorum* and *L. rubellum* came from the comparison of C-bands and PI–DAPI bands. In *L. longiflorum*, C-bands and DAPI bands appeared on chromosomes 1, 3, and 9, C-bands and PI bands appeared on chromosomes 4 and 7, and the remaining C-bands appeared on chromosomes 7, 8, 11, and 12, without deviant PI–DAPI
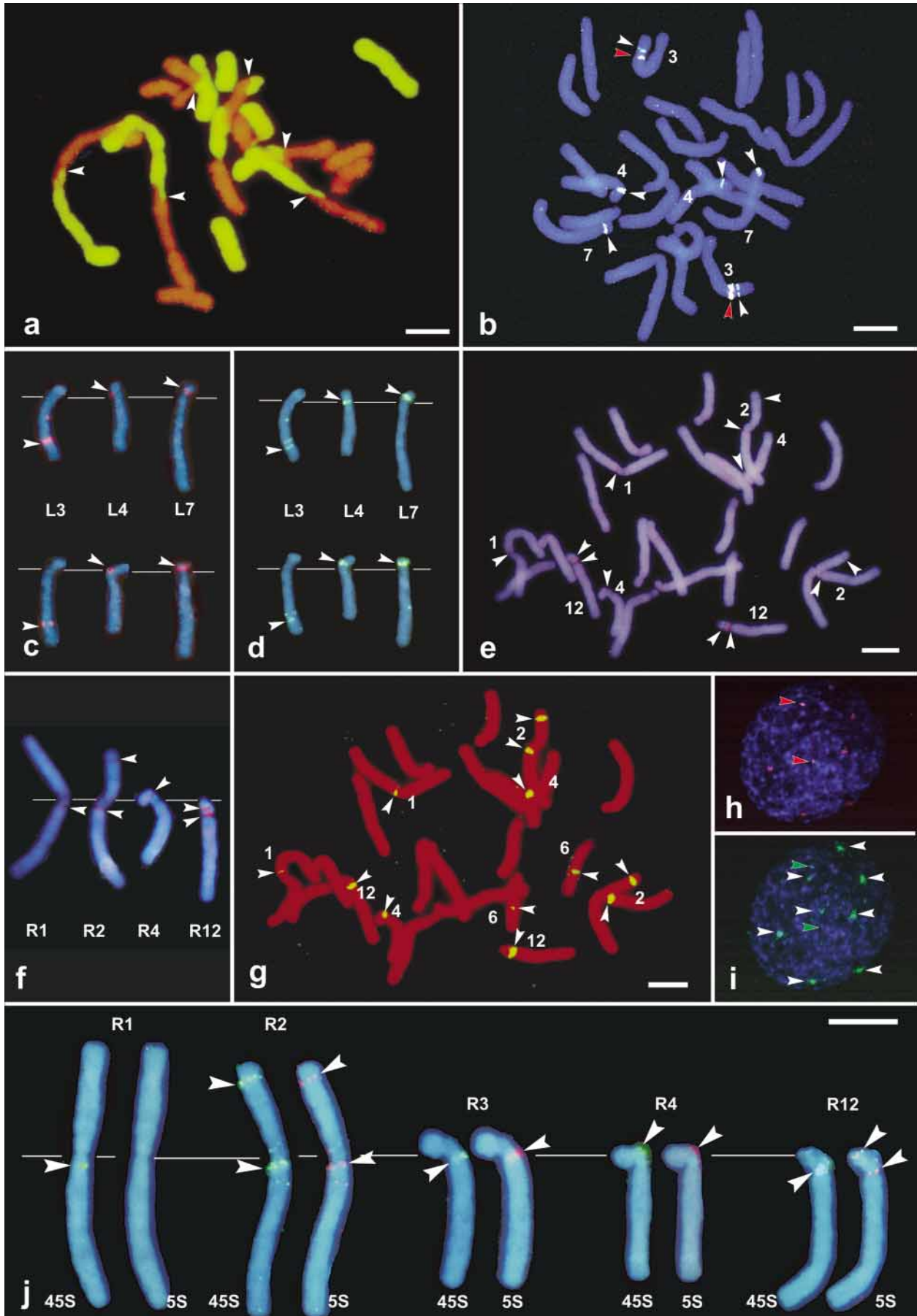
Fig. 3. (*a*) Genomic in situ hybridization on the metaphase I complement of an interspecific hybrid between *Lilium longiflorum* and *L. rubellum* shows a range of bivalent formation, indicating some homology in DNA sequence between the two species. (*b*) Simultaneous FISH detection of 5S and 45S rDNA probes (white arrowheads) in *L. longiflorum*. The red arrowheads represent the 5S rDNA probe, which has a stronger signal on chromosome 3 than the 45S rDNA probe. (*c* and *d*) Karyotypes of 5S rDNA (*c*) and 45S rDNA (*d*) on *L. longiflorum* chromosomes. (*e*) Simultaneous staining with PI and DAPI of *L. rubellum* chromosomes. Reverse PI–DAPI bands are visible and are indicated by arrowheads. (*f*) Detailed karyotype of the reverse PI–DAPI bands in *e*. (*g*) Detection of 45S rDNA on *L. rubellum* chromosomes. (*h* and *i*) Simultaneous detection of 5S and 45S rDNA. There are stronger 5S rDNA signals (*h*, red arrowheads) than 45S rDNA signals (*i*, green arrowheads) on an interphase nucleus of *L. rubellum*. (*j*) Karyotype of rDNA in *L. rubellum*. Scale bars = 10 μm.

fluorescence (Fig. 1). A comparable situation—three C-heterochromatin classes—was found for *L. rubellum* chromosomes, although different chromosomes were involved.

FISH of rDNA repeats allowed the following classes to be distinguished: (1) 45S + 5S rDNAs, as on *L. longiflorum* chromosomes 4 and 7 and *L. rubellum* chromosomes 2, 3, 4, and 12; (2) only 45S rDNA, as on *L. longiflorum* chromosome 3 and *L. rubellum* chromosomes 1 and 6; and (3) only 5S rDNA, as on *L. longiflorum* chromosome 3 and *L. rubellum* chromosome 12 (Figs. 3*c*, 3*d*, and 3*j*). The 45S rDNA signals, which occurred mainly in the secondary constrictions, were mostly larger and brighter than the 5S rDNA signals. However, on chromosome 3 in both *L. longiflorum* and *L. rubellum*, the 5S rDNA signal was stronger than the 45S rDNA signal (Figs. 3*b*–3*d* and 3*j*). All 45S rDNA sites were found to co-localise with the simultaneously stained reverse PI–DAPI bands, with the exception of the long-arm sites on chromosome 3 in *L. longiflorum* (Figs. 3*c* and 3*d*) and chromosomes 1 and 6 in *L. rubellum* (Fig. 3*g*). Lima-de-Faria (1976) and Schulz-Schaeffer (1980) observed that satellites are generally attached to the short arm of a NOR chromosome. The present results confirm that the satellite repeats (reverse PI–DAPI band) appear in the secondary constriction of a short arm or, when in a long arm, appear very near the centromere (Fig. 1).

Lima-De-Faria (1976) analysed the nucleolus-organising citrons in over 700 species and reported that, in 87% of cases, the nucleolus was located on the short arm of the chromosome. Such striking conservatism in karyotype morphology suggests some molecular or physical constraint for chromosome arms to associate with the nucleolus. Surprisingly, the sites with ribosomal genes in *Lilium* spp. are far more variable than those observed for most other plant species, exhibiting various NORs on both long and short chromosome arms (Fig. 1). One of the explanations is that the large genome size allows greater variability in ribosomal gene distribution along the short arms and so permits chromosomal rearrangements that involve moving the NORs to proximal long arm positions (see Fig. 1). It is still not known whether such long-arm sites of 45S rDNA became silenced.

DAPI is known to bind preferentially to A–T rich heterochromatic regions or to act as a dye that is specific for double-stranded DNA (Schweizer and Nagl 1976; Trask 1999). PI intercalates between the bases of either single- or double-stranded nucleic acid molecules (Heslop-Harrison and Schwarzacher 1996). NORs are composed of tandemly repeated G–C sequences (Macgregor and Kezer 1971; Yasmineh and Yunis 1971; Ingle et al. 1975). Therefore, staining simultaneously with PI and DAPI can give rise to a red band, a so-called reverse PI–DAPI band, at NOR posi-

tions. The same type of reverse PI–DAPI band has been demonstrated in species of the genera *Lycopersicon* and *Oryza* (Peterson et al. 1999; Andras et al. 2000). A similar case of reverse PI–DAPI bands was seen in tandemly repeated regions such as NORs in the chromosomes of *Lilium* species (Lim et al. 2000). This band position could represent DAPI negative bands in G–C rich regions. We found that repetitive bands such as reverse PI–DAPI bands are located mostly at the same positions as rDNA sites, not only on the short arm but also on the long arm adjacent to the centromere (see Fig. 3*f*).

Our study revealed a few major differences in the distribution of heterochromatin, rDNA sites, and several tandem repeats between *L. longiflorum* and *L. rubellum*. As genome painting of *L. longiflorum* × *L. rubellum* hybrids (Lim et al. 2000) also demonstrates large-scale differences in total genomic DNA between parental species, it is likely that Ty/*copia* and (or) related dispersed repeat families diverged recently during the evolution of *Lilium* spp. For the breeder and geneticist, it is more important to know whether these changes in chromosome morphology, banding pattern, and molecular organisation also reflect large-scale chromosomal rearrangements like translocations and inversions. Observation of regular bivalent formation in metaphase I microsporocytes of a *L. longiflorum* × *L. rubellum* hybrid seem to suggest that the two homoeologous genomes retain extensive correspondence (Fig. 3*a*).

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