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Homoeologous pairing and recombination in backcross derivatives of tomato somatic hybrids [*Lycopersicon esculentum* (+) *L. peruvianum*]

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Abstract Genomic in situ hybridization (GISH) was used to examine genome interactions in two allohexaploid (2n = 6x = 72) *Lycopersicon esculentum* (+) *L. peruvianum* somatic hybrids and their seed progenies originated from subsequent backcrosses to *L. esculentum*. The ability of GISH to distinguish between chromatin derived from two closely related species, *L. esculentum* and *L. peruvianum* (both 2n = 2x = 24), allowed the precise chromosomal constitution of somatic hybrids and their backcross progenies to be unequivocally established. This enabled the interaction of species genomes to be observed at meiosis, providing clear evidence of strictly regular homoeologous pairing and the high degree of homoeologous recombination in allodiploid plants (2n = 2x = 24) of the BC_1 generation. In segmental allodiploids of the BC_2 and BC_3 generations, the recombinant chromosomes continued to pair with a homoeologous partner (in the absence of a homologous one), and therefore could be stably incorporated into gametes. Chiasmata were found almost exclusively in more distal, rather subterminal, chromosome segments. A considerable proportion of meiotic recombination was detected in subterminal heterochromatic regions, often involving distal euchromatin, located in close proximity. GISH also supplied information on the extent of the overall sequence homology between the genomes of *L. esculentum* and *L. peruvianum*, indicating that despite their different breeding systems, these species may not be differentiated to a high degree genetically. The present study has demonstrated that somatic hybridization between two such closely related, but sexually incompatible or difficult to cross species, provides a way of transferring genes, via homoloeogous crossing-over and recombination, across the incompatibility barriers. Indeed, such hybrids may offer the preferred route for gene transfer, which subsequently results in more stable gene introgression than other methods.

Key words *Lycopersicon esculentum* · *L. peruvianum* · Somatic hybrids · Backcross (BC) progeny · Homoeologous pairing · Homoeologous recombination · Genomic in situ hybridization (GISH)

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Introduction

Homoeologous recombination represents one of the most reliable ways of achieving stable gene introgression (Koebner and Shepherd 1986; Islam and Shepherd 1992). It delivers desirable genes within a segment of DNA whose physical environment, in terms of chromatin structure and configuration, matches that of the recipient homoeologous genome to a high extent. The resulting recombinant chromosomes usually have a hom(oe)ologous pairing partner at the next meiosis and, therefore, can be stably incorporated into gametes. However, a high degree of overall sequence homology between the genomes of the two parental species in a hybrid is required to allow homoeologous crossing-over and recombination.

A number of observations suggest that, despite specialized breeding barriers between *L. esculentum* and *L. peruvianum*, their genomes may not be differentiated to a high degree genetically. Karyotypes of these species are remarkably similar, as established from their pachytene morphology (Barton 1950). Moreover, their

genomes are of a similar size; the 2C DNA values for diploid *L. esculentum* and *L. peruvianum* are 2.3 and 2.5 pg, respectively (Bennett and Leitch 1995). It is likely that the morphological organization of their chromosomes has been highly conserved during the course of evolution.

In allotetraploid hybrids between closely related diploid species, the four hom(oe)ologous chromosomes form quadrivalents when they exchange partners during pairing. The frequency of quadrivalents at meiotic metaphase I is often used as an index to analyse the affinity between chromosomes of the component genomes (Sybenga 1975). Recently, Sybenga et al. (1994) compared the hom(oe)ology between chromosomes of *L. esculentum* and *L. peruvianum* in allotetraploid (2n = 4x = 48) somatic hybrids by studying pairing behaviour at pachytene and metaphase I. The pairing affinity between these two species was estimated as to be slightly lower than the affinity within the species. Conventional cytological analysis of meiosis in two allohexaploid tomato somatic hybrids (2n = 6x = 72) comprising one diploid genome from *L. esculentum* and two diploid genomes from *L. peruvianum* showed the presence of hexavalents, along with rare pentavalents, thus indicating that homoeologous as well as homologous chromosome pairing exists at meiosis in these hybrids (Giddings and Rees 1992).

Breto et al. (1993) analysed isozyme variation among species of *Lycopersicon* in an attempt to establish an overview of their genetic relationships. Significantly, isozymes of *L. esculentum* were, in general, the ones most frequently found in *L. peruvianum*. This analysis placed *L. peruvianum*, with a self-incompatible breeding system, near the middle of the graphic representation of *Lycopersicon* species, showing that it has a slightly higher affinity to the group of self-compatible cultivated species (including *L. esculentum*, *L. pimpinellifolium* and *L. cheesmanii*). Moreover, these results suggest that the *peruvianum*-like genotype may have been involved in the ancestry of the *Lycopersicon* species.

Taken together the above observations indicate that *L. esculentum* and *L. peruvianum* may share a high degree of overall genome homology and that the genetic loci in these species may be largely homosequential, as has been demonstrated for the potato and tomato genomes (Gebhardt et al. 1991; Tanksley et al. 1992) . In addition, unequivocal evidence of recombination has been detected genetically for nearly all marker genes studied in sesquidiploid hybrids of *L. esculentum* (×) *S. lycopersicoides* and their progenies derived from backrosses to *L. esculentum* through *L. pennellii* (Rick et al. 1988). Evidence was not found limiting recombination to certain chromosomes.

Genomic in situ hybridization (GISH) provides a new tool for effective parental genome analysis in both sexual and somatic hybrids. GISH, which utilizes total genomic DNA from one of the parental species as a probe, allows chromosomes from different parental origins to be "painted" in different colours in the nuclei of interspecific hybrids (Schwarzacher et al. 1989; Parokonny et al. 1992a,b; Schwarzacher et al. 1992; Jiang and Gill 1994). Intergenomic recombinant chromosomes containing chromosome segments from both parental species can be readily identified, and their behaviour and transmission can be monitored through meiosis into the progeny of subsequent backcrosses (Parokonny et al. 1992b; 1994; Schwarzacher et al. 1992). Moreover, the ability of GISH to discriminate between genomes of closely related species enables the re-evaluation of how accurately karyotype and meiotic pairing reflect species genome relationships (Parokonny et al. 1992a). GISH applied to meiotic cells also has the advantage that it provides valuable information on homoeologous chromosome pairing and allows the analysis of meiotic recombination between homoeologous chromosomes (Bailey et al. 1993).

Somatic hybrids obtained by the fusion of protoplasts from two closely related, but sexually incompatible or difficult to cross species may provide excellent starting material for speeding up introgressive hybridization through homoeologous crossing-over and recombination. A number of somatic hybrids between self-compatible *L. esculentum* (2n = 2x = 24) and its wild relative, self-incompatible *L. peruvianum* (2n = 2x = 24), as well as their backcross derivatives, have been produced (Kinsara et al. 1986; Patil et al. 1993). These accessions have been used to analyse, with the GISH technique, both the feasibility and the possible advantages of the former approach for introgressive hybridization of *L. peruvianum* in the somatic hybrids involving tomato, and then over several backcross generations. The primary aim of the work was to test whether GISH offers a useful tool for the unequivocal discrimination of chromatin derived from two species, and if so, to use GISH to examine the extent of species genome interaction in somatic hybrids and their backcross derivatives.

Materials and methods

Two allohexaploid somatic hybrids (SH6 and SH18, 2n = 6x = 72) were generated following the fusion of leaf mesophyll protoplasts of *L. esculentum* with cell suspension-derived protoplasts of *L. peruvianum* (Kinsara et al. 1986). Subsequently, these somatic hybrids were used to obtain backcross progenies (Patil et al. 1993). The origin of the somatic hybrids and their backcross derivatives used in the present study are summarized in Table 1.

The methods for isolation of total genomic DNA, labelling of total genomic DNA by nick translation, chromosome preparations from root-tip meristem and meiotic pollen mother cells (PMCs) and GISH were generally as described by Parokonny et al. (1992a, b; 1994).

Preliminary GISH experiments utilizing standard stringency conditions (detailed later) revealed complete hybridization of probe DNA to the chromosomes of both parents, indicating that the

Table 1 Origin and chromosome constitution of the somatic hybrids and their backcross progeny

Somatic hybrids/ Backcross progenies (code number)	Somatic and sexual hybrids[a]	Chromosome number (2n)	Number of *L. esculentum* chromosomes[b]	Number of *L. peruvianum* chromosomes
Somatic hybrids				
SH6	*L. esculentum* AC (+) *L. peruvianum* S37/68	72	24	48
SH18	*L. esculentum* AC (+) *L. peruvianum* S37/68	72	24	48
Backcross progeny	Pollen parent (×) Maternal parent			
BC_1-1	SH6 (×) *L. esculentum* MM	24	12 (0)	12
BC_1-2	SH6 (×) *L. esculentum* MM	24	12 (0)	12
BC_1-3	SH6 (×) *L. esculentum* MM	24	12 (0)	12
BC_1-4	SH18 (×) *L. esculentum* MM	24	12 (0)	12
BC_1-5	SH18 (×) *L. esculentum* MM	24	12 (0)	12
BC_1-6	SH18 (×) *L. esculentum* MM	24	12 (0)	12
BC_1-7	SH18 (×) *L. esculentum* MM	24	12 (0)	12
BC_2-2	BC_1-1 (×) *L. esculentum* R	24	20 (5)	4
BC_2-5	BC_1-1 (×) *L. esculentum* PR	24	20 (4)	4
BC_2-14	BC_1-5 (×) *L. esculentum* PR	24	14 (0)	10
BC_2-17	BC_1-6 (×) *L. esculentum* PED	24	16 (4)	8
BC_3-1	BC_2-1 (×) *L. esculentum* PED	24	21 (3)	3
BC_3-3	BC_2-16 (×) *L. esculentum* S10	24	22 (4)	2
BC_3-5	BC_2-16 (×) *L. esculentum* PED	24	20 (4)	4

[a] *L. esculentum* cultivars: AC, 'Ailsa Craig'; MM, 'Moneymaker'; R, 'Roma'; PR, 'Pusa Ruby'; PED, 'Pusa Early Dwarf'; S10, 'Selection 10'
[b] Number of *L. esculentum* recombinant chromosomes is indicated in parentheses

nuclear genomes of *L. esculentum* and *L. peruvianum* share a high degree of sequence homology. Therefore, the GISH protocol was refined to eliminate most of the cross-hybridization between total genomic DNAs from the two species.

The stringency of the in situ hybridization reaction was increased by raising the formamide concentration in the hybridization mixture from 50% to 60% (v/v). The stringency conditions of the post-hybridization washes employed in the initial GISH experiments [10 min, 50% (v/v) formamide in 2 × SSC, 42°C] allowed sequences with 80–85% homology to remain hybridized. Accordingly, the stringency was increased to 90–95% by raising the formamide concentration to 60% in 2 × SSC, the temperature was raised from 42°C to 43°C and the duration of the formamide wash increased from 10 to 15 min. The hybridization specificity of probe DNA was further increased by including, in the hybridization mixture, a large excess of unlabelled (blocking) DNA from the other parent. The amount of blocking DNA required to discriminate between the genomes of *L. esculentum* and *L. peruvianum* was determined in a series of test assessments. Optimum results were obtained when blocking DNA exceeded the concentration of probe DNA by one hundred fold.

Results

The somatic karyotypes of *L. esculentum* and *L. peruvianum* are morphologically almost identical. Hence, most somatic chromosomes cannot be distinguished in their hybrids using conventional cytological procedures such as aceto-orcein, Feulgen or even C-banding. Only nucleolus organizer regions (NORs), which are associated with secondary constrictions of satellite chromosomes (chromosome 2 of both species), can serve as useful physical markers. The *L. peruvianum* satellite chromosome contains a significantly larger satellite than its *L. esculentum* equivalent (see Figs. 1 and 2, marked with wide and narrow arrows, respectively). In GISH experiments, the total genomic DNA from one *Lycopersicon* species cross-hybridized to the NOR regions of the other (e.g. the *L. esculentum* NOR regions, cross-hybridized to the total genomic DNA from *L. peruvianum*, are marked with narrow arrows in Figs. 1 and 2). These regions, containing 25S, 18S and 5.8S ribosomal genes, are highly conserved among plant species and can be distinguished readily from species-specific hybridization using the rDNA-specific probe pTa71 (Gerlach and Bedbrook 1979).

Chromosome constitution and meiosis of tomato somatic hybrids SH6 and SH18

As determined by GISH with biotinylated total genomic DNA either from *L. peruvianum* or *L. esculentum*, in both somatic hybrids (SH6 and SH18) the somatic chromosome number (2n = 6x = 72) comprised a diploid chromosome set (2n = 2x = 24) from *L. esculentum* and a tetraploid chromosome set (2n = 4x = 48) from *L. peruvianum* (Fig. 1A). GISH revealed no evidence of chromosome structural rearrangements that might have resulted from somatic recombination, which is often the case in somatic hybrids (Parokonny et al. 1992b). Although each hybrid contained four homologous haploid sets (n = x = 12) from *L. peruvianum*, analysis of chromosome configurations at diakinesis and metaphase I in orcein-stained PMCs revealed that the majority of configurations were bivalents (up to 85%). One possible

explanation for such an anomalous pattern of pairing could be the cryptic tetraploid nature (2n = 4x = 24) of the *Lycopersicon* species. There was no significant difference in the distribution of chromosome configurations at diakinesis between the two hybrids. These consisted of a higher proportion of ring (closed) bivalents with two chiasmata to rod (open) bivalents with one chiasma. In both somatic hybrids, the chiasma frequency at diakinesis was very similar, with an overall mean estimated to comprise 1.54 chiasmata per bivalent. All chiasmata were located in the distal chromosome segments. Apart from rare univalents and pentavalents, quadrivalents and hexavalents were also detected at frequencies of 13% and 9%, respectively. The presence of hexavalents and pentavalents indicates that both homoeologous and homologous chromosome pairing occurred at diakinesis and metaphase I. Chromosome segregation at anaphases I and II was regular in approximately 80–85% of the nuclei, resulting in the equal segregation of 36 chromosomes to each pole. However, some chromosome lagging at these stages reduced chromosome numbers at second telophase, and one or two micronuclei in tetrads were frequently seen in both hybrids. These results agree with previous observations by Giddings and Rees (1992) on similar tomato somatic hybrids (SH2 and SH17) originating from the same protoplast fusion experiments (Kinsara et al. 1986). Somatic hybrids SH6 and SH18 were fertile, setting up to 50% of viable seeds upon self-pollination.

Chromosome constitution and meiosis of the backcross progenies

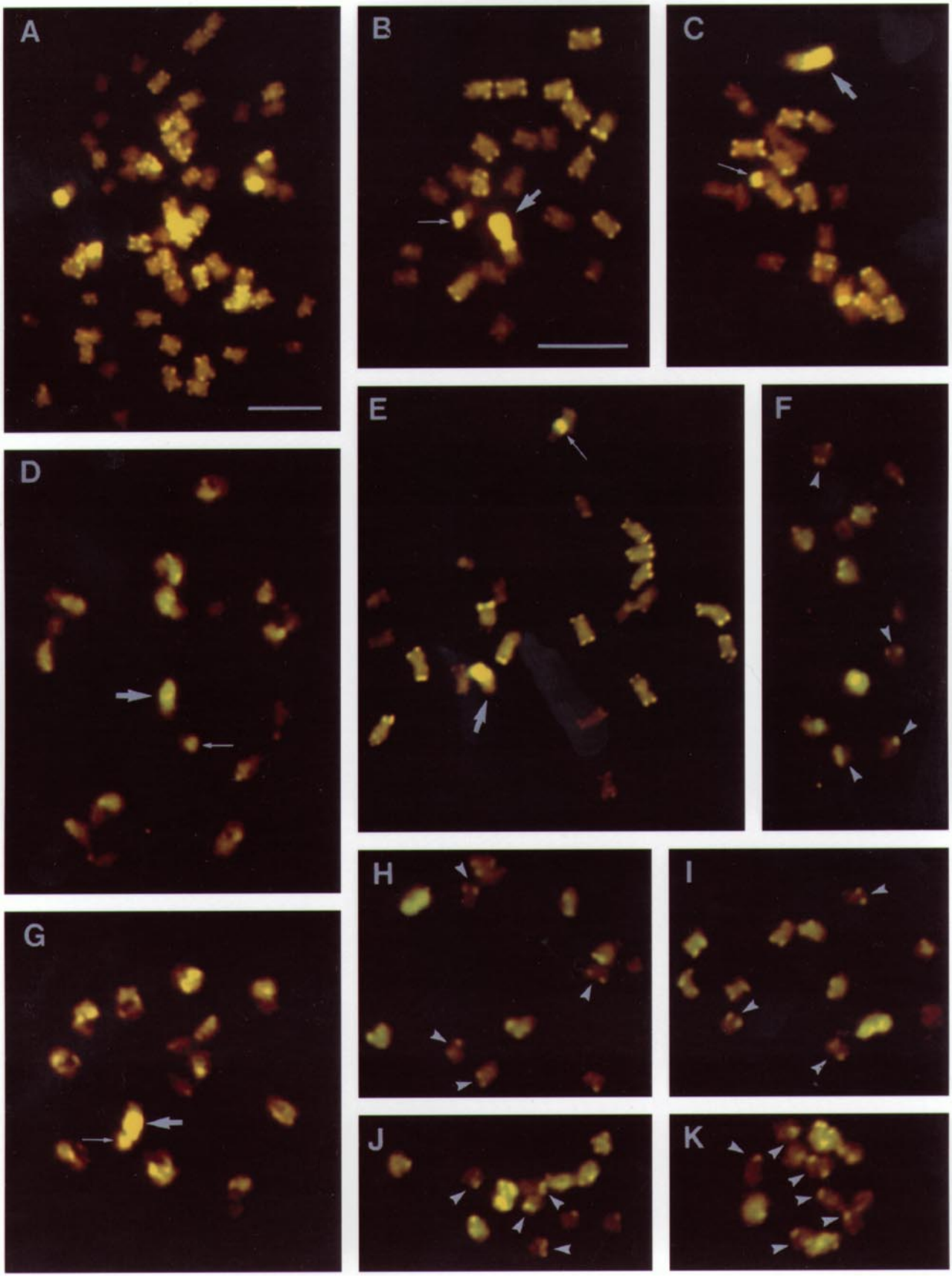
Allohexaploid somatic hybrids SH6 and SH18 were initially used as pollen parents for backcrossing with diploid (2n = 2x = 24) *L. esculentum* cv 'Moneymaker' (MM). Seven BC_1-generation plants were recovered via embryo culture. Subsequently, these were employed as pollen parents in further backcrossings to produce the BC_2 and BC_3 generations. The crossing scheme is given in Table 1.

The somatic chromosome number in root-tip cells and chromosome pairing in meiotic PMCs for all BC progenies were analysed with both conventional aceto-orcein staining and after GISH with biotinylated total genomic DNA either from *L. esculentum* or *L. peruvianum*. Table 1 summarizes the chromosome constitutions revealed by GISH in the different backcrosses examined.

The somatic chromosome complement of allodiploid (2n = 2x = 24) plants of the BC_1 generation consisted of a haploid chromosome set (n = x = 12) from both *L. esculentum* and *L. peruvianum* (Fig. 1B, C and E; Table 1). As mentioned earlier, in SH6 and SH18 somatic hybrids, tetrad nuclei often contained one or two micronuclei along with a reduced (in most cases nearly haploid) number of chromosomes. This indicates that gametic, rather then zygotic, selection may be the cause of non-transmission of the two haploid chromosome sets from the allohexaploid somatic hybrids to the allodiploid BC_1 progeny. Interestingly, all BC_1 plants were recovered via embryo culture, the latter isolated from pre-selected immature seeds with minimum necrosis of the endosperm. Perhaps seeds containing larger necrotic regions in the endosperm, and possibly tetraploid embryos, were selected against prior to embryo rescue.

Meiosis in allodiploid BC_1 plants was strictly regular. Twelve bivalents per PMC were consistently detected at diakinesis and metaphase I, whereas univalents were not observed (Fig. 1D and G; wide and narrow arrows indicate, respectively, the satellite chromosomes of *L. peruvianum* and *L. esculentum* forming a homoeologous bivalent). Similarly, no irregularities were seen at anaphase I or II. In all PMCs examined, chromosome segregation was equal at telophase of both the first and second divisions. However, the mean chiasma frequency (19.8 per cell, 1.65 per bivalent) was slightly lower than those for *L. esculentum* (21.12 per cell, 1.76 per bivalent) and *L. peruvianum* (21.96 per cell, 1.83 per bivalent). This difference reflected a lower proportion of ring bivalents with two chiasmata to rod bivalents with one chiasma in BC_1 plants compared to the situation in the parental species. Chiasmata were

Fig. 1A–K GISH with total genomic DNA from *L. peruvianum* to chromosomes of somatic and meiotic nuclei of the *L. esculentum* (+) *L. peruvianum* somatic hybrids and BC_1-generation plants. *L. peruvianum* chromatin fluoresces yellow-green, indicating hybridization to the probe. Unlabelled chromatin from *L. esculentum* fluoresces red with the non-specific propidium iodide counterstain. *Wide* and *narrow arrows* indicate hybridization of the *L. peruvianum* total genomic DNA to the rDNA sites on satellite chromosomes of *L. peruvianum* and *L. esculentum*, respectively. *Solid arrowheads* mark *L. esculentum* recombinant chromosomes containing terminal (yellow-green) segments of the *L. peruvianum* chromatin. **A** Root-tip metaphase of the allohexaploid (2n = 6x = 72) somatic hybrid SH18, comprising one diploid chromosome set (2n = 2x = 24) from *L. esculentum* and two diploid chromosome sets (2n = 2x = 24) from *L. peruvianum*. **B**, **C** and **E** Root-tip metaphases from allodiploid (2n = 2x = 24) plants of BC_1, BC_1-2 (**B**), BC_1-4 (**C**) and BC_1-1 (**E**), comprising one haploid chromosome set (n = x = 12) from each species. The subterminal heterochromatic regions of the *L. peruvianum* chromosomes are easily distinguished from the adjacent euchromatic regions by their brighter yellow-green fluorescence. The rDNA cross-hybridization sites at NOR regions of the *L. peruvianum* satellite chromosomes (*broad arrows*) are significantly larger than the *L. esculentum* counterparts (*narrow arrows*). **D** and **G** Late diakinesis in PMCs of the allodiploid (2n = 2x = 24) plant, BC_1-7, showing 12 bicoloured homoeologous bivalents. The *L. peruvianum* and *L. esculentum* satellite chromosomes, forming homoeologous bivalents, are marked with wide and narrow arrows, respectively. **F** and **H–K** Meiotic telophase I in PMCs of plant BC_1-7, showing a number of *L. esculentum* recombinant chromosomes (marked with *solid arrowheads*) originating from homoeologous crossing-over and recombination. Bar in **A** (10 μm) applies also to **D** and **G**. Bar in **B** (10 μm) applies also to **C**, **E**, **F** and **H–K**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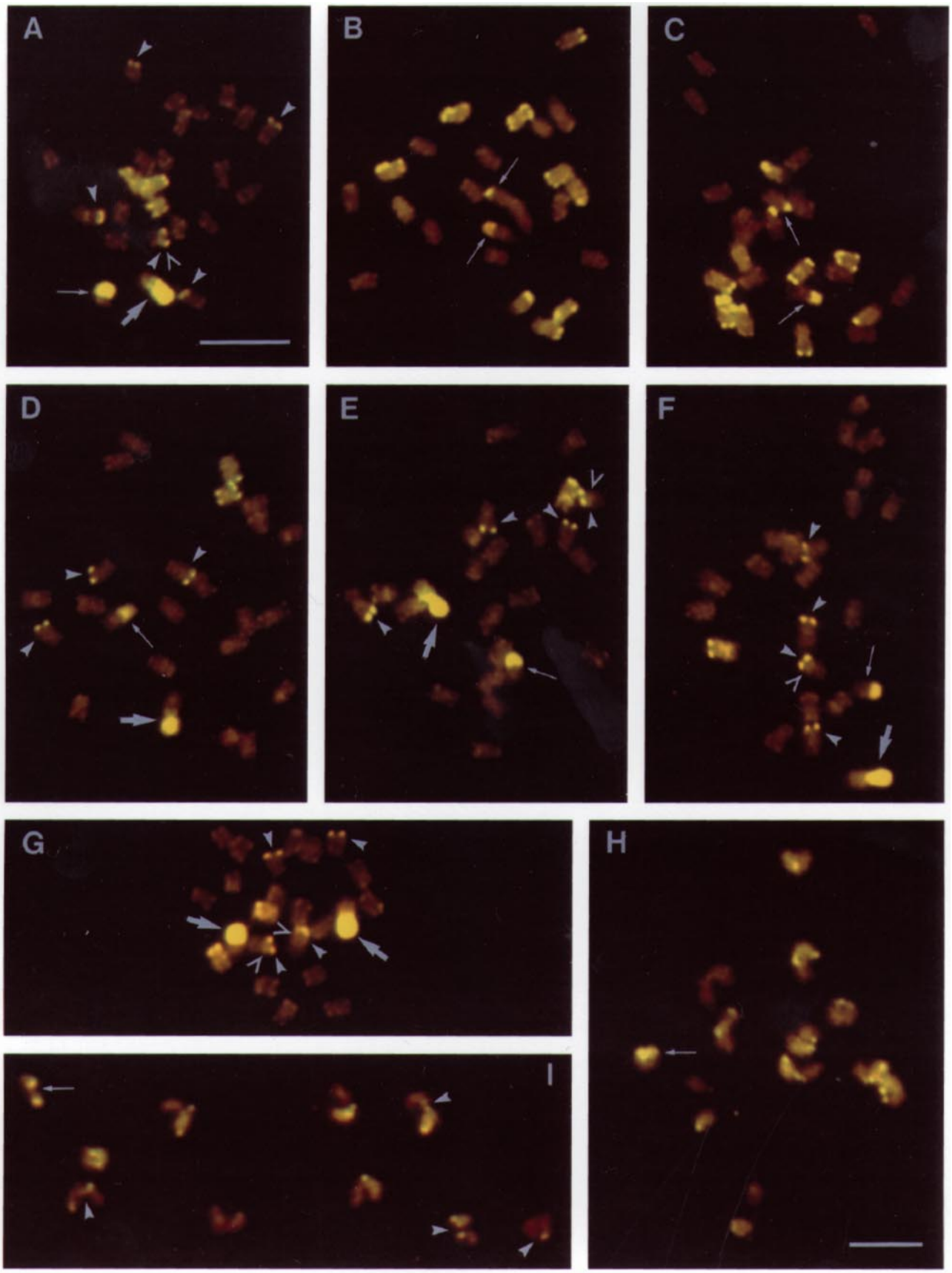
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located exclusively in the more distal, and even subterminal, chromosome segments. Thus, most homoeologous recombination was apparently restricted to the subterminal heterochromatic segments, which are composed of the clustered highly repetitive DNA sequences in both species. After GISH, these segments can be easily distinguished from adjacent euchromatic regions by their brighter fluorescence (Fig. 1B, C and D; the yellow-green chromosomes from *L. peruvianum* contain brightly fluorescent subterminal segments).

GISH analysis of recombinant chromosomes segregating at telophase I in BC_1-generation plants indicated that homoeologous crossing-over and recombination seem unlikely to be restricted to only certain pairs of homoeologues, as in some cases they occurred in up to eight pairs per PMC [see Fig. 1F, H, I, J and K, where solid arrowheads indicate the recombinant *L. esculentum* chromosomes with subterminal (yellow-green) segments from *L. peruvianum*].

Segmental allodiploids ($2n = 24$) of the BC_2 and BC_3 generations were characterized by a gradual reduction in the numbers of *L. peruvianum* chromosomes – 2 chromosomes in individual BC_3-3 (Fig. 2D and e; Table 1) – and by the number of recombinant chromosomes, varying from 0 in BC_2-14 to 5 in BC_2-2 [Fig. 2A-G; the *L. esculentum* recombinant chromosomes containing subterminal segments of the *L. peruvianum* DNA (yellow-green fluorescence) are marked with solid arrowheads; see also Table 1]. Most recombinant chromosomes contained subterminal heterochromatic regions from *L. peruvianum*. However, in several cases, small segments of distal euchromatin adjacent to these regions were also identified (these recombinants are indicated with open arrowheads in Fig. 2A, E, F and G).

Twelve regular bivalents (Fig. 2H and I) were consistently detected at diakinesis and metaphase I in all BC_2 and BC_3 plants tested. No irregularities were revealed at anaphase I and anaphase II. Chromosome segregation at telophases I and II was regular in all of the cells examined, resulting in an equal segregation of 12 chromosomes to each pole. The mean chiasma frequency (per cell and per bivalent) was very similar to that of *L. esculentum*, especially in plants with fewer *L. peruvianum* chromosomes.

Importantly, recombinant chromosomes paired regularly with the available hom(oe)ologous partner (Fig. 2I; the hom(oe)ologous bivalents involving the *L. esculentum* recombinant chromosomes are marked with solid arrowheads) and segregated normally to the poles, thus incorporating stably into dyad and tetrad nuclei. Such chromosomes would probably be subject to further recombination at meiosis in subsequent generations.

Fig. 2A–J GISH with total genomic DNA from *L. peruvianum* to chromosomes of somatic and meiotic nuclei in segmental allodiploids of BC_2 and BC_3. The *L. peruvianum* chromatin fluoresces yellow-green, indicating hybridization to the probe. Unlabelled chromatin from *L. esculentum* fluoresces red with the non-specific propidium iodide counterstain. *Solid arrowheads*, *wide* and *narrow arrows* have the same significance as in Fig. 1. *Open arrowheads* mark *L. esculentum* recombinant chromosomes containing both subterminal heterochromatic and distal euchromatic segments from *L. peruvianum*. **A–C** Root-tip metaphases of individual BC_2 plants, BC_2-2 (**A**) and BC_2-14 (**B** and **C**). **D–G** Root-tip metaphases of individual BC_3 plants, BC_3-1 (**D**), BC_3-3 (**E** and **F**) and BC_3-5 (**G**). **H** and **I** Diakinesis in PMCs of plant BC_2-17. *Solid arrowheads* in **I** indicate the hom(oe)ologous bivalents involving the *L. esculentum* recombinant chromosomes (two bivalents are missing). *Narrow arrows* mark homologous bivalents involving the *L. esculentum* satellite chromosomes. Bar in **A** (10 μm) applies to **B–G** and *bar* in **H** (10 μm) applies also to **I**

Discussion

Genome differentiation as revealed by GISH

The ability of GISH to discriminate rapidly and unequivocally between the genomes of closely related species makes it a valuable tool in studies of species' genome relationships. Its success as an indicator of species' genome differentiation relies on the ability of a detection system to resolve small differences in sequence homology between total genomic DNA from the two species.

The standard GISH protocol uses stringency conditions that normally allow duplexes to be formed and persist between single-stranded DNAs sharing at least 80–85% sequence homology (Britten et al. 1974; Schwarzacher et al. 1989). If the overall sequence homology between two genomes exceeds 85%, the specificity of the standard GISH procedure may be insufficient to discriminate between two such genomes. The hybridization specificity of probe DNA can be increased by including, in the hybridization mixture, a large excess of unlabelled, total genomic DNA from one of the species that has been sheared to about 100- to 200-bp fragments by autoclaving. Repetitive sequences in the unlabelled (blocking) DNA saturate sites of common sequence homology, leaving only probe-specific sites available for hybridization. The approximate percentage of sequences correctly matched between the probe and target single-stranded DNAs is largely determined by the stringency conditions of the in situ hybridization reaction and post-hybridization washes. An increase in stringency conditions, combined with the use of blocking DNA, allowed fine-tuning of the GISH procedure, enabling discrimination between the *Lycopersicon* genomes with more than 90–95% of overall sequence homology.

Karyotype morphology and meiotic analysis are useful as indicators of species' genomes relationships at, and below, the generic level. The comparison of genomes using meiotic analysis of novel hybrids has

increased the application of chromosome studies for assessing genomic affinities between *Lycopersicon* species (Rick 1979; Giddings and Rees 1992; Sybenga et al. 1994). However, the extent to which karyotype similarity and meiotic pairing reflects species' genome relationships may often be difficult to determine using conventional methods (Parokonny et al. 1992a).

The advent of GISH has allowed a re-evaluation of how accurately karyotype and meiotic pairing reflect species' relationships. Questions re-examined include, for example, how do identical karyotypes in related species indicate a similar genome organization and close molecular homology, and to what extent are homoeologous meiotic pairing partners homologous in molecular terms?

A recent example of how GISH can shed new light on species' genome relationships was the study of two allopatric sibling species, *Gibasis karwinskyana* and *G. consobrina* (Parokonny et al. 1992a: Kenton et al. 1993). These occur north and south, respectively, of the trans-Mexican volcanic belt. Both were once included in *G. karwinskyana* and both have relatively large genome sizes (2C DNA values in diploids, 20 and 22 pg, respectively, Bennett and Smith 1991). As with the *Lycopersicon* species studied here, *Gibasis* karyotypes were observed to be almost identical after Feulgen staining. Experimental F_1 hybrids were then made at both the diploid and tetraploid levels. Allodiploid hybrids showed little or no meiotic pairing between parental chromosomes and were sterile. In allotetraploid hybrids, parental chromosomes paired autosyndetically.

This pairing failure could have been caused by genetic control (the effect of a single gene or a group of genes) limiting pairing to strictly homologous chromosomes, or there could have been a general lack of linear homology between the morphologically similar homoeologues from the two species. GISH utilizing the standard stringency conditions and only a 1 : 10 ratio of labelled probe DNA to unlabelled blocking DNA readily distinguished parental genomes in the *Gibasis* hybrids, revealing approximately 70–80% sequence homology between *G. consobrina* and *G. karwinskyana* that was uniformly distributed throughout the karyotype. Southern hybridization with biotinylated total genomic DNAs under different stringency conditions also suggested that the two species have a similar level of sequence homology (Parokonny et al. 1992a; Kenton et al. 1993). Thus, the near identical karyotypes of the two species appeared to mask overall differences in sequence homology, which, in turn, could well be the cause of pairing failure between homoeologues. The molecular differentiation of *G. consobrina* and *G. karwinskyana* was consistent with their geographical separation and was almost certainly due to the lack of opportunity for sequence homogenization to occur between two species.

In the present study on tomato, GISH using standard stringency conditions failed to differentiate the nuclear genomes of *L. esculentum* and *L. peruvianum* in the context of their somatic hybrids. Probe DNA hybridized completely to the chromosomes from both parents, indicating that the nuclear genomes of *L. esculentum* and *L. peruvianum* share a very high degree of overall sequence homology. However, and importantly, GISH was able to distinguish these genomes when the stringency conditions were increased (see Materials and methods), and unlabelled blocking DNA was added to the hybridization mixture at a concentration exceeding that of the labelled probe DNA by a factor of 100. GISH revealed more than 95% overall sequence homology between *L. esculentum* and *L. peruvianum* uniformly distributed throughout their chromosome complements. Thus, unlike the situation with the two *Gibasis* species, similar karyotypes of *L. esculentum* and *L. peruvianum* appeared to coincide well with a similar genome organization and close molecular homology, which, furthermore, allowed for a strictly regular homoeologous pairing and recombination in allodiploid backcross progenies.

Interestingly, it was relatively easy, compared to the tomato somatic hybrids described here, to discriminate tomato and potato genomes in their somatic hybrids by GISH (Jacobsen et al. 1995) using conditions similar to those developed for *Gibasis*. This was consistent with the results obtained by Ganal et al. (1988), which indicated that highly repetitive DNA sequences found in tomato have undergone rapid divergence since the separation of *Lycopersicon* from *Solanum*.

The breeding systems of *Lycopersicon* species range from autogamy to self-incompatibility (Rick 1987). As the genomes of the self-incompatible *L. peruvianum* and self-compatible *L. esculentum* share a very high degree of overall sequence homology, this implies that evolution of the breeding systems and molecular differentiation did not follow the same pattern. Within the genus *Lycopersicon* adaptation of the breeding systems to specific habitats, rather than molecular differentiation, has played the primary role in speciation.

Genome differentiation by GISH and genome size

In tomato, highly repetitive DNA sequences account for about 22% of the total nuclear DNA as was estimated under high stringency conditions (Ganal et al. 1988). These comprised ribosomal DNA, satellite DNA, interspersed repeats with clustering at some chromosomal regions and interspersed repeated DNA sequences. The latter two forms of repeats are interspersed throughout most of the tomato genome. For example, the TGRII repeat occurs approximately every 133–170 kb of DNA, similar to the *Alu*I sequence in humans (Ganal et al. 1988). Moreover, restriction fragment length polymorphism (RFLP) analysis of single-copy nuclear DNA probes also indicated that a large

fraction of the tomato genome is comprised of single and low-copy sequences located in both, hetero- and euchromatin (Zamir and Tanksley 1988; Miller and Tanksley 1990; Peterson et al. 1996). The success of GISH in discriminating the genomes of *L. esculentum* and *L. peruvianum* would, therefore, rely largely on an ability to resolve minor differences between the above types of DNA sequences.

The present work bears usefully on a general question concerning the possible limits of applicability of GISH in relation to genome size. DNA 1C values in angiosperms vary considerably in magnitude from about 0.1 pg in *Arabidopsis thaliana* to 125 pg in *Fritillaria assyriaca* (Bennett and Smith 1976). As already noted, the resolving of genomes using GISH depends mainly on differences in non-coding interspersed DNA sequences, whose proportion is known to decrease with genome size. Thus, it was questionable whether GISH could be used to resolve angiosperm taxa with very low genome sizes (Bennett 1995).

Until recently most of the success in using GISH to distinguish angiosperm genomes concerned taxa with small-to-intermediate genome sizes in the range 1C = approximately 5–20 pg. The present work on *Lycopersicon* used two of the smaller plant genomes (1C = approximately 1 pg) examined in detail. Thus, the present results are important in showing: (1) that GISH can clearly distinguish such small genomes, and (2) that genome-specific labelling was distributed throughout the entire length of most chromosomes of this material where the 1C DNA amount per chromosome is approximately 0.1 pg.

In this respect, our present work strongly supports results from several recent investigations on tomato (+) potato somatic hybrids (Wolters et al. 1994; Ramulu et al. 1996) or sexual hybrids between *Solanum* species (Wilkinson et al. 1995), which showed that GISH can distinguish entire genomes, chromosomes or chromosome segments in several dicot materials with 1C values of about 1–2 pg. Moreover, work on a monocot hybrid, *Saccharum officinarum* (×) *Ericanthus arundinaceus*, [*S. officinarum* (2n = 8x = 80, 1C = appro, 4 pg; Bennett and Leitch 1995)], with similarly small chromosomes to *Lycopersicon*, also showed entire parental chromosomes distinguishable by GISH (D'Hont et al. 1995).

It should be noted that other recent investigations on *Brassica* hybrids (1C = approx. 0.5 pg) and on *Arabidopsis* species (1C = approx. 0.1 pg), with some of the smallest angiosperm genomes, showed that while ISH, using families of repeated sequences as probes, could resolve such genomes, genome-specific labelling was limited to heterochromatic segments near the centromere and did not extend to other chromosome segements (Maluszynska and Heslop-Harrison 1993; Kamm et al. 1995). Thus, the genome and chromosome sizes in *Lycopersicon* may be near the lower limit for GISH in terms of resolving whole chromosomes over their entire lengths.

Homoeologous pairing and recombination as revealed by GISH

In the present study, GISH has unequivocally revealed a strictly regular homoeologous pairing and a high frequency of homoeologous recombination in backcross derivatives of the somatic hybrids *L. esculentum* (+) *L. peruvianum*. It also indicated that homoeologous crossing-over and recombination are probably not restricted to only certain pairs of homoeologues. Indeed, they some times occurred in up to eight pairs per single PMC in allodiploid BC_1 plants. In segmental allodiploids of the BC_2 and BC_3 generations, the recombinant chromosomes paired regularly with the available hom(oe)ologous partners, segregated normally to the poles and were stably incorporated into gametes. Importantly, the allodiploid nature of the BC_1-generation plants allowed for entire haploid sets of each species to engage in homoeologous crossing-over and recombination. It also facilitated and explained why it is possible to rapidly recover almost the entire *L. esculentum* genome, along with the main tomato cultivar characteristics (fruit size, colour), by BC_3 (Patil et al. 1993). This has important implications for the breeding of tomato and perhaps of related crop species.

In *L. esculentum* and *L. peruvianum*, as in several other Solanaceous species, pericentromeric heterochromatin extends to almost half the length of the arms of most chromosomes and is similar in size to distal euchromatic segments (Barton 1950; Ramanna and Prakken 1967; Peterson et al. 1996). Comparatively small regions of subterminal heterochromatin consist mostly of clustered, highly repetitive DNA sequences (for example, TGR1 in *L. esculentum*; Ganal et al. 1988). According to Peterson et al. (1996), most of the tomato nuclear DNA (perhaps up to 77%) is located in pericentromeric and subterminal heterochromatin that is assumed to be genetically inactive. The remaining tomato nuclear genome is located in the distal euchromatic segments of the chromosome arms, which are supposedly gene-rich. In both species and in backcross progenies of their somatic hybrids chiasmata were found almost exclusively in the more distal, even rather subterminal, chromosome segments. Our results clearly demonstrate that a considerable proportion of meiotic recombination occurs in these subterminal heterochromatic segments, although often it can also involve the distal euchromatin, which is located in close proximity.

Irrespective of the species combination, somatic hybridization frequently results in the generation of individuals that deviate from the expected alloploid chromosome complement. Such individuals, even if fertile, are often not regarded by breeders as appropriate input resources for further refinement by backcrossing as part of a conventional breeding programme. Importantly, this study of tomato has clearly

shown that this assumption is incorrect. Interspecific somatic hybrids of a high ploidy status and, as in the case of tomato, with imbalanced parental genomes can, following a first backcross, lead directly to the recovery of fertile, allodiploid progeny coupled with the introgression of foreign chromosomes. Such BC_1 progeny derived from complex somatic hybrid germplasms provide precisely the spectrum of allodiploid individuals required in conventional breeding strategies.

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