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ORIGINAL PAPER

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Transmission and recombination of homeologous *Solanum sitiens* chromosomes in tomato

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Abstract The goal of the present experiments was to transfer the chromosomes of *Solanum sitiens* (syn. *Solanum rickii*) into cultivated tomato (*Lycopersicon esculentum*). By crossing an allotetraploid *L. esculentum* × *Solanum sitiens* hybrid to sesquidiploid *L. esculentum* × *S. lycopersicoides*, a trigenomic hybrid (2n+14=38) was obtained. Analysis of the latter by GISH (genomic in situ hybridization) indicated it contained a full set of 12 *S. sitiens* chromosomes, plus two extras from *S. lycopersicoides*. This and other complex hybrids were pollinated with *Lycopersicon pennellii*-derived bridging lines to overcome unilateral incompatibility. A total of 40 progeny were recovered by embryo rescue, including diploids and aneuploids (up to 2n+8). In order to determine the origin of chromosomes and the location of introgressed segments, progeny were genotyped with RFLP markers. *S. sitiens*-specific markers on all chromosomes, except 6 and 11, were detected in the progeny. Several *S. sitiens* chromosomes were transmitted intact, either through chromosome addition (i.e., trisomics) or substitution (i.e., disomics). Recombination between *S. sitiens* and *L. esculentum* was detected on most chromosomes, in both diploid and aneuploid progeny. A monosomic alien addition line for *S. sitiens* chromosome 8 was identified, and the extra chromosome was stably transmitted to approximately 13% of the backcross progeny. This study demonstrates the feasibility of gene transfer from *S. sitiens* to *L. esculentum* through chromosome addition, substitution, and recombination in the progeny of complex aneuploid hybrids.

Keywords *Solanum sitiens* · Tomato · Sesquidiploids · Monosomic alien addition lines · GISH

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Introduction

Wild relatives of many crop plants have been important resources for plant breeding (Harlan 1976; Tanksley and McCouch 1997; Jarvis and Hodgkin 1999). Cultivated tomato (*Lycopersicon esculentum*) is cross compatible to varying degrees with each of the nine wild *Lycopersicon* species, which have been valuable sources of economic traits such as disease and insect resistance, fruit quality and environmental-stress tolerance (Rick and Chetelat 1995). The much larger *Solanum* genus (approximately 1,250 spp, Nee 1999) includes four tomato-like nightshades, *Solanum lycopersicoides*, *Solanum sitiens*, *Solanum juglandifolium* and *Solanum ochranthum* (Rick 1988). Their morphology, ecology, distribution and crossing-relationships all suggest these species represent two pairs of sister taxa, of which the first (*S. lycopersicoides* and *S. sitiens*) are most closely related to *Lycopersicon* (Rick 1988; Child 1990). Crosses between *S. lycopersicoides* and *S. sitiens* result in fertile interspecific hybrids, while only *S. lycopersicoides* is cross compatible with *L. esculentum* (Rick 1951, 1979; Pertuzé et al. 2002). However, *S. sitiens* can be indirectly hybridized with cultivated tomato using *L. esculentum* × *S. lycopersicoides* derivatives as a bridge (see below).

First discovered by Johnston (1929), *S. sitiens* was described again by Correll (1961) as *Solanum rickii*, a synonymy pointed out by Marticorena and Quezada (1977). Collection-site information from genebank accessions and herbarium specimens indicate that *S. sitiens* is restricted to a small area of the Atacama Desert in northern Chile, a region of extreme aridity. Populations of *S. sitiens* are found only in a narrow altitudinal belt (about

2,500–3,000 meters above sea level) in a minor cordillera (Cord. de Domeyko) of the Andes. Precipitation in this region is strongly dependent on elevation, and is generally <5 cm/year below 3,000 m (Alpers and Brimhall 1988). Tolerance of extreme aridity, most-likely exceeding that found in any *Lycopersicon*, can therefore be inferred from conditions in the native habitat of *S. sitiens* (Rick 1988). Other useful traits, such as low temperature tolerance and/or disease resistances, are likely to be discovered when this species is more thoroughly evaluated.

The genome of *S. sitiens* is mostly colinear with that of *Lycopersicon* (Pertuzé et al. 2002). The only large-scale rearrangement is a paracentric inversion encompassing most of the long arm of chromosome 10. The *S. sitiens* configuration on 10L is shared with its sibling species *S. lycopersicoides*, as well as several other Solanaceous species, including potato, eggplant and pepper (Livingstone et al. 1999; Doganlar et al. 2002). On the basis of this inversion, as well as chromosome affinities during meiosis of interspecific and intergeneric hybrids, we recognize two genomes among cultivated tomato and its immediate wild relatives: the L genome, shared by all *Lycopersicon* spp., and the S genome of *S. sitiens* and *S. lycopersicoides*.

Direct hybridizations between *S. sitiens* (genome herein designated S^s) and *L. esculentum* (L^e) were unsuccessful in either direction (Rick 1979, 1988). Although this sexual incompatibility can be avoided by somatic hybridization, the only fusion products reported to-date were apparently sterile (O'Connell and Hanson 1986). In the case of *S. lycopersicoides* (S^l), treatment of the intergeneric hybrid (L^eS^l) with colchicine resulted in allotetraploids ($L^eL^eS^lS^l$) with sufficient pollen fertility for further backcrosses to diploid tomato. This resulted in sesquidiploid hybrids, containing two genomes of *L. esculentum* and one of *S. lycopersicoides* ($L^eL^eS^l$, Rick et al. 1986). During meiosis of $L^eL^eS^l$, the *S. lycopersicoides* chromosomes are mostly unpaired and are eliminated. As a result, this genotype serves as a convenient donor of the *L. esculentum* genome, with greater compatibility to *S. sitiens* (DeVerna et al. 1990). From $L^eL^eS^l \times S^sS^s$ crosses, the first diploid L^eS^s hybrids were obtained. Chromosome doubling produced allotetraploid hybrids ($L^eL^eS^sS^s$), which exhibited preferential pairing between homologous chromosomes and significant pollen fertility (DeVerna et al. 1990).

The objectives of the present experiments were to transfer the chromosomes of *S. sitiens* into cultivated tomato and to monitor recombination between their homeologous genomes. The $L^eL^eS^sS^s$ allotetraploid hybrid provided a means to derive sesquidiploid, aneuploid and recombinant diploid derivatives, in theory similar to what was accomplished previously for *S. lycopersicoides*. Special attention was given to the development of monosomic alien addition lines and recombinant diploids, as these are particularly useful in breeding programs and genetic studies.

Materials and methods

Plant material and pollinations

The following stocks used in this study were obtained from the Tomato Genetics Resource Center (TGRC), Department of Vegetable Crops, University of California, Davis: *L. esculentum* cv 'Vendor Tm-2^a'; *L. pennellii* accession LA0716, collected by Donovan Correll near Atico, Arequipa, Peru; *S. lycopersicoides* LA1964, collected by Charles Rick, 5 km above Palca, Tacna, Peru; and *S. sitiens* LA1974, collected by Carlos Ochoa at Chuquicamata, Region II (Antofagasta), Chile. Clones of intergeneric hybrids, including an allotetraploid ($L^eL^eS^sS^s$, plant GH2754-4*x*) and sesquidiploid ($L^eL^eS^l$, plant GH266) were provided by Joe DeVerna, formerly at the Campbell Soup Company.

L. pennellii-derived bridging lines were used to overcome unilateral incompatibility of *L. esculentum* × *S. sitiens* hybrids. Effective bridging lines were selected in segregating populations using RFLP markers for loci on chromosomes 1, 6 and 10 determining compatibility of pollen with *L. esculentum* × *S. lycopersicoides* hybrids (Chetelat and DeVerna 1991). In addition, the compatibility reactions of bridging lines were evaluated phenotypically by observation of pollen tube growth in pistils of a putative sesquidiploid hybrid (plant 90L4190-1). At 48 h post-pollination, pistils were removed, fixed and stained with aniline blue to reveal pollen tubes by epifluorescence microscopy as described by Martin (1959). At least three pistils were observed per cross. A cross was judged compatible if pollen tubes reached the ovaries. Conversely, if pollen tube growth was arrested in the style (usually the upper half) the cross was considered incompatible.

Selected bridging lines were used as male parents in crosses to 90L4190-1 and aneuploid derivatives (Fig. 1). Later progenies were backcrossed to *L. esculentum* cv Vendor-Tm-2^a whenever possible, unless prevented by unilateral incompatibility, in which case the bridging lines were employed as pollen parents for an additional

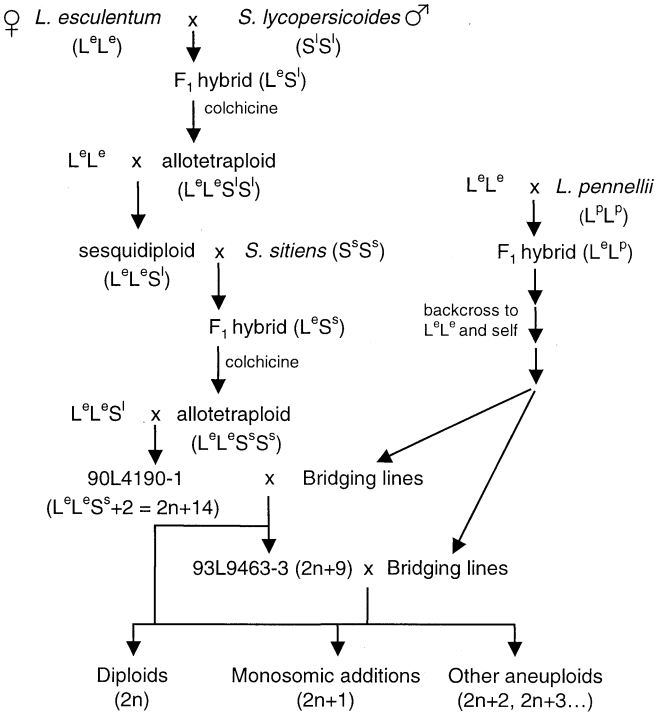

Fig. 1 Crossing scheme employed for hybridization of *L. esculentum* and *S. sitiens*. The ploidy and genomic constitution of each hybrid is represented by symbols: *L=Lycopersicon, S=Solanum, e=esculentum, p=pennellii, s=sitiens*, and *l=lycopersicoides*

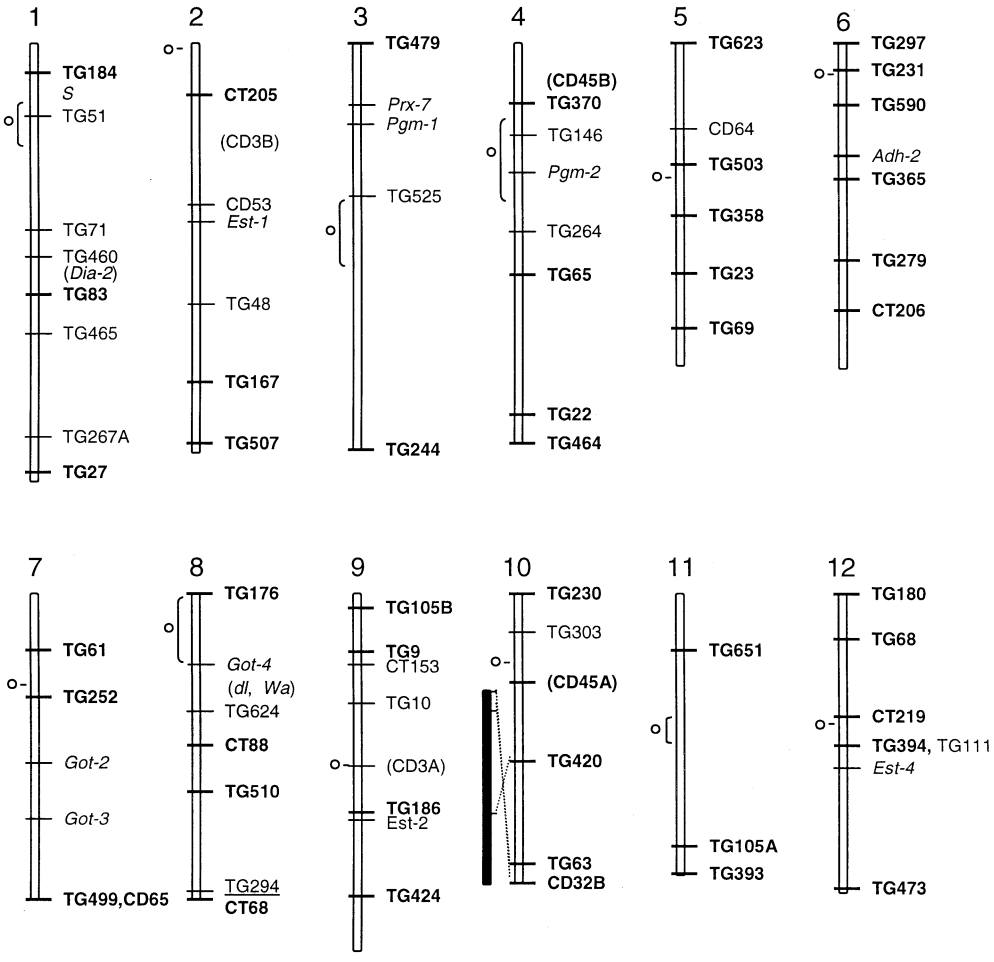

Fig. 2 Genetic map of markers used to genotype *S. sitiens* derivatives. The approximate positions of marker loci and centromeres are from the tomato RFLP map (Pillen et al. 1996), with additional loci (*in parentheses*) from Chetelat et al. (2000). The location of a paracentric inversion on chromosome 10 (*solid segment*, from Pertuzé et al. 2002) that distinguishes *S. sitiens* and *S. lycopersicoides* from the *Lycopersicon* spp., is also shown. *Markers in bold* were scored for most derivatives, others only in a subset (see Fig. 4)

generation. All pollinations were performed in the greenhouse (approximately 25°C by day, 15°C by night) at UC-Davis.

Prior to cross pollination, each *S. sitiens* derivative was evaluated for pollen viability (PV). Pollen was collected from up to four flowers per plant, and each flower was squashed in a drop of acetocarmine (1% in 50% glacial acetic acid) and observed under the microscope. The percentage of stainable (i.e., viable) pollen grains was estimated from at least 100 grains per flower, and values averaged across the replicate flowers. Plants with high PV, inserted stigmas and/or evidence of fruit set from self-pollination were emasculated prior to controlled pollinations. However, on plants with low PV, shrunken anthers and exserted stigmas, emasculation were not performed. The number of controlled pollinations and resulting fruit set were recorded for each cross.

Embryo culture

Embryo culture was used to improve the survival rate of *S. sitiens* derivatives. At approximately 30 to 45 days after pollination, fruit were surface-sterilized with 70% alcohol (2 min) and 25% bleach (10 min), rinsed and opened under sterile conditions. Swollen ovules were dissected, and embryos plated either on the HLH medium (Neal and Topoleski 1983) if poorly developed (e.g., heart or torpedo stages), or on Gamborg's B-5 medium with minimal organics (Sacks et al. 1997) if they were more advanced stages (e.g., walking stick to mature). Embryos on HLH medium were transferred to B-5 1–2 weeks later. The number of embryos or seeds per fruit was recorded for each cross. Once large enough, plants were transplanted to soil, acclimatized, then transferred to the greenhouse.

Chromosome observations

Chromosome counts of *S. sitiens* derivatives were performed by the acetocarmine squash method (Khush and Rick 1963). Immature flower buds were collected, dissected to remove calyx and corolla, and fixed in 3:1 95% EtOH : glacial acetic acid with $FeCl_3$. Fixative was replaced after the first 1–2 h, buds were fixed overnight, then transferred to 70% EtOH, and stored at 4°C. Anthers were squashed in a drop of acetocarmine and chromosomes were observed under the microscope using phase-contrast optics. Chromosomes were counted and their pairing relationships were analyzed from meiocytes at diakinesis or metaphase-I. In order to help determine the number and origin of chromosomes in a putative sesquidiploid (90L4190-1), genomic in situ hybridization (GISH) was performed on mitotic and meiotic chromosomal preparations as described (Ji and Chetelat 2003).

Marker analysis

RFLP, isozyme, and morphological markers were used to genotype the *S. sitiens* derivatives. A total of 148 RFLP probes combined with 5–6 restriction enzymes (REs) were evaluated for polymorphism between the four species involved in this study (*L. esculentum, L. pennellii, S. sitiens* and *S. lycopersicoides*). From these, 142 loci were polymorphic between at least the *Lycopersicon* and the *Solanum* species, with one or more REs. RFLP probes were chosen based on their known map locations in tomato (Tanksley et al. 1992) and *S. sitiens / S. lycopersicoides* (Pertuzé et al. 2002), as well as the level of polymorphism that they provided. RFLP analysis, including DNA isolations, digestions, electrophoresis,

blotting and hybridizations were performed as previously described (Pertuzé et al. 2002). DNA was digested with the following restriction enzymes: *Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I, *Dra*I and *Pst*I. Each *S. sitiens* derivative was genotyped at a minimum of 48 RFLP loci distributed amongst all 12 chromosomes. Each informative marker was polymorphic between the *Lycopersicon* and the *Solanum* species, and in most cases also distinguished between *S. sitiens* and *S. lycopersicoides* (Fig. 2). In addition, a subset of plants were scored for up to 11 isozyme markers, according to previously described protocols (Chetelat et al. 1997).

Results

Synthesis of aneuploid and diploid derivatives

The allotetraploid *L. esculentum* × *S. sitiens* hybrid ($L^eL^eS^sS^s$) was crossed as pollen parent to a *S. lycopersicoides* sesquidiploid ($L^eL^eS^l$) to obtain a putative *S. sitiens* sesquidiploid ($L^eL^eS^s$, plant 90L4190-1) by embryo culture (Fig. 1). This plant was the source of all other *S. sitiens* derivatives. Initial chromosome counts by acetocarmine staining indicated plant 90L4190-1 was approximately triploid (2n, ≅36). Since three species were involved in its generation, genomic in situ hybridization (GISH) was used to distinguish chromosomes of *L. esculentum* from those of the two *Solanum* spp., and to determine the precise chromosome number (see Fig. 3). The results of GISH on meiocytes at diakinesis or metaphase indicated this plant had 24 *Lycopersicon* and 14 *Solanum* chromosomes (2n+14=38). From the pedigree of 90L4190-1, the origins of these nightshade chromosomes could be inferred: a full set of *S. sitiens* chromosomes transmitted from the $L^eL^eS^sS^s$ allotetraploid, plus two *S. lycopersicoides* chromosomes inherited from the $L^eL^eS^l$ sesquidiploid. RFLP analysis confirmed this hypothesis and permitted identification of each extra *S. lycopersicoides* chromosome (see below).

This 2n+14 plant was used as the female parent to generate additional aneuploids with reduced numbers of chromosomes by pollination with *L. pennellii*-derived bridging lines. A total of more than 8,000 flowers were pollinated, yielding five plants by embryo culture (Table 1). Only one of these progeny (plant 93L9463-3,

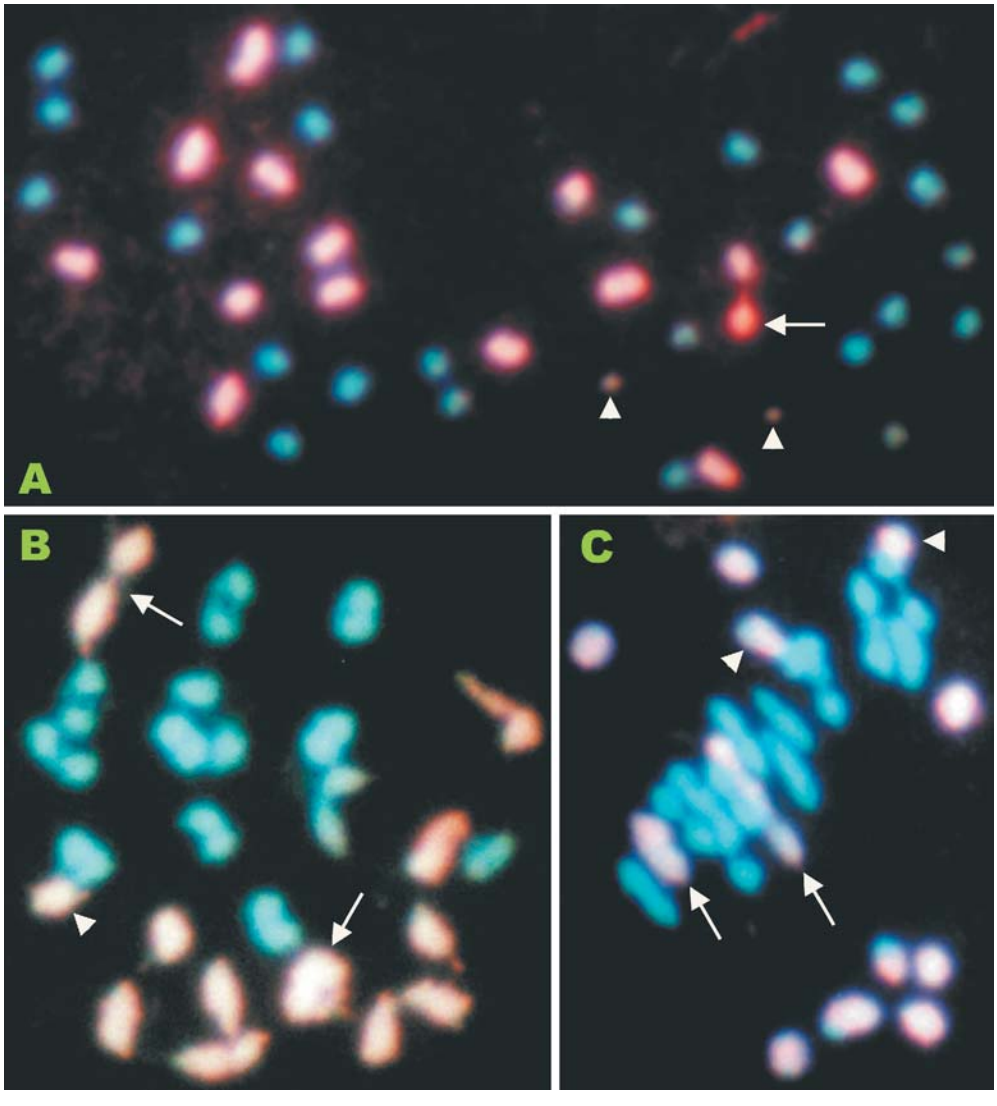

Fig. 3A–C Genomic in situ hybridization of a putative sesquidiploid plant (90L4190-1) showing its chromosome constitution. **A** Mitotic chromosome spread showing 14 *Solanum* (red) and 24 *L. esculentum* (*blue*) chromosomes, with the nucleolar organizers (NOR) marked (*arrow* for *Solanum*, arrowheads for *L. esculentum*). **B** Meiotic cell at diakinesis in which the 14 *Solanum* chromosomes (*red*) form two bivalents (*arrows*), one trivalent involving the *L. esculentum* homeologues (*arrowhead*), and nine univalents. **C** Meiotic cell at metaphase-I in which the 14 *Solanum* chromosomes are present as two bivalents (*arrows*), two trivalents with their respective *L. esculentum* homeologues (*arrowheads*), and eight univalents

Table 1 Results of pollinations and embryo culture of aneuploid *L. esculentum* × *S. sitiens* hybrids. Intergeneric hybrids were pollinated with *L. pennellii*-derived bridging lines, pure *L. pennellii*, or pure *L. esculentum*

Female parent	Male parent (generation)	Flowers pollinated	Fruit harvested	Embryos cultured	Viable plants	Plants/ 100 poll's
90L4190-1	Bridge line (BC_5F_2)	7,146	1,235	231	4	0.06
(2n+14)	Bridge line (BC_5F_3)	29	6	0	0	0.00
	Bridge line (BC_6F_3)	320	85	3	0	0.00
	Bridge line (BC_3)	652	232	22	1	0.15
		8,147	1,558	256	5	0.06
90L4190-1	*L. pennellii*	319	131	36	7	2.19
93L9463-3	Bridge line (BC_5F_2)	155	89	24	4	2.61
(2n+9)	Bridge line (BC_5F_3)	92	21	8	2	2.17
	Bridge line (BC_6F_3)	82	6	1	1	1.22
	Bridge line (BC_3)	268	66	56	27	10.07
		597	182	89	34	5.70
98L8983-1 (2n+8)[a]	*L. esculentum*	73	17	1	1	1.37

[a] Approximate chromosome number

2n+9) survived long enough to produce additional derivatives. The 2n+9 plant was more fecund, yielding a total of 34 plants from about 600 pollinations with the *L. pennellii*-derived bridging lines (Table 1), constituting most of the progeny analyzed in the present experiments. Among the progeny of 93L9463-3 was another higher-order aneuploid (2n+8, 98L8983-1), from which additional progeny were obtained.

The pollen viability of these three higher-order aneuploids was so low (2.5%, 0.0% and 12% PV for the 2n+14, 2n+9 and 2n+8 plants, respectively), that they could only be used as female parents. As a result, the *L. pennellii*-derived bridging lines were required to overcome unilateral incompatibility in all but one of them (98L8983-1). The greater fecundity of the 2n+9 than the 2n+14 aneuploid may have been due to greater female fertility or a weakened incompatibility response in the former. To distinguish these possibilities, the female fertility of 90L4190-1 was evaluated by pollination with pure *L. pennellii* (Table 1). This wild species was assumed to have the maximum number of compatibility factors (i.e., the greatest bridging ability) required to overcome the unilateral incompatibility of this 2n+14 hybrid. The yield of progeny per pollination was approximately 2% using pure *L. pennellii* (Table 1). In contrast, the *L. pennellii*-derived bridging lines yielded progeny at a rate of only 0.06%, which suggests that the bridging lines did not carry all the necessary pollen-compatibility loci.

The bridging lines had been selected for their ability to cross to F_1 *L. esculentum* × *S. lycopersicoides* and derivatives in early backcrosses from *L. pennellii* to *L. esculentum*. Selection for compatibility with *S. sitiens* derivatives was only performed in the last backcross-generation, by which time one or more compatibility gene(s) may have been eliminated. In support of this hypothesis, earlier generation (BC_3) bridging plants selected for compatibility with 90L4190-1 resulted in a higher-progeny yield (Table 1).

In addition to incomplete pollen compatibility, a low rate of embryo survival reduced the efficiency of these crosses. A total of nearly 350 embryos were rescued from these three aneuploids following pollination with bridging lines or *L. esculentum*. Of these, only 40 viable plants were recovered, a success rate of only about 12%. This low yield was apparently due to a strong tendency of cultured embryos to abort in vitro, rather than a lack of culturable embryos. A large number of cultured embryos failed to develop—many were abnormally small or deformed, or were surrounded by a degenerate endosperm. Most embryos that did produce viable plants were well-developed, later-stage embryos (i.e., walking stick or later) at the time of culture.

Ploidy and fertility of *S. sitiens* derivatives

Chromosomes counts were performed on most of the progeny of 90L4190-1 and 93L9463-3 (Table 2). For some plants only approximate chromosome numbers could be determined by cytology, either because they produced too few flowers, were sterile, or the right meiotic stages were not observed. For several plants, chromosome numbers were inferred from RFLP marker genotypes and/or morphology. Most of the derivatives originated from the 2n+9 aneuploid, and therefore had nine or fewer *Solanum* chromosomes. Diploids were the most common category (37% of all derivatives), followed by the trisomics (26%), with higher numbers of extra chromosomes becoming increasingly rare (Table 2).

Fecundity of the derivatives was inversely correlated with the chromosome number (Table 2). In general, a greater number of extra *Solanum* chromosomes was associated with lower pollen fertility and seed production. Diploids had the highest pollen fertility (average 57.6% PV), although some were quite sterile, followed by 2n+1 (34.5%) and 2n+2 (18%). As a result of their low pollen viability, the aneuploids generally failed to produce seeds from self pollinations. Fortunately, a majority of the

Table 2 Ploidy, chromosome pairing, and fertility of individual *S. sitiens* derivatives. Plant numbers of parental hybrids are underlined and progeny indented to indicate their relationships. Ploidy values are based on chromosome counts, supplemented by marker data and morphology in some cases. Pairing configurations represent the average number of univalents (I), bivalents (II), and trivalents (III) observed in the indicated number of pollen mother cells (PMCs). PV, % pollen viability. Seed set is from self-pollination or backcrossing (BC) to male *L. esculentum*, unless otherwise noted

Plant #	Ploidy	Average Pairing Configuration	# PMCs	PV (%)	Seed Set	
					Self	BC
90L4190-1	2n+14	13.3II+9.9I+0.5III	132	2.5	No	No
99L1120-1	2n+8[a]	–	–	–	No	No
93L9463-3	2n+9	–	–	0.0	No	No
98L8983-1	2n+8[a]	–	–	11.5	No	Yes
99L1094-1	2n+8[a]	6.0II+2.0I+6.0III	9	3.8	No	Yes
00L3196-8	2n+3–4[b]	–	–	–	No	No
00L3196-1	2n+2–3[b]	–	–	7.5	No	Yes
00L2568-5	2n+2	11.2II+1.2I+0.8III	6	39.6	No	Yes
00L2568-4	2n+2	11.3II+1.3I+0.7III	3	21.6	No	Yes
99L1137-1	2n+2	12.0II+2.0I	1	7.5	No	Yes
00L3076-2	2n+2	11.0II+1.0I+1.0III	1	2.4	No	No
99L1118-1	2n+1	12.0II+1.0I	3	78.4	Yes	Yes
00L2648-3	2n+1	10.0II+2.0I+1.0III	3	60.3	Yes	Yes
00L2568-2	2n+1	11.5II+1.1I+0.3III	11	18.9	No	No
00L2568-3	2n+1	12.0II+1.0I	3	18.3	Yes	Yes
00L3196-7	2n+1	11.6II+0.6I+0.4III	7	15.4	No	No
00L3074-1	2n+1[b]	–	–	7.0	No	No
95L2026-1	2n+1	–	–	–	Yes	Yes
99L1239-1	2n	12.0II	14	85.7	Yes	No
00L2568-1	2n	11.8II+0.5I	4	85.6	Yes	No
00L3196-6	2n	12.0II	10	75.8	Yes	Yes
00L3196-4	2n	12.0II	6	74.2	Yes	Yes
00L3196-5	2n	12.0II	1	69.9	Yes	Yes
00L3073-1	2n	–	–	43.0	No	Yes[c]
00L3076-5	2n	12.0II	3	29.0	Yes	Yes
99L1138-2	2n[b]	–	–	28.3	No	Yes
99L1138-1	2n	11.8II+0.4I	15	12.3	Yes	Yes
95L2026-2	2n	–	–	–	No	Yes

[a] Approximate number
[b] Based on marker analysis and/or parental genotypes only
[c] Backcrossed as male parent to *L. esculentum*

aneuploids had sufficient female fertility to produce seed following pollination with *L. esculentum* or the bridging lines.

Marker analysis and chromosome transmission

More than 700 probe × RE combinations (148 probes and 5–6 REs) were analyzed to find polymorphisms among *L. esculentum, L. pennellii, S. sitiens* and *S. lycopersicoides*. Almost all probes differentiated the *Lycopersicon* from the *Solanum* spp. with at least one of the REs analyzed, but only a subset of these probes were polymorphic between the two *Lycopersicon* (77%) or the two *Solanum* (53%) congeners. Three-way polymorphisms, which distinguished the *Lycopersicon* spp. from *S. sitiens* and *S. lycopersicoides* were even less common (23% of probes). Based on this survey information, the *S. sitiens* derivatives were genotyped with RFLP markers, and a few isozymes and morphological loci, spanning most of the genome (Figs. 2 and 4). The marker genotypes indicated which *Solanum* chromosomes or segments thereof were transmitted to each progeny, and their species of origin.

GISH analysis of the 2n+14 derivative (90L4190-1) showed consistent bivalent formation between two pairs of *Solanum* chromosomes, as well as ten *Solanum* univalents (Fig. 3). Therefore, this plant was expected to show marker genotypes indicating a total of 14 *Solanum* chromosomes in addition to a complement of 24 *L. esculentum* chromosomes. The results of the marker analysis (Fig. 4) were consistent with this prediction, and indicated a complete set of 12 *S. sitiens* chromosomes and two intact *S. lycopersicoides* chromosomes (numbers 4 and 10). Additionally two *L. esculentum* chromosomes had recombinant *S. lycopersicoides* segments.

Marker analysis and chromosome counts of 93L9463-3 suggested this plant was trisomic for chromosomes 1, 2, 3, 4, 5, 7, 8, 9 and 10 (Fig. 4). Intact *S. sitiens* chromosomes 1, 2, 3, 7 and 8 were indicated by the marker data. All the other extra chromosomes (4, 5, 9 and 10) were recombinant. In addition, chromosome 12, present in the disomic condition, was also recombinant for *S. sitiens* and *L. esculentum* segments.

Among all the descendents of the 2n+14 plant, *S. sitiens* chromosomes 1, 2, 3, 7, 8 and 12 were transferred intact to at least one derivative, in most cases along with segments from other chromosomes (Fig. 4). Chromosomes 4, 5, 9, 10 and 12 were represented only as recombinant segments of different sizes. Only *S. sitiens* chromosomes 6 and 11 were not transmitted in any form to the progeny. *S. sitiens* markers from chromosome 2 were transmitted at a much higher rate (69%) than those on all other chromosomes. Considering both the *S. sitiens* and the *S. lycopersicoides* alleles together, more than 80% of the progeny showed *Solanum* markers on chromosome

Fig. 4 Graphical genotypes of *S. sitiens* derivatives based on marker analysis. Plant numbers of parental hybrids are underlined and their progeny are indented to indicate their relationships. Estimates of ploidy are based on chromosome counts and/or marker data. Chromosomes are oriented with the short arm to the *left*. The order and approximate positions of marker loci used in this study are represented in the header row. The subset of markers scored in each plant are indicated by *tick marks* on the corresponding chromosomes. *L. esculentum* chromosomes, if present, are shown below any *Solanum* or recombinant chromosomes. In a given marker interval, the genotype of each plant is *color-coded* according to the schema presented in the legend

2. Also remarkable was the high level of recombination on this chromosome evident in the progeny genotypes: only one plant (00L3076-5) inherited an intact *S. sitiens* chromosome 2. Furthermore, the latter plant was diploid, indicating the *S. sitiens* chromosome had substituted for one of its *L. esculentum* homeologues, probably as a result of pairing and chiasma formation.

S. sitiens chromosomes 1 and 12 were also transmitted at relatively high frequencies: about 30% of the progeny carried some *S. sitiens* alleles on each of these chromosomes. Chromosome 1 was transmitted more often as recombinant segments than as the parental chromosome. In the case of chromosome 12 (and number 5), the 2n+9 plant carried a recombinant *S. sitiens* segment on one arm of the chromosome; this segment was transmitted intact, undergoing little further recombination in the progeny. *S. sitiens* chromosomes 3, 7 and 9 were transmitted to approximately 20% of the derivatives, both in the form of recombinant segments and intact chromosomes. Chromosomes 8 and 10 were transmitted at a low rate (13%), the former always intact, and the latter chromosome always recombinant for *S. lycopersicoides* and *S. sitiens* segments.

Trisomics

Among the *S. sitiens* derivatives, seven putative trisomic plants were identified by chromosome counts and morphology (Table 2). Only two plants (00L2568-2 and 95L2026-1) carried intact extra *S. sitiens* chromosomes (numbers 1 and 8, respectively) and hence constituted monosomic alien addition (MA) lines. MA-1 was highly sterile and incompatible with *L. esculentum* pollen, and could not be transmitted via seed. Fortunately, a recombinant diploid (00L3073-1) carried a nearly intact *S. sitiens* chromosome 1, and could be backcrossed to *L. esculentum* (however only as the male parent, see below). Many other trisomics carried recombinant *Solanum* chromosomes, as well as additional *S. sitiens* markers elsewhere in the genome, primarily on chromosome 2.

Diploids

Of the nine putative diploids analyzed with RFLP markers, all but one carried some *S. sitiens* markers as a result of recombination or chromosome substitution (Fig. 4). In different proportions, *S. sitiens* chromosomes 1, 2, 3, 8 and 12 were represented among the diploids. In two plants (00L3076-5 and 99L1138-1), intact *S. sitiens* chromosomes 2 or 8 were substituted for one of the *L. esculentum* homeologues. As with the aneuploid progeny, alleles from *S. sitiens* chromosome 2 were transmitted far more than any other, being present in five out of the eight diploid plants. The average pollen viability of the diploid plants was higher than for any of the aneuploid derivatives and all produced seed, either by backcrosses to *L. esculentum* or by selfing (Table 2).

Genetic basis of bridging ability

Almost all the *S. sitiens* derivatives carried *L. pennellii* alleles near previously mapped pollen-compatibility loci on chromosomes 1, 6 and 10 (Chetelat and DeVerna 1991). *L. pennellii* markers on chromosomes 1 and 6 were transmitted to all the progeny of the 2n+14 and 2n+9 hybrids, suggesting that loci on these chromosomes were required to overcome unilateral incompatibility of the *S. sitiens* hybrids. In addition, *L. pennellii* markers on chromosomes 7 and 10 were transmitted at a high rate, indicating that important compatibility factors are also located on these chromosomes. However, some *L. pennellii* alleles may have been transmitted through the female parent, independent of selection for bridging ability, because the 2n+9 hybrid was already heterozygous for *L. pennellii* alleles on several chromosomes. As a result, homozygosity was observed for *L. pennellii* markers among some progeny of this *S. sitiens* hybrid. Plants with fewer chromosomes from either of the *Solanum* species tended to have a weakened unilateral incompatibility, and most produced backcross seed following pollinations with pure *L. esculentum* (Table 2). The only plants that produced neither self nor backcross seeds were those that had three or more *Solanum* chromosomes (or segments thereof), or plants that carried *S. sitiens* markers near the *S* locus on chromosome 1 (Fig. 2).

A monosomic addition for chromosome 8

To study the transmission of the extra *S. sitiens* chromosome in more-advanced backcross progeny, a monosomic alien addition line for chromosome 8 (MA-8) was backcrossed as female parent to *L. esculentum.* Sixty backcross plants were genotyped with three RFLP markers (TG176, TG624 and TG294) covering the *S. sitiens* chromosome. The RFLP data revealed eight putative trisomic plants (13.3%) that were heterozygous for all three *S. sitiens* markers and showed morphological features of *S. lycopersicoides* MA-8 (Chetelat et al. 1998): white, dialytic anthers (genes *Wa* and Dl^s), exserted stigmas, leaves curved downward at the margins and small, whitish-green fruits. The trisomic nature of these plants was confirmed by relative-band intensity on autoradiograms and chromosome counts on selected individuals (data not shown). All other plants were presumably diploid, and no plants with recombinant segments were found.

Chromosome pairing in diploids and aneuploids

The frequency of various chromosome pairing configurations was estimated for many of the derivatives (Table 2). Though based on a limited number of meiocytes in some cases, the results suggest a relatively normal meiosis in the diploids, whereas substantial

pairing failure was observed in the aneuploids. In the former, chromosomes at diakinesis or metaphase-I of pollen mother cells tended to form 12 bivalents. This included diploids with relatively large chromosome segments from *S. sitiens* (e.g., 00L3076-5 and 99L1239-1). However, occasional univalents were detected in two diploid progeny, one of which contained a substituted *S. sitiens* chromosome 8 (99L1138-1), the other a large introgression on chromosome 12 (00L2568-1). Chromosomes of the trisomics formed primarily 12 bivalents and one univalent at meiosis. However, many also formed trivalents at a substantial rate, indicating recombination between homeologous chromosomes. Meiosis in the higher-order aneuploids (2n+2 or greater) was disrupted to a greater extent, with fewer bivalents, and more univalents and trivalents observed.

Discussion

The present study represents the first successful gene transfer from *S. sitiens* to cultivated tomato. This was achieved through use of a pseudo-sesquidiploid (2n+14) plant carrying a full set of *S. sitiens* chromosomes (plus 2 from *S. lycopersicoides*) in the background of *L. esculentum*. Analysis of meiosis in this intergeneric hybrid using GISH cytology showed that chromosomes of the two-nightshades pair readily with one another, and less frequently with their *L. esculentum* homeologues. The same tendency for preferential pairing among homologous chromosomes was observed in the allotetraploid hybrid ($L^eL^eS^sS^s$, DeVerna et al. 1990), as well as in true sesquidiploids representing *S. lycopersicoides* ($L^eL^eS^l$, Rick et al. 1986). From the latter hybrids, we obtained monosomic additions (Chetelat et al. 1998) and recombinant diploids (Rick et al. 1986), suggesting that similar stocks might be synthesized for *S. sitiens*.

Unilateral incompatibility

Strong unilateral incompatibility (UI) observed in the 2n+14 hybrid towards the pollen of *L. esculentum* necessitated use of bridging lines derived from *L. pennellii*. UI is generally observed in crosses between self-incompatible species (i.e., *S. sitiens* or its hybrids in this case) used as female parents, and related self-compatible taxa (i.e., *L. esculentum*) used as pollen donors (see review by De Nettancourt 1977). Incompatibility can be avoided in such combinations by using the self-compatible species as female parent. However, in the present study, pollen viability of the 2n+14 hybrid and many of its aneuploid derivatives was so low that they were not functional pollen parents. Therefore, the only means of producing progeny was to develop suitable bridging lines.

However, the bridging lines used in this study were only partially compatible with the 2n+14 hybrid. Whereas the yield of progeny using the backcrossed bridging lines was quite low, pollinations with pure *L. pennellii* were much more successful. Therefore, female fertility of the 2n+14 hybrid did not appear to be the limiting factor in determining success of this cross. The use of earlier generation *L. pennellii*-derived bridging lines increased the progeny recovery rate, presumably because they contained a greater number of pollen-compatibility factors. Noteworthy in this regard was the high transmission rate observed for *L. pennellii* markers on chromosome 7. This may indicate a compatibility locus important in crosses to *S. sitiens* hybrids that was not detected in our earlier studies with *S. lycopersicoides* (Chetelat and DeVerna 1991). Therefore, selection of more-effective bridging lines in early backcross generations could substantially improve prospects for gene transfer from *S. sitiens* to tomato.

UI was weakened or eliminated in most of the derivatives with fewer extra chromosomes. This will simplify elimination of residual genetic material from *L. pennellii*, since backcrosses to *L. esculentum* are productive, even in derivatives with relatively low male fertility. However, UI persisted in 2n and 2n+1 plants (00L3073-1 and 00L2568-2, respectively) containing most or all of *S. sitiens* chromosome 1, which includes the *S*-locus controlling specificity of the self-incompatibility system (Tanksley and Loaiza-Figueroa 1985). Similarly, the *S. lycopersicoides* monosomic addition for chromosome 1 was also incompatible with *L. esculentum* pollen (Chetelat et al. 1998). In the case of the diploid derivative (00L3073-1), pollen fertility was sufficient to allow backcrossing as male parent to *L. esculentum*. However, this solution would not be effective for the trisomic individual (00L2568-2), since male transmission of extra chromosomes is relatively low in tomato due to strong gametophytic selection against n+1 pollen (Khush 1973).

Aneuploidy

Progeny from the 2n+14 hybrid included aneuploids with up to nine extra chromosomes. This far-exceeds the maximum aneuploidy (2n+4) observed previously in a much-larger progeny array (459 plants) obtained from the *S. lycopersicoides* sesquidiploids (Chetelat et al. 1998). This discrepancy might be related to the slightly higher ploidy of the intergeneric hybrid representing *S. sitiens* (2n+14) than *S. lycopersicoides* (2n+12). Regular association in the former hybrid between the two pairs of nightshade chromosomes would result in more-frequent transmission than expected from a true sesquidiploid. However, this provides only a partial explanation, since most of the *S. sitiens* derivatives were descendants of the 2n+9 plant.

The relatively low success rate of crosses involving these *S. sitiens* hybrids implies a strong selection at pre-fertilization and/or post-syngamic stages. In this context, our results are all the more surprising, since selection would normally favor gametes or zygotes with fewer extra chromosomes. In the progeny of tomato triploids,

for example, a maximum number of three extra chromosomes was observed, and this category of aneuploids represented less than 1% of the total (Rick and Barton 1954). The fact that embryo rescue in the present experiments was performed relatively late in development may have allowed weak embryos (i.e., those with higher ploidy levels) to mature. This interpretation is consistent with our observations that most of the viable embryos were more advanced (walking sticks or later) at the time of culture.

Of the aneuploid progeny, the trisomics are potentially the most useful as they are more fertile and may include monosomic addition lines. The original goal was to produce a complete set of monosomic additions for each of the 12 *S. sitiens* chromosomes. However, the rate of plant recovery from the 2n+14 plant was extremely low. As a result, certain chromosomes were not transmitted. Although a greater number of progeny were obtained from the 2n+9 plant, it contained only a partial set of *S. sitiens* chromosomes. This further-limited opportunities for transmission of some chromosomes.

Intact *S. sitiens* chromosomes 1, 2, 3, 7, 8 and 12 were identified in the aneuploid progeny. Although all 2n+1 plants had *S. sitiens* markers on more than one chromosome, their relatively high fecundity should enable recovery of true monosomic additions in later generations. This has already been accomplished for MA-8, the first monosomic addition isolated in this study. In contrast, *S. sitiens* chromosome 1 could not be transmitted due to its pollen sterility and incompatibility. These results are identical to our experience with the *S. lycopersicoides* monosomic additions, wherein MA-8 was among the first to be recovered (DeVerna et al. 1987) and MA-1 was impossible to maintain for the same reasons (Chetelat et al. 1998).

In progeny of MA-8, the extra *S. sitiens* chromosome transmitted well but did not recombine. The frequency of trisomic progeny, though somewhat lower than observed for the corresponding *S. lycopersicoides* chromosome (13% vs 22%, Chetelat et al. 1998), was sufficient for maintenance purposes. However, no recombinant chromosomes were found, either among the diploid or the trisomic plants. Although the population size was not large (n=60), the results do suggest a much lower rate of recombination in the backcrossed monosomic addition than in the earlier generation aneuploids. A similar suppression of recombination following introgression into *L. esculentum* was observed for the *S. lycopersicoides* monosomic additions (Ji and Chetelat 2003). For this reason, selection for recombinant genotypes would be more efficient in the earliest descendants of the intergeneric hybrid.

Evidence of recombination

All but one of the diploids carried genetic material from *S. sitiens*, usually in the form of recombinant chromosome segments. Extensive recombination was also detected in the aneuploid progeny. The frequency of recombinant genotypes in these derivatives appears to be at least as high as that observed previously in similar stocks containing *S. lycopersicoides* chromosomes in tomato (Rick et al. 1988; Chetelat et al. 1998). Evidence for chromosome substitution was also detected, as one plant contained an intact chromosome 8. Heterozygous substitution lines of this type are also useful sources of recombinants. Since they lack homologous partners, homeologous chromosomes recombine at relatively high frequencies (Ji and Chetelat 2003).

Although recombination was detected on all chromosomes except 6 and 11, the frequency of recombinant genotypes was highest for chromosome 2. This could indicate a greater degree of homology between the genomes on this chromosome, or selection for recombinant gametes. However, a more-likely explanation is that the 2n+9 aneuploid, from which most of the derivatives were obtained, itself contained a recombinant chromosome 2 with a large *S. lycopersicoides* segment. As a result, this chromosome could pair and recombine in homologous regions on both the *L. esculentum* and the *S. sitiens* homeologues.

Future prospects

Finally, we conclude that prospects are good for transmission of a wide range of variation found in *S. sitiens*. Despite the relatively limited number of progeny obtained, our results demonstrate the feasibility of gene transfer via aneuploids and recombinant diploids. With additional crosses and marker-assisted selection, a more complete series of monosomic additions could be obtained. Such pre-bred stocks would provide a convenient means for preserving the genome of *S. sitiens* in a more accessible form, for chromosomal assignment of dominant genes identified in the nightshade, and eventual transfer of useful traits into horticultural tomatoes. From progeny of the recombinant diploids, homozygous introgression lines (ILs) could be selected. Libraries of ILs have already been synthesized for the *L. pennellii* and *L. hirsutum* genomes (Eshed and Zamir 1995; Monforte and Tanksley 2000), and have many advantages for breeding and genetic studies (Zamir 2001). Recent synthesis of an IL library for *S. lycopersicoides* (Chetelat and Meglic 2000; Canady 2002) suggests similar genotypes for *S. sitiens* would be viable. Thus, our results so far bode well for expanding the gene pool of cultivated tomato to include this interesting desert nightshade.

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