Comparative Oligo-FISH Mapping: An Efficient and Powerful Methodology To Reveal Karyotypic and Chromosomal Evolution

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ABSTRACT Developing the karyotype of a eukaryotic species relies on identification of individual chromosomes, which has been a major challenge for most nonmodel plant and animal species. We developed a novel chromosome identification system by selecting and labeling oligonucleotides (oligos) located in specific regions on every chromosome. We selected a set of 54,672 oligos (45 nt) based on single copy DNA sequences in the potato genome. These oligos generated 26 distinct FISH signals that can be used as a "bar code" or "banding pattern" to uniquely label each of the 12 chromosomes from both diploid and polyploid (4× and 6×) potato species. Remarkably, the same bar code can be used to identify the 12 homeologous chromosomes among distantly related *Solanum* species, including tomato and eggplant. Accurate karyotypes based on individually identified chromosomes were established in six *Solanum* species that have diverged for >15 MY. These six species have maintained a similar karyotype; however, modifications to the FISH signal bar code led to the discovery of two reciprocal chromosomal translocations in *Solanum etuberosum* and *S. caripense*. We also validated these translocations by oligo-based chromosome painting. We demonstrate that the oligo-based FISH techniques are powerful new tools for chromosome identification and karyotyping research, especially for nonmodel plant species.

KEYWORDS chromosome identification; karyotype; oligo-FISH; chromosome painting; translocation

THE karyotype of a eukaryotic species represents the number, size, and shape of all chromosomes in the nucleus. Karyotype has long been used as the most general description of the basic genetic makeup of individual eukaryotic species. In most lineages, closely related species share a similar karyotype. For example, gorilla (*Gorilla gorilla*) diverged from the human/chimpanzee (*Pan troglodytes*) lineages >10 MYA and human and chimpanzee have been separated by 7–8 MY (Langergraber *et al.* 2012). These three species, however, have maintained a similar karyotype, except that human chromosome 2 was fused from two different chromosomes, resulting in the reduction of chromosome number from $2n = 48$ in chimpanzee and gorilla to $2n = 46$ in humans (Jauch *et al.* 1992).

Karyotype analysis relies on the identification of individual chromosomes and has been a challenge for most nonmodel plant and animal species, especially those with polyploidy and/or those with a large number of small chromosomes. Chromosome banding and fluorescence *in situ* hybridization (FISH) were two milestone techniques in the history of chromosome identification and karyotype analysis. Unfortunately, only a few plant species with large chromosomes have benefited from the chromosome banding techniques (Friebe *et al.* 1996). G-banding, which is commonly used in

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karyotyping in mammalian species, does not generate bands on chromosomes from most plants (Greilhuber 1977; Anderson *et al.* 1982); while FISH can be universally applied in plant species (Schwarzacher *et al.* 1989; Lim *et al.* 2000; Mandakova *et al.* 2010; Szinay *et al.* 2012; Weiss-Schneeweiss and Schneeweiss 2013). Various types of DNA probes can be used in FISH, including repetitive DNA sequences (Mukai *et al.* 1993; Fransz *et al.* 1998; Kato *et al.* 2004) and large-insert genomic DNA clones (Jiang *et al.* 1995; Dong *et al.* 2000; Kulikova *et al.* 2001; Kim *et al.* 2002). However, it is often a major challenge to establish a FISH-based chromosome identification system in a nonmodel species because of the lack of chromosome-specific DNA probes. Although karyotypes have been described in many plant species, individual chromosomes were not identified in most of these reported karyotypes. Such karyotypes, therefore, are not comparable among related species and cannot be used for evolutionary studies.

The Solanaceae is an important plant family comprising >3000 species. One of the genus, *Solanum*, contains several major food crops, including potato, tomato, and eggplant. Solanaceae species were derived ~40 MYA from an ancestral diploid species with $2n = 24$ chromosomes. Nearly all diploid family members have maintained this chromosome number (Wu *et al.* 2006). However, this identical basic chromosome number does not indicate maintenance of genomic synteny of the 12 homeologous chromosomes among the solanaceous species. Although both potato and tomato genomes have been sequenced (The Potato Genome Sequencing Consortium 2011; The Tomato Genome Consortium 2012), the karyotypes, genomes, and their evolution in other solanaceous species are largely unknown.We developed a novel chromosome identification system using solanaceous species as a model. We selected a set of 54,672 oligonucleotides (oligos) from the single copy sequences associated with 26 specific chromosome regions in the potato genome. These oligos were massively synthesized *de novo* in parallel and were labeled as FISH probes (Han *et al.* 2015). The pooled oligos produced 26 distinct FISH signals, which can be used as a "bar code" or a "banding pattern" to identify all 12 potato chromosomes. Strikingly, this bar code has been maintained among distantly related *Solanum* species, including tomato and eggplant, which diverged from potato ~5–8 and 15 MYA, respectively (Y. Wang *et al.* 2008; Wu and Tanksley 2010; Sarkinen *et al.* 2013). Modifications to this bar code in different species can be inferred as potential rearrangements of the associated chromosome(s) during evolution. We demonstrate that the oligo-FISH-based techniques are powerful new tools for chromosome identification and karyotyping research in nonmodel species.

Materials and Methods

Plant materials

Seven diploid species were used in FISH mapping, including the doubled monoploid *Solanum tuberosum* Group Phureja clone DM1-3 516 R44 [doubled monoploid (DM)], *S. bulbocastanum* (PI 498223; Oaxaca, Mexico), tomato (*S. lycopersicum*) variety Micro Tom, *S. etuberosum* (E genome, PI 558306; O'Higgins, Chile), *S. melongena* (eggplant) (PI 665010, cultivar Black Beauty), *S. caripense* (PI 243342, Costa Rica), and pepper (*Capsicum annuum* var. annuum ACE F1). Tetraploid potato cultivar "Katahdin" and hexaploid species *S. demissum* (PI 225711; Boyaca, Colombia) were also used in FISH mapping.

Oligo-FISH probe design

The oligo probes were designed using Chorus software (https://github.com/forrestzhang/Chorus) with only minor modifications (Han *et al.* 2015). Briefly, the repetitive sequences in the potato genome (The Potato Genome Sequencing Consortium 2011; Hardigan *et al.* 2016) were filtered and remaining sequences were then divided into oligos (45 nt) in a step size of 5 nt. Each oligo was aligned to the potato genome to filter out those with duplicates in the genome (>75% similarity over all 45 nt). Oligos within the centromeric regions (Gong *et al.* 2012) were also excluded. Oligos with dTm >10 [dTm = melting temperature (Tm) − hairpin Tm] were kept to build a probe database. Oligo sequences that were homologous to the tomato genome were preferentially selected for chromosome painting probes. We adjusted the number of oligos across the chromosomes to ensure that the painting probes produce uniform signals on the entire chromosomes. For the bar code oligo probes, we first selected target regions with a relatively high density of oligos based on the density distribution profile on the entire chromosome. We then selected oligos that show >90% homology with tomato sequences. The oligos were synthesized by Arbor Biosciences (Ann Arbor, MI) were labeled following published protocols (Han *et al.* 2015).

Oligo-FISH

To prepare mitotic metaphase chromosomes, root tips were harvested from greenhouse-grown plants and treated with nitrous oxide at a pressure of 160 psi (~10.9 atm) for 20–50 min. The root tips were then fixed in fixative solution (3 ethanol:1 acetic acid) and kept at −20°. An enzymatic solution with 3% cellulase (Yakult Pharmaceutical, Tokyo, Japan), 1.5% pectinase (Plant Media), and 1% pectolyase (Sigma Chemical, St. Louis, MO) was used to digest the root tips for 50 min at 37°, and slides were prepared using a stirring method. Briefly, root tips were put on a microscope slide and macerated with a needle in 20 μl of 45% acetic acid. Then, the suspension was spread with a needle on a hot plate at 50° for 2 min. Chromosomes were fixed by adding 200 μl of ethanol:acetic acid (3:1) fixative solution on a hot plate at 50° for 10 sec. Afterward, an additional 200 μl of ethanol: acetic acid (3:1) fixative solution was dropped on the tilted slide, which was dried at room temperature. Slides were also prepared using the dropping method (Kato *et al.* 2004) for chromosome painting experiments.

FISH was performed following published protocols (Dong *et al.* 2000). The hybridization mixture (500 ng of each labeled probe of single-stranded DNA, 50% formamide, 10% dextran sulfate, 2× SSC) was applied directly to denaturated chromosome slides and incubated for 2 days at 37°. Approximately 2000 ng of sheared genomic DNA (with average size of 100 bp) prepared from *S. etuberosum* and *S. caripense* was used as blocking DNA in chromosome painting experiments. The hybridization mixture for chromosome painting was denatured at 95° for 8 min and incubated at 37° for 2 hr before being applied to denatured chromosome slides. Biotin- and digoxigenin-labeled probes were detected by anti-biotin fluorescein (Vector Laboratories, Burlingame, CA) and anti-digoxigenin rhodamine (Roche Diagnostics, Indianapolis, Indiana), respectively. Chromosomes were counterstained with DAPI in VectaShield antifade solution (Vector Laboratories). FISH images were captured using a QImaging Retiga EXi Fast 1394 CCD camera attached to an Olympus BX51 epifluorescence microscope. Images were processed with Meta Imaging Series 7.5 software. The final contrast of the images was processed using Adobe Photoshop CS3 software.

Karyotyping

The short (S) and long (L) arms of individual chromosomes were measured from 10 complete metaphase cells for each species using the computer application MicroMeasure version 3.3 (Reeves and Tear 2000). The chromosomal arm measurements were used to calculate the total length of each chromosome ($tl = S + L$), total length of entire set of chromosomes ($TL = \sum tl$), arm ratio ($AR = L/S$) of each chromosome, and relative length of each chromosome ($RL = tl/TL \times 100$).

Synteny analysis of potato and tomato DNA sequence

Potato genome (V404) (The Potato Genome Sequencing Consortium 2011) and tomato genome (SL3.0) (The Tomato Genome Consortium 2012) were aligned using MUMmer 3 -(Kurtz *et al.* 2004). The parameters used for mummer were "-mum -n -c -b -l 30" and the parameters used for gaps were "-l 60 -f .12 -s 1000." Synteny blocks between potato and tomato genome were identified using DAGchainer (Haas *et al.* 2004) with parameters "-o -0f -e-2f -A 10." The positions of potato and tomato centromeres were determined as the major peaks of CENH3 chromatin immunoprecipitation-sequencing reads for each chromosome. For chromosomes with unassembled centromeric/pericentromeric sequences, the centromere positions were determined by analyzing the distribution of centromeric repeats, transposable elements, and sequencing gaps in the chromosomes.

Data availability

Supplemental Material, Table S1 in File S1 contains all information about the number and locations of oligos associated with each of the 26 individual FISH signals generated by the two bar code FISH probes. The Chorus software used for oligo-FISH probe design is freely available (https://github.com/forrestzhang/Chorus).

Results

Development of oligo-based FISH probes for chromosome identification in Solanum species

We developed two oligo-FISH probes: PB9446 (green) and PB8495 (red). These two probes contain 27,306 and 27,366 oligos (45 nt), respectively, and are derived from 26 different regions on the 12 potato chromosomes (Table S1 in File S1). These two probes were designed to produce 26 distinct FISH signals, which can be used as a bar code or banding pattern to uniquely label each of the 12 potato chromosomes (Figure 1). Each chromosomal region is covered by 2000–2250 oligos (Table S1 in File S1) that were selected using our oligo-FISH probe development pipeline (Han *et al.* 2015). The oligos were selected from single copy sequences in the potato genome (The Potato Genome Sequencing Consortium 2011; Hardigan *et al.* 2016). The oligos associated with each of 26 FISH signals spanned a genomic region ranging from 184 to 707 kb (Table S1 in File S1). Some chromosomal arms contained two signals, which were separated by at least 7 Mb (Table S1 in File S1) to ensure the separation of the two signals on the same arm.

A total of 54,672 oligos were included in the two probes. Sequence analysis showed that 33,911 oligos (62%) are associated with annotated potato genes, including 16,489 with coding sequences, 13,354 with introns, and 4068 with 5′ and 3′ UTRs. The remaining oligos were derived from intergenic regions. We analyzed the sequence similarity of these potato oligos with the tomato genome sequence (The Tomato Genome Consortium 2012). Only 3023 oligos (11%) were identical to the corresponding tomato sequences. In addition, 19,033 oligos (35%) showed one to four mismatches (>90% homology) with the tomato sequences.

Chromosome identification in diploid and polyploid potato species

The two oligo-FISH probes were labeled and hybridized to the somatic metaphase chromosomes prepared from *S. tuberosum* Group Phureja clone DM1-3 516 R44 ($2n = 2x = 24$) (DM), which is a homozygous clone and has been fully sequenced (The Potato Genome Sequencing Consortium 2011). The green and red FISH signals derived from the two probes (Figure 2A) matched to the predicted patterns (Figure 1). The signals formed a bar code that uniquely labels the 12 chromosomes. Chromosome 2 is the only nucleolus organizer (Nor) chromosome in the potato genome (Dong *et al.* 2000). The 45S ribosomal RNA genes were located at the distal end of the short arm, which is distinctly decondensed and stained faintly by DAPI (Figure S1 in File S1). Karyotyping analysis revealed that most potato chromosomes are metacentric or submetacentric (except for chromosome 2) with an arm ratio ranging from 2.67 to 1.19 (Table 1). Chromosomes 1 and 2 (without

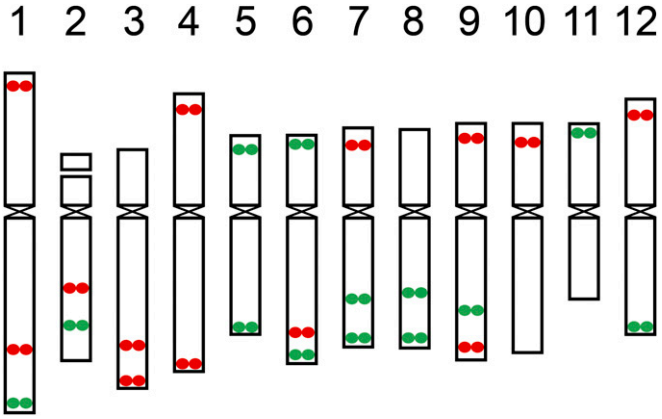


Figure 1 Predicted locations of the oligo-FISH signals on 12 potato chromosomes. Oligos were selected from a total of 26 chromosomal regions (13 red regions and 13 green regions). The 12 chromosomes can be distinguished from each other based on number and location of the red/green signals. The centromere positions on the 12 chromosomes in the potato reference genome were based on the locations of sequences associated with CENH3 nucleosomes (Gong *et al.* 2012).

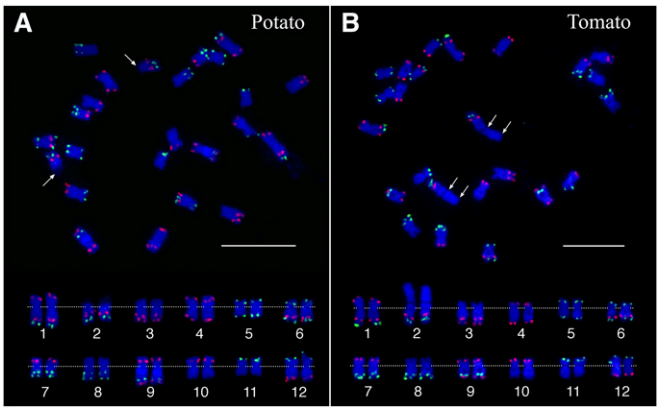


Figure 2 FISH mapping of potato and tomato chromosomes using two oligo-FISH probes. (A) FISH mapping of DM potato. Arrows point to the 45S rDNA regions associated with chromosome 2 (FISH mapping of the 45S rDNA on the same metaphase cell is shown in Figure S1 in File S1). The rDNA region is distinctly decondensed compared to the rest of the chromosome. (B) FISH mapping of tomato. The double arrows indicate the extent of the 45S rDNA regions (FISH mapping of the 45S rDNA on the same metaphase cell is shown in Figure S1 in File S1). The rDNA region is similarly condensed compared with the rest of the chromosome. The top panels show a complete metaphase cell from potato and tomato, respectively. Homologous chromosomes in the bottom panel were digitally excised from the same cells and paired. The centromeres of the chromosomes are aligned by a dotted line. Bar, 10 μm.

including the 45S rDNA region) represent the largest and smallest chromosomes, respectively (Table S2 in File S1).

We then performed FISH on cultivated potato (*S. tuberosum*, $2n = 4x = 48$), an autotetraploid species. We observed four identical copies of each of the 12 chromosomes from potato cultivar Katahdin (Figure 3A). *S. demissum* ($2n = 6x = 72$) was recognized as an allohexaploid species based on traditional chromosome pairing analyses of hybrids between *S. demissum* and various *Solanum* species (Matsubayashi 1991). The consensus conclusion from traditional cytogenetic studies was that *S. demissum* contains two similar genomes that differ from the third genome (Matsubayashi 1991). We identified 6 copies of each of the 12 potato chromosomes in *S. demissum* (Figure 3B). The FISH signal patterns from the six homologous/homeologous chromosomes were identical to those from DM potato. Interestingly, two of the six copies of chromosome 2 lack the 45S ribosomal gene arrays (Figure S2 in File S1).

Comparative karyotyping of potato and tomato

DNA sequence-based analysis suggested that tomato and potato have diverged for ~5–8 MY (Y. Wang *et al.* 2008; Sarkinen *et al.* 2013). Chromosome synteny between the potato and tomato has been well maintained based on comparative genetic linkage mapping and comparative cytogenetic mapping (Tanksley *et al.* 1992; Iovene *et al.* 2008; Tang *et al.* 2008; Gaiero *et al.* 2017). We conducted DNA sequence-based synteny analyses between the 12 pairs of pseudomolecules from potato and tomato genomes. Multiple inversions in different sizes were found to be associated with all 12 homeologous chromosome pairs (Figure S3 in File S1), which revealed abundant intrachromosomal rearrangements, but no interchromosomal arrangement, occurred during the divergence of these two species.

The two oligo-FISH probes generated an identical signal bar code on tomato and potato chromosomes (Figure 2). Two tomato chromosomes showed distinct morphology compared to the potato homeologues. The tomato 45S ribosomal RNA genes were also located at the distal region of the short arm of chromosome 2 (Figure 2B and Figure S1 in File S1). However, the 45S ribosomal DNA (rDNA) region was as condensed as the rest of the tomato chromosome 2 (Figure S1 in File S1), which was consistently observed in all metaphase cells. This unique condensation pattern of the 45S rDNA region makes chromosome 2 the longest chromosome in tomato (Figure 2B). Chromosome 4 from the two species showed a distinct difference in arm ratios. Potato chromosome 4 is a submetacentric chromosome with an arm ratio of 1.50; while tomato chromosome 4 appeared to be a subtelocentric (or acrocentric) chromosome with an arm ratio of 2.21 (Figure 2 and Table 1). At least two inversions in the long arms, each spanning several megabases of DNA, distinguished the two chromosomes (Figure S3 in File S1). By contrast, no inversion was detected in the short arms of the two chromosomes. It is not clear whether the different arm ratios of these two chromosomes were caused by an inversion that spanned the centromere of the chromosome in one species or by some other chromosomal rearrangement events.

Comparative karyotyping of Solanum species that are distantly related to potato

To reveal the karyotype evolution of the *Solanum* species, we performed comparative oligo-FISH in five additional species using the two probes developed in potato. These species have diverged variously from potato, including *S. bulbocastanum*

Table 1 Arm ratio of individual chromosomes in six *Solanum* species

Chromosome	*S. tuberosum* (potato)	*S. bulbocastanum*	*S. lycopersicum* (tomato)	*S. etuberosum*	*S. caripense* (tzimbalo)	*S. melongena* (eggplant)
1	1.80 ± 0.46	2.20 ± 0.38	1.57 ± 0.25	1.71 ± 0.63	2.56 ± 0.60	1.44 ± 0.27
2[a]	3.63 ± 0.61	3.94 ± 0.73	3.31 ± 1.37	2.89 ± 0.60	3.32 ± 1.13	2.58 ± 0.76
3	2.67 ± 0.49	2.29 ± 0.64	2.96 ± 0.49	1.76 ± 0.31	2.83 ± 0.68	1.37 ± 0.18
4	1.50 ± 0.22	1.64 ± 0.25	2.21 ± 0.37	1.21 ± 0.14	1.43 ± 0.43	1.46 ± 0.24
5	1.30 ± 0.13	1.32 ± 0.22	1.17 ± 0.12	1.25 ± 0.14	1.23 ± 0.24	1.35 ± 0.21
6	1.98 ± 0.29	1.78 ± 0.49	2.11 ± 0.34	1.63 ± 0.27	2.53 ± 0.64	1.63 ± 0.25
7	1.85 ± 0.32	1.75 ± 0.21	1.67 ± 0.32	1.27 ± 0.28	2.40 ± 0.69	1.20 ± 0.13
8	1.90 ± 0.25	2.04 ± 0.49	1.84 ± 0.38	2.37 ± 0.54	2.61 ± 0.55	1.14 ± 0.11
9	1.96 ± 0.29	1.55 ± 0.18	1.81 ± 0.29	1.26 ± 0.22	1.58 ± 0.48	1.47 ± 0.20
10	1.38 ± 0.18	1.50 ± 0.33	1.44 ± 0.20	1.51 ± 0.19	1.52 ± 0.32	1.48 ± 0.20
11	1.19 ± 0.15	1.16 ± 0.10	1.55 ± 0.17	1.17 ± 0.11	1.50 ± 0.34	1.22 ± 0.28
12	1.43 ± 0.27	1.28 ± 0.21	1.32 ± 0.21	1.53 ± 0.32	1.49 ± 0.65	1.12 ± 0.09

Measurement was conducted on each chromosomal arm in 10 metaphase cells.
[a] The 45S rDNA on the short arm of chromosome 2 was not included in the measurement.

(a wild species closely related to potato), *S. etuberosum*, *S. caripense* (tzimbalo), *S. melongena* (eggplant), and *C. annuum* (pepper), which are more distantly related to potato than tomato is to potato (Lou *et al.* 2010).

S. bulbocastanum: The FISH signals generated on *S. bulbocastanum* chromosomes were identical to those from potato (Figure 4). The arm ratio (Table 1) and relative length (Table S2 in File S1) of individual *S. bulbocastanum* chromosomes were also highly similar to the homeologous potato chromosomes.

S. etuberosum: *S. etuberosum* is a nontuberizing wild species that has been used in potato breeding due to its resistance to various potato diseases (Dong *et al.* 1999; Novy *et al.* 2002, 2007). Phylogenetically, *S. etuberosum* is more distantly related to potato than tomato is to potato (Lou *et al.* 2010). The FISH signals on most *S. etuberosum* chromosomes were identical to those on potato chromosomes. Chromosome 2 is the sole Nor chromosome (Figure S4 in File S1). However, signal modifications were observed on chromosomes 2 and 7. The long arm of chromosome 2 lost its distal green signal and gained an additional red signal ("b" in Figure 4). By contrast, the short arm of chromosome 7 lost its distal red signal but gained a green signal ("c" in Figure 4). A reciprocal translocation between chromosomes 2 and 7 would explain the observed FISH signal pattern changes (Figure 5A). The distal red signal on *S. etuberosum* chromosome 2 is more close to the end of the chromosome compared to the distal green signal on potato/tomato chromosome 2 (Figure 4). This can be explained by the fact that the green signal on chromosome 2 is 9.3 Mb away from the end, while red signal on chromosome 7 is only 4 Mb away from the end (Figure 5A). The other 10 *S. etuberosum* chromosomes showed a similar arm ratio and relative length to the homeologous potato chromosomes (Table 1 and Table S2 in File S1).

S. caripense: *S. caripense*, also known as tzimbalo, is an evergreen shrub native to South America and is grown for its edible fruit. The *S. caripense* chromosomes were visibly larger than potato chromosomes. Phylogenetically, *S. caripense* is more distantly related to potato than *S. etuberosum* is to potato (Lou *et al.* 2010). Overall *S. caripense* showed a similar karyotype as potato and tomato. However, we observed distinct FISH signal patterns on chromosomes 4 and 11, respectively. The red signal on the long arm of chromosome 4 was replaced by a green signal ("d" in Figure 4). On the other hand, the green signal on the short arm of chromosome 11 was replaced by a red signal ("e" in Figure 4). A reciprocal translocation between chromosomes 4 and 11 would explain this signal pattern change (Figure 5B). The rest of the *S. caripense* chromosomes showed a similar arm ratio and relative length to the homeologous potato chromosomes (Table 1 and Table S2 in File S1).

Eggplant (S. melongena): Eggplant diverged from a common ancestor of potato/tomato ~15.5 MYA (Wu and Tanksley 2010). The two oligo-FISH probes generated uniform but generally weak background signals on all eggplant chromosomes. Surprisingly, the patterns derived from the major FISH signals matched those from potato and tomato chromosomes (Figure 4). Eggplant chromosome 8 is a metacentric chromosome with an arm ratio of 1.14. However, chromosome 8 from the other five *Solanum* species have subtelocentric morphology with an arm ratio ranging from 1.84 to 2.61 (Figure 4 and Table 1). Since the two green signals on the long arm of chromosome 8 of *S. melongena* were clearly closer to the centromere than those on chromosome 8 of other *Solanum* species ("f" in Figure 4), chromosome 8 of *S. melongena* likely resulted from an inversion spanning the centromere, and a large fragment from the long arm was moved to the short arm due to the inversion. Similarly, a pericentric inversion is also likely involved in chromosome 10, which would explain the red signal at the distal region on the long arm ("g" in Figure 4), which is located on the short arms of chromosome 10 in other species (Figure 4).

Pepper (C. annuum): Pepper diverged from a common ancestor of potato/tomato ~19.6 MYA (Wu and Tanksley

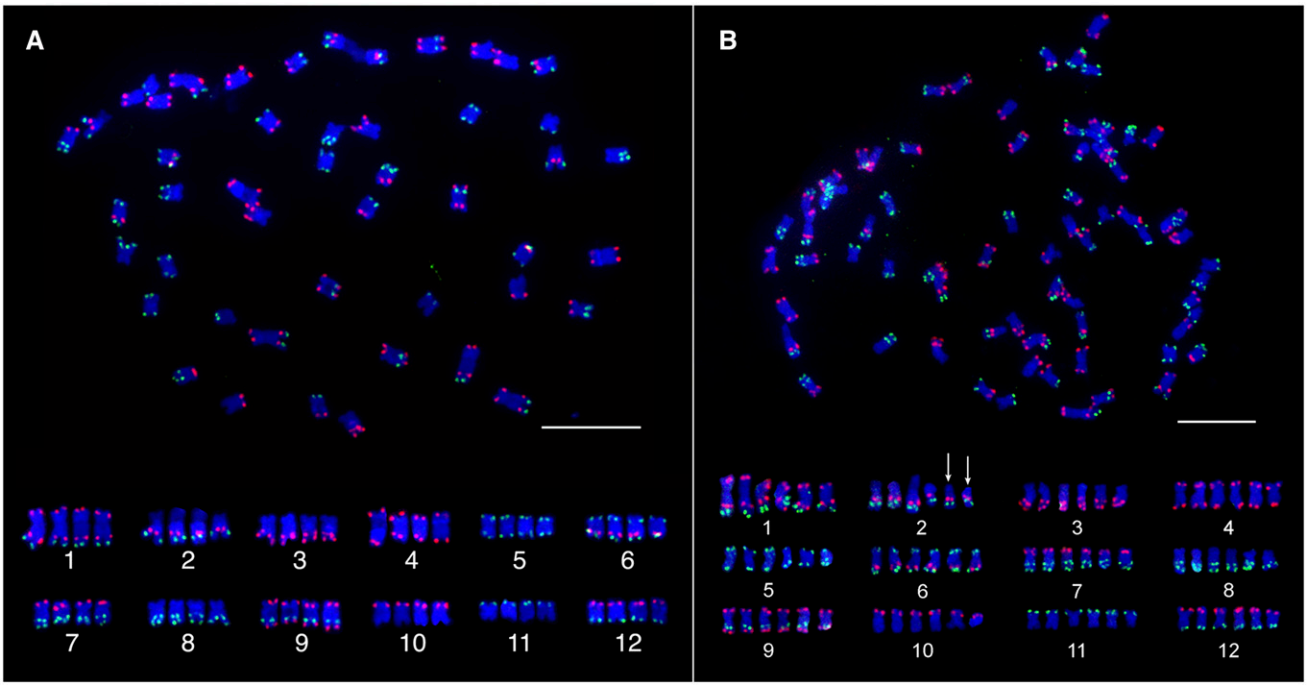


Figure 3 Chromosome identification in polyploid *Solanum* species. (A) Chromosome identification of potato cultivar Katahdin. The top panel shows a complete metaphase cell hybridized with two oligo-FISH probes. The bottom panel shows the 4 homologous chromosomes of each of the 12 potato chromosomes digitally excised from the same cell. (B) Chromosome identification in the hexaploid species *S. demissum*. The top panel shows a complete metaphase cell hybridized with two oligo-FISH probes. The bottom panel shows the 6 homologous chromosomes of each of the 12 potato chromosomes digitally excised from the same cell. The two arrows indicate the two copies of chromosome 2 that are not associated with 45S rDNA (FISH mapping of the 45S rDNA is showed in Figure S2 in File S1). Bar, 10 μm.

2010). The two oligo-FISH probes produced massive background signals on pepper chromosomes (Figure S5 in File S1). Punctuated major signals were observed on every chromosome. However, most of the pepper chromosomes cannot be unambiguously identified based on the signal patterns on potato chromosomes, suggesting that major structural arrangements have occurred between most potato and pepper chromosomes. The sizes of the pepper chromosomes appeared to be at least twice that of potato chromosomes. The current sequence assemblies estimate 3000 Mb for the pepper genome (https://www.ncbi.nlm.nih.gov/assembly, GCA_000512255.1), which is significantly larger than the potato genome (~800 Mb). These results suggest that the pepper genome has undergone major expansion and rearrangements during evolution.

Confirmation of interchromosomal translocation by oligo-based chromosome painting

We developed oligo-based chromosome painting probes to validate the interchromosomal translocations in *S. etuberosum* and *S. caripense*, which were predicted based on bar code FISH signal modifications relative to potato chromosomes (Figure 5). Oligos unique to a single potato chromosome were computationally identified and synthesized in parallel (Han *et al.* 2015). We selected 27,392 oligos for both potato chromosomes 2 and 7. The chromosome 7 probe generated uniform FISH signals on DM chromosome 7 (Figure 6A2). However, the chromosome 2 probe generated weak signals on the short arm and the proximal region on the long arm of DM chromosome 2 (Figure 6A3). These two probes, especially that for chromosome 2, generated very weak signals in the pericentromeric regions of chromosome 2 and 7 of *S. etuberosum* (Figure 6B3). This is likely caused by divergence of the DNA sequences located in the pericentromeric regions. Nevertheless, chromosome painting clearly showed that a small chromosome 7 segment was translocated to chromosome 2 (2^{7}). In contrast, a relatively large chromosome 2 segment was translocated to chromosome 7 (7^{2}) (Figure 6, B1 and B4). Thus, the chromosomal painting results matched to the predicted reciprocal translocation based on the modification to the bar code (Figure 5A).

Similarly, we developed painting probes for potato chromosomes 4 and 11, each containing 27,392 oligos. Both probes generated uniform FISH signals on DM chromosomes with only limited hybridization background (Figure 6, C2 and C3). The painting probes, however, produced unambiguous hybridization signals only at the distal ends of chromosomes 4 and 11 of *S. caripense* (Figure 6, D1 and D4). Only background-level FISH signals were detected in the pericentromeric regions of the homeologous chromosomes in *S. caripense* (Figure 6D3). Nevertheless, chromosome painting in *S. caripense* clearly revealed the reciprocal translocation between chromosome 4 and 11, resulting in chromosomes 4^{11} and 11^{4}, respectively (Figure 6, D1 and D4). The

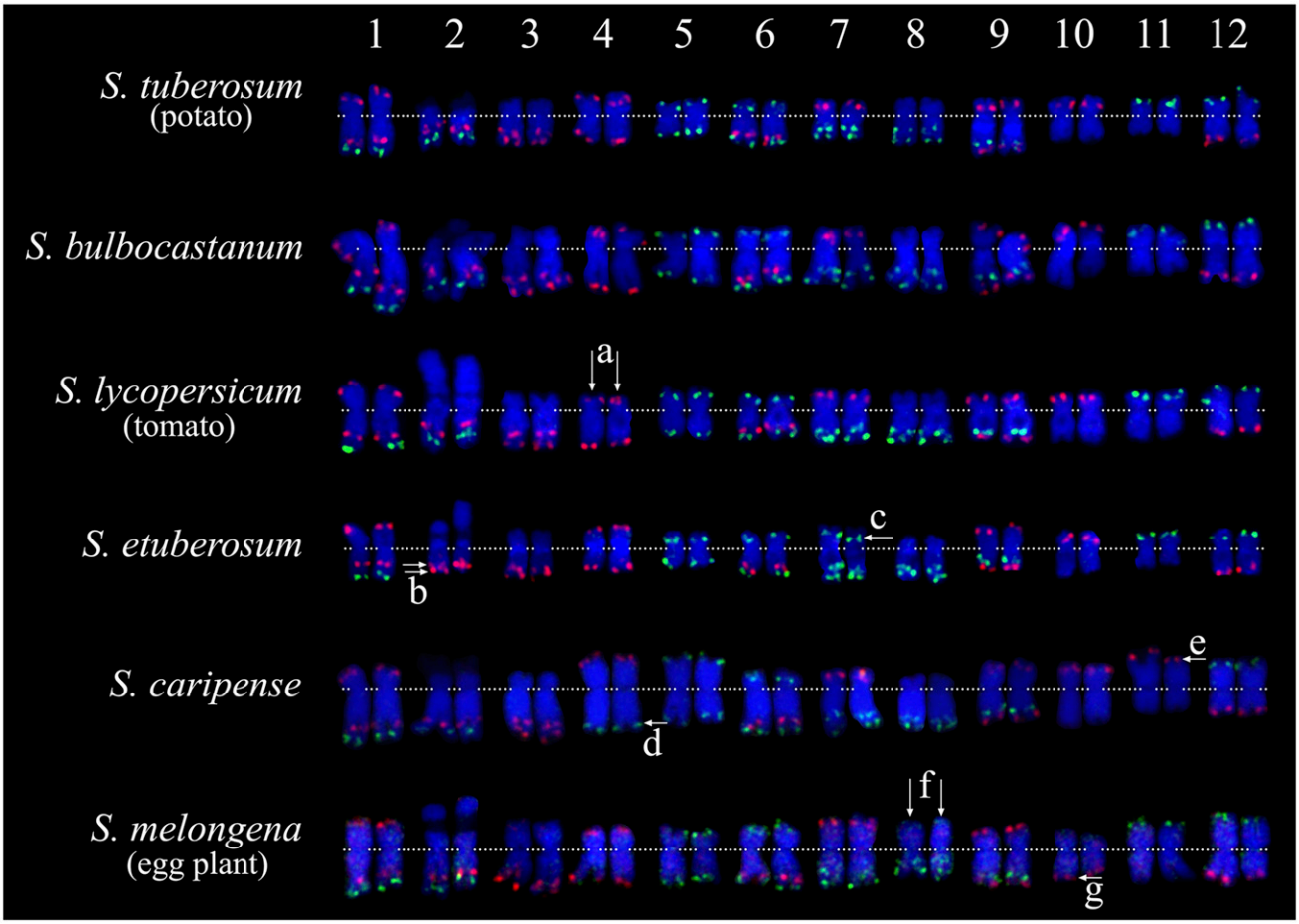


Figure 4 Comparative karyotyping of six diploid *Solanum* species. Chromosomes 1–12 from each species are arranged from left to right. Karyotypes of potato and tomato were developed from the same metaphase cells in Figure 2. Karyotypes of the remaining four species are developed from the same metaphase cells in Figure S4 in File S1. (a) Double arrows point to the two copies of tomato chromosome 4, which have a distinct arm ratio compared to chromosome 4 from other species. (b) Double arrows point to two closely linked red signals on *S. etuberosum* chromosome 2, the bottom red signal is predicted to be derived from the short arm of chromosome 7. For comparison, we used the karyotype of potato as our reference, see switches between red and green signals among these two species. (c) Arrow indicates the green signal on the short arm of *S. etuberosum* chromosome 7, which is predicted to be derived from the long arm of chromosome 2. (d) Arrow points to the green signal on the long arm of *S. caripense* chromosome 4, which is predicted to be derived from the short arm of chromosome 11. (e) Arrow points to the red signal on *S. caripense* chromosome 11, which is predicted to be derived from the long arm of chromosome 4. (f) Double arrows point to the two copies of eggplant chromosome 8, which have a distinctly large short arm compared to chromosome 8 from other species. (g) Arrow indicates the location of the red signal on the long arm of eggplant chromosome 10. This signal is located at the short arm of chromosome 10 from other species.

exchanged chromosomal segments from the two chromosomes showed a similar size (Figure 6D4). Thus, the chromosomal painting results in *S. caripense* also matched the predicted reciprocal translocation based on the modifications to the oligo-FISH bar code (Figure 5B).

Discussion

Oligo-FISH bar code: a new chromosome identification methodology

FISH is the most important technique for chromosome identification in plants (Jiang and Gill 1994, 2006). Repetitive DNA sequences were commonly used as probes in FISH-based chromosome identification (Mukai *et al.* 1993; Kato *et al.* 2004). However, it is often challenging to find a repeat that would produce distinct FISH signals on individual chromosomes in a plant species. More importantly, the FISH signals from repetitive DNA probes can potentially be highly polymorphic among different varieties and accessions, which may prevent consistent identification of individual chromosomes (Jiang and Gill 2006). Alternatively, large-insert genomic DNA clones, such as bacterial artificial chromosome (BAC) clones, can be used as FISH probes for chromosome identification (Jiang *et al.* 1995). However, this approach is dependent on the availability of a large-insert genomic DNA library as well as a major effort to isolate clones specific to every chromosome (Dong *et al.* 2000; Cheng *et al.* 2001; Kulikova *et al.* 2001; Kim *et al.* 2002; K. Wang *et al.* 2008). In addition, BACs from plant species with large and complex genomes often contain high proportions of repetitive DNA

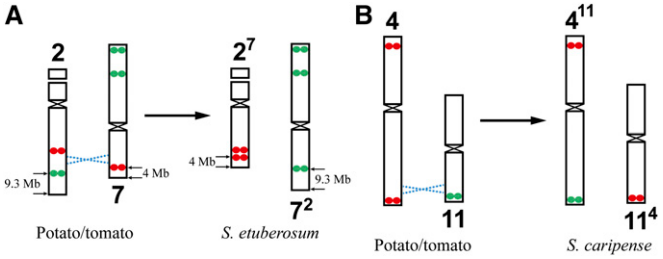


Figure 5 Predicted reciprocal chromosomal translocations identified in *Solanum* species. (A) A reciprocal translocation between chromosomes 2 and 7 in *S. etuberosum*. Chromosomes 2 and 7 from potato/tomato are hypothesized to be the ancestral types. A reciprocal translocation (dashed blue lines) is predicted based on the modifications to the oligo-FISH bar code, which result in the two translocation chromosomes 2^7 and 7^2, respectively, in *S. etuberosum*. (B) A reciprocal translocation between chromosomes 4 and 11 in *S. caripense*. The chromosomes 4 and 11 from potato/tomato are hypothesized to be the ancestral types. A reciprocal translocation (dashed blue lines) is predicted based on the modifications of the oligo-FISH bar code, which result in the two translocation chromosomes 4^{11} and 11^4, respectively, in *S. caripense*.

sequences and do not produce chromosome-specific FISH signals (Zhang *et al.* 2004; Janda *et al.* 2006).

We demonstrate that oligo-FISH bar codes provide a powerful and efficient technique for plant chromosome identification. It has several major advantages compared to the repeat- or BAC-based FISH probes: (1) Oligo-based FISH probes can be designed in any species with a sequenced genome, which has been demonstrated in several animal and plant species (Boyle *et al.* 2011; Yamada *et al.* 2011; Beliveau *et al.* 2012; Han *et al.* 2015). Thus, a single or few oligo pools can be designed to identify all chromosomes in a plant species with a sequenced genome. If the majority of oligos are associated with genic sequences, the same bar code can be expected from different varieties and accessions in the same species. (2) We demonstrate that a bar code probe can potentially be used to identify homeologous chromosomes among distantly related species, which allow for evolutionary studies. (3) Oligos can be selected from multiple regions from the same chromosome. Such a cocktail oligo probe will generate a unique hybridization pattern that resembles FISH signal patterns generated from multiple BACs derived from a single chromosome (Iovene *et al.* 2008; Szinay *et al.* 2008, 2012; Tang *et al.* 2008). An unlimited number of possible patterns can be designed for each chromosome. (4) Each oligo-based probe can be used for nearly 1,000,000 FISH experiments (Han *et al.* 2015). Thus, such bar code oligo-FISH probes are cost effective and can be maintained as a permanent resource.

The total number of FISH signals will be the most important factor in designing an oligo-FISH bar code. Oligos spanning 30–50 kb of single copy sequences can generate a strong FISH signal on metaphase chromosomes. However, it may be difficult to identify such long stretches of single copy sequences in some plant genomes. If multiple signals are designed on a single chromosome arm, the groups of oligos should be separated by a sufficient distance to ensure separate FISH signals. We demonstrate that 7 Mb is sufficient to consistently separate two FISH signals on potato metaphase chromosomes. However, a longer distance (>10 Mb) should be considered for plant species with chromosomes much larger than those of potato.

Chromosomal inversion and translocations in Solanum species

Chromosomal evolution of the solanaceous species has been investigated traditionally using pairwise comparative genetic linkage mapping (Wu and Tanksley 2010). Since genetic linkage maps and DNA markers were best developed in tomato (Tanksley *et al.* 1992), most of the pairwise mapping was performed between tomato and other solanaceous species, including potato, eggplant, pepper, and *Nicotiana* species (Bonierbale *et al.* 1988; Tanksley *et al.* 1992; Livingstone *et al.* 1999; Doganlar *et al.* 2002; Wu *et al.* 2009, 2010). Comparative FISH mapping has also been conducted among *Solanum* species using common sets of BACs isolated from potato or tomato (Iovene *et al.* 2008; Tang *et al.* 2008; Lou *et al.* 2010; Szinay *et al.* 2012; Gaiero *et al.* 2017). These comparative studies showed that inversions were the most common cause of chromosomal rearrangements among the solanaceous species. Translocations were also reported in some comparisons, for example, tomato and eggplant were found to differ by 24 inversions and 5 translocations based on eggplant linkage mapping using a set of 232 tomato-derived DNA markers (Wu *et al.* 2009).

The resolution of linkage mapping is restricted by the number of markers used. Genotyping or mapping errors, caused by wrong marker order or population size, may result in misidentified chromosomal rearrangements, such as inversion. In addition, population-based linkage mapping is an expensive and time-consuming approach; it has mostly been conducted in crops or economically important plant species. Although translocations were reported in some of the comparative mapping investigations among *Solanum* species, no cytological evidence was provided for any of the predicted translocations. For example, linkage mapping suggested that eggplant chromosome 5 is an equivalent of a fusion of the short arm of chromosome 5 with the long arm of chromosome 12 in tomato. Similarly, eggplant chromosome 11 is an equivalent of a fusion of the short arm of chromosome 11 with the short arm of chromosome 4 in tomato (Wu *et al.* 2009). However, our comparative oligo-FISH does not indicate whole-arm translocations associated with eggplant chromosomes 4, 5, 11, and 12 (Figure 4). We cannot exclude the possibility that the interchromosomal translocations are specific to the eggplant accession used by Wu *et al.* (2009). Thus, application of additional eggplant genotypes in oligo-FISH mapping may explain the discrepancy of results based on genetic linkage mapping and comparative oligo-FISH mapping.

It is intriguing that chromosomal inversions are highly common among the *Solanum* species (Figure S3 in File S1). By contrast, chromosomal translocations are relatively rare. Interestingly, we discovered reciprocal translocations in

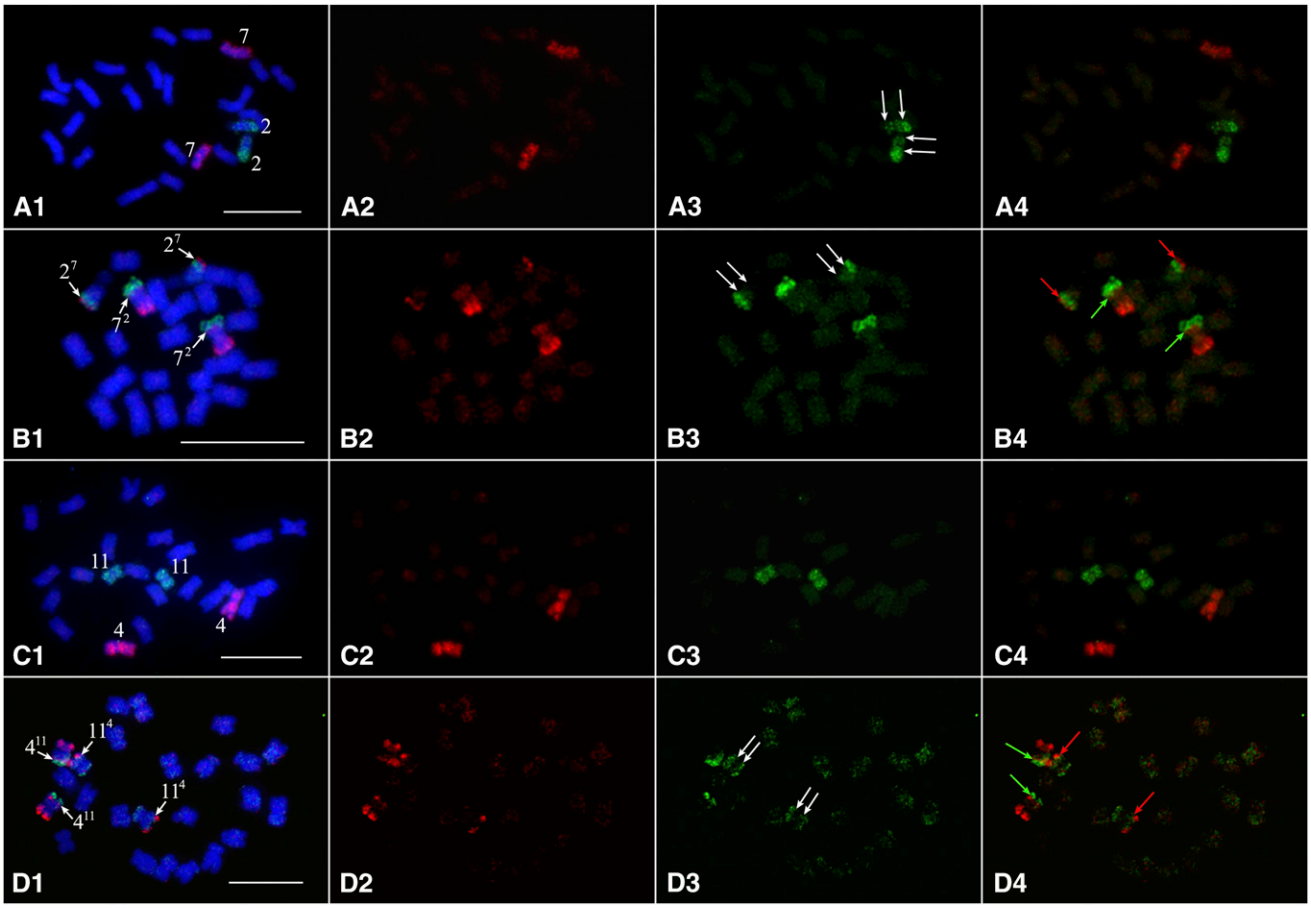


Figure 6 Validation of chromosomal translocations by chromosome painting. (A1–A4) Painting of chromosomes 2 (green) and 7 (red) of DM potato. Red (A2), green (A3), and both red and green (A4) fluorescence signals were digitally separated from (A1). Double white arrows in (A3) indicate relatively weak FISH signals that span the short arm and proximal region of the long arm of chromosome 2. (B1–B4) Painting of chromosomes 2 (green) and 7 (red) in *S. etuberosum*. Red (B2), green (B3), and both red and green (B4) fluorescence signals were digitally separated from (B1). Double white arrows in (B3) indicate very weak or background level FISH signals that span the short arm and proximal region of the long arm of chromosome 2. Red arrows in (B4) point to the breakpoint where a small chromosome 7 fragment attached to chromosome 2 (2^{7}). Green arrows in B4 point to the breakpoint where a large chromosome 2 fragment attached to chromosome 7 (7^{2}). (C1–C4) Painting of chromosomes 4 (red) and 11 (green) of DM potato. Red (C2), green (C3), and both red and green (C4) fluorescence signals were digitally separated from (C1). (D1–D4) Painting of chromosomes 4 (red) and 11 (green) in *S. caripense*. Red (D2), green (D3), and both red and green (D4) fluorescence signals were digitally separated from (D1). Double white arrows indicate background level FISH signals that span pericentromeric region of chromosome 11. Red arrows in (D4) point to the breakpoint where a chromosome 4 fragment attached to chromosome 11 (11^{4}). Green arrows in (B4) point to the breakpoint where a chromosome11 fragment attached to chromosome 4 (4^{11}). Bar, 10 μm.

S. etuberosum and *S. caripense*, and both are wild species. Strikingly, the oligo-FISH probes generated nearly identical signal patterns on chromosomes from potato and eggplant (Figure 4), which have diverged for ~15.5 MY (Wu and Tanksley 2010). A recent study in humans showed that a translocation can change the spatial position of the translocated chromosome fragment in the nucleus and, thus, alter the expression of the associated genes (Harewood *et al.* 2010). Since potato, tomato, and eggplant are crop species, selection in breeding practice may have eliminated chromosomal variants that may have negatively affected the fitness of the species due to the altered gene expression associated with the chromosomal rearrangement. Translocations have previously been reported to be rare in wheat cultivars but common in their wild ancestors (Badaeva *et al.* 1995). Analysis of the presence of the translocations in multiple populations of *S. etuberosum* and *S. caripense* will reveal if these chromosomal variants have been fixed in these wild species.

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Author contributions: J.J. conceived the research, G.T.B. and L.H. conducted FISH experiments. T.Z. and H.Z. designed oligo-FISH probes. K.S. and J.-M.R. synthesized probes and provided reagents. G.T.B., L.H., G.A.T., and J.J. analyzed data. J.J. wrote the article.

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