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Collinearity between potato (*Solanum tuberosum* L.) and wild relatives assessed by comparative cytogenetic mapping

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Abstract: A major bottleneck to Introgressive hybridization is the lack of genome collinearity between the donor (alien) genome and the recipient crop genome. Structural differences between the homeologs may create unbalanced segregation of chromosomes or cause linkage drag. To assess large-scale collinearity between potato and two of its wild relatives (*Solanum commersonii* and *S. chacoense*), we used BAC-FISH mapping of sequences with known positions on the RH potato map. BAC probes could successfully be hybridized to the *S. commersonii* and *S. chacoense* pachytene chromosomes, confirming their correspondence with linkage groups in RH potato. Our study shows that the order of BAC signals is conserved. Distances between BAC signals were quantified and compared; some differences found suggest either small-scale rearrangements or reduction/amplification of repeats. We conclude that *S. commersonii* and *S. chacoense* are collinear with cultivated *S. tuberosum* on the whole chromosome scale, making these amenable species for efficient introgressive hybridization breeding.

Keywords: multicolour BAC-FISH, macrosyteny, introgressive hybridisation, potato, *Solanum commersonii*, *Solanum chacoense*

Introduction

Potato (*Solanum tuberosum* L.) is the third most important food crop worldwide (after rice and wheat) and is number one among vegetables (Birch et al. 2012; Jansky et al. 2013). As with other crops, potato growers face severe yield losses by pests and diseases (Haynes and Lu 2005), encouraging breeders to select for traits that confer specific resistance and tolerance within the wild relative gene pool (Jansky 2000). Strategies have been developed to overcome hybridization barriers and perform the necessary manipulations to introgress such resistances into potato cultivars (Jansky 2000; Jansky 2006). However, only few resistance genes have been successfully introgressed into cultivars, leaving most wild resources to various pests and disease resistances untapped (Jansky 2000; Solomon-Blackburn and Barker 2001; Simko et al. 2009; Birch et al. 2012). One of the reasons for this underutilization is lack of knowledge on the physical (co-)localization of genetic loci between cultivated potato and its close relatives that are potential donors for introgressive hybridization breeding initiatives. If there are physical differences among the chromosomes of potato and its donor relatives they can hinder homoeologous recombination, cause problems in segregation during the meiosis of hybrids and/or produce linkage drag (Young and Tanksley 1989). Such changes hamper introgression of selected traits into the recipient crop rendering the ultimate aim unachievable (Verlaan et al. 2011).

Genome-wide collinearity within Solanaceae has been established mostly by comparative genetic analysis using high-density molecular marker maps. More recently, cytogenetic studies have explored the use of Fluorescence *in situ* Hybridisation (FISH)-based chromosome staining to establish structural and numerical comparisons of their karyotypes (Tang et al. 2008; Iovene et al. 2008; Szinay et al. 2008; Wu and Tanksley 2010; Anderson et al. 2010; Szinay et al. 2010; Lou et al. 2010; Verlaan et al. 2011;

Szinay et al. 2012). With the development of high-throughput DNA sequencing, genome assemblies for tomato (The Tomato Genome Consortium 2012), potato (The Potato Genome Sequencing Consortium 2011) and several of their wild relatives (Bolger et al. 2014; Aflitos et al. 2014; Aversano et al. 2015) have become available. Concerted genomics and bioinformatics efforts have improved genome assemblies (Sharma et al. 2013; Shearer et al. 2014). However, only a few studies have utilized sufficient high-quality physical maps needed to reveal structural differences related to introgression barriers (Peters et al. 2012; Aflitos et al. 2014; Aflitos et al. 2015; de Boer et al. 2015). Cytogenetic information obtained by high-resolution FISH maps on extended chromosomes can provide important information on structural rearrangements.

There is a long history to the study of potato chromosome morphology, with a starting point being the detailed descriptions of gross morphology of pachytene bivalents (Yeh and Peloquin 1965; Ramanna and Wagenvoort 1976) for dihaploid clones derived from different potato cultivars. Their studies enabled full identification of all chromosome pairs through morphological features such as length, centromere positions and heterochromatin patterns. The few discrepancies between their observations suggest intraspecific variation among different *Solanum tuberosum* genotypes (Yeh and Peloquin 1965; Ramanna and Wagenvoort 1976). However, since these studies are extremely laborious, such pachytene karyotypes are only available for one of the wild potato relatives, namely *Solanum clarum* (Marks 1969). Other types of structural genomic information for wild potato diploid species in Section Petota were initially obtained through meiotic pairing and fertility studies of their F1 hybrids. They share a common haploid genome (A), with the exception of species in series Etuberosa. Across most of the species, this A genome is only differentiated by cryptic variations, which are so small that pairing behaviour appears regular, or slightly more differentiated by

definite structural differences, that are evidenced by meiotic irregularities (Matsubayashi 1991). More detailed and higher resolution chromosomal studies are needed to look at the genomes of potato wild relatives and assess whether these differences will have an effect on chromosome pairing, recombination and segregation. Such data can be obtained using light microscopy of long pachytene chromosomes (Tang et al. 2008; Iovene et al. 2008; Lou et al. 2010; Verlaan et al. 2011; Szinay et al. 2012), by electron microscopy of synaptonemal complexes (Anderson et al. 2010) or by comparative chromosomal painting.

Fluorescence *In situ* Hybridization with Bacterial Artificial Chromosomes as probes (BAC-FISH) has proven to be a powerful diagnostic tool for high resolution genomic studies and introgression breeding (Verlaan et al. 2011). Various previously unknown inversions could be described in the *Solanum* crops using BAC-FISH analyses (Tang et al. 2008; Wu and Tanksley 2010; Szinay et al. 2010; Lou et al. 2010; Szinay et al. 2012). This strategy was also used to identify potato chromosomes using a set of 12 chromosome-specific cytogenetic DNA markers (CSCDM) selected from a *S. bulbocastanum* library (Dong et al. 2000) and also to explain the suppression of recombination of an introgressed segment harbouring resistance to TYLCV in tomato (Verlaan et al. 2011). Using multi-colour fluorescence microscopy it is possible to hybridize several probes in a single experiment, simplifying the examination of any *Solanum* species chromosome set to only a few experiments (Tang et al. 2009; Szinay et al. 2010). Moreover, hybridization under lower stringency conditions allows cross-species painting of tomato or potato probes to display homoeologous chromosomal positions in related *Solanum* species. For example, the RHPOTKEY BAC library was developed for diploid potato clone RH89-039-16 (Borm 2008) and anchored to AFLP markers in the ultrahigh density (UHD) genetic map (van Os et al. 2006). From this

library, 60 BACs were selected as a cytogenetic painting set with anchors for genetic map positions on the potato chromosomes, thus providing a useful tool to study collinearity between potato and its wild relatives (Tang et al. 2009).

For our study we focus on the diploid *Solanum commersonii* and *S. chacoense* ($2n = 2x = 24$), two potato relatives adapted to a variety of environmental and climate conditions (Hawkes 1990). Both are widely distributed in Brazil, Argentina and Uruguay, whereas *S. chacoense* is also distributed in Paraguay and Bolivia. Taxonomically they are included in section Petota, which comprises all tuber-bearing species including cultivated potatoes (Hawkes 1990). *Solanum commersonii* belongs to the potato tertiary gene pool, because it can be hybridized to potato through unreduced gametes and bridge crosses, whereas *S. chacoense* belongs to its secondary gene pool and can be directly crossed with tetraploid potato through unreduced gametes (Jansky 2006). Both species are excellent resources for potato breeding as they harbour resistances to many biotic stresses such as *Phytophthora infestans* (agent of late blight, the most important potato disease) (Micheletto et al. 2000), *Ralstonia solanaceum* (agent of bacterial wilt, the second most important potato disease) (González et al. 2013) and other severe potato pathogens, such as *Pectobacterium*, *Verticillium*, *Alternaria*, X and Y viruses (Laferriere et al. 1999). Additionally, they have been described as good reservoirs for broadening the genetic base to improve adaptation to cold and drought (Chen et al. 1999). A few accessions from these species have been used in potato introgression breeding (Carputo et al. 1997; Laferriere et al. 1999; Chen et al. 1999; Carputo et al. 2000; Chen et al. 2013; González et al. 2013). However, their full potential has not been realized due to lack of genetic and genomic information and the potential for homeologous incorporation without linkage drag. Although the genome sequence of *S. commersonii* has been published (Aversano et al. 2015), its fragmented assembly does

not allow robust structural comparisons with potato. The aim of this work was to build a cytogenetic map of *Solanum commersonii* and *S. chacoense* with respect to cultivated potato (*Solanum tuberosum* L.) to discover large-scale translocations and/or inversions, if any, that could hamper introgressive breeding using these wild relatives as donors and could assist in the assembly of their genome sequences.

Materials and methods

Plant material and slides preparation

We used three clones of *Solanum commersonii* (02.04.1 from Rocha, southeast Uruguay, 04.02.3 from Colonia, southwest Uruguay and 05.05.2.4 from Canelones, southern Uruguay) and one clone of *S. chacoense* (07.01.7 from Salto, northwest Uruguay). In addition, we studied two full-sib triploid interspecific hybrids (*S. commersonii* (2n) x *S. tuberosum* Group Phureja (n)) developed by González (2010). Flower buds containing anthers at meiotic prophase I were collected in the morning (11-12 am), fixed in absolute ethanol: acetic acid (3:1) for one day and then stored in 70 % ethanol at 4° C until further use. Pachytene spreads preparation followed the procedure described in (Szinay et al. 2008) with few minor modifications.

Probes, blocking and Fluorescence in situ Hybridization

We used the RHPOTKEY potato BAC library constructed from the RH clone RH89-039-16, previously selected by Tang et al. (2009), for each of the twelve potato linkage groups. BAC DNA was isolated with the QIAGEN (Valencia, CA) plasmid minikit and amplified with the REPLI-g minikit (QIAGEN, Valencia, CA). For direct labelling we used Cyanine 3-dUTP (Enzo Life Sciences), Cyanine 3.5-dCTP (GE Healthcare, Sweden) or Diethylaminocoumarin-5-dUTP (Perkin Elmer Inc), and for indirect

labelling biotin-16-UTP or digoxigenin-11-dUTP, all of them incorporated in the probe DNA by standard nick translation reaction (Roche Diagnostic, Indianapolis). Probes from the 45S rDNA from the pTa71 plasmid (Gerlach and Bedbrook 1979) and 5S rDNA from the pCT4.2 plasmid (Campbell et al. 1992) were used as hybridization controls. We performed FISH experiments as described previously (Zhong et al. 1996) with minor modifications. We carried out incubations of more than three days to obtain better hybridization of the BAC probe DNA. Hybridization of repetitive sequences in the BAC DNA was suppressed by adding unlabelled *Cot*100 (50x probe concentration) which was prepared from genomic *S. commersonii* and *S. tuberosum* DNA as described by Tang et al. (2008). To enhance hybridization efficiency, we carried out post-hybridization washes under low stringency conditions for 3 x 5 min in 20% formamide, 2x SSC at 42° C. Chromosomes were counterstained with 5 $\mu\text{g.mL}^{-1}$ DAPI in Vectashield anti-fade (Vector Laboratories).

Image acquisition and processing

We examined slides under a Zeiss Axioplan 2 imaging photomicroscope (<http://www.zeiss.com/>) with epifluorescence illumination and filter sets for 4',6-diamidino-2-phenylindole (DAPI), DEAC (blue), FITC (green), Cy3 (orange), Cy3.5 (red), and Cy5/Alexa Fluor 647 (far-red) fluorescence. Only late pachytene complements were considered. Selected images were captured using a Photometrics Sensys 1305 x 1024 pixel CCD camera (Photometrics, <http://www.photomet.com>). Image thresholding was performed using Genus image analysis software (Applied Imaging Corporation, <http://www.aicorp.com>). We performed image adjustments with Adobe® Photoshop® software as follows: DAPI images were displayed in light grey and sharpened using a 7 x 7 pixel Hi-Gauss high-pass spatial filter to accentuate minor details and the heterochromatin morphology of the chromosomes. The remaining

fluorescence images were pseudo-coloured in blue (DEAC), green (FITC), orange (Cy3), red (Cy3.5, Texas Red) and purple (Cy5), and overlaid in multichannel mode. Brightness and contrast adjustments were performed using the Levels tool in Adobe Photoshop affecting all pixels equally.

Distance measurements and analysis

We straightened the images of late pachytene chromosomes using ImageJ (<http://rsb.info.nih.gov/ij/plugins/straighten.html>) and the cubic spline interpolation straightening plug-in of (Kocsis et al. 1991). We measured the total length of pachytene chromosomes, the centromere index (length of the short arm/total chromosome length) $\times 100$ (Levan et al. 1964), the size of the pericentromere heterochromatin, the size and position of interstitial heterochromatin knobs and the relative position of BAC signals also using ImageJ. We used the chromosome numbering system employed by Tang et al. (2009), which correspond to the potato linkage groups based on the molecular markers assigned to the UHD genetic map (van Os et al. 2006). We also followed Tang et al. (2009) in the alignment of chromosomes in the karyotype. For chromosome pairs 5, 7, 10, 11 and 12, we aligned the short arms to the south arms in the genetic map, contrary to the convention. We measured bivalents from different cell spreads and slides for each chromosome pair. In the case of *S. commersonii*, we analysed bivalents coming from different individuals, to account for intraspecific variation. Average lengths and standard deviations were calculated for all measurements.

We calculated the cytological position of each BAC clone as $(S/T) \times 100$, where S is the distance (in μm) from the FISH site to the end of the short chromosome arm and T is the total length of the chromosome (in μm). Because no genetic map is available for either *S. commersonii* nor *S. chacoense*, we could not convert positions to fraction

lengths (FL) for a direct comparison with the physical positions of BACs found in Tang et al. (2009). Thus, we converted the data in their work to relative positions as stated above.

Results

Morphology of pachytene chromosomes in S. commersonii and S. chacoense

For each chromosome we measured the lengths in 2 to 12 different cell complements from *S. commersonii* and *S. chacoense* (see examples in Figure 1). Lengths ranged from $75.8 \pm 12.1 \mu\text{m}$ (chromosome pair 1, chr 1) to $40.2 \pm 5.6 \mu\text{m}$ (chr 11) in *S. commersonii* and from $66.5 \pm 12.7 \mu\text{m}$ (chr 6) to $37.5 \pm 6.0 \mu\text{m}$ (chr 10) in *S. chacoense*, from a total complement length of $660.9 \pm 116.0 \mu\text{m}$ and $602.7 \pm 113.1 \mu\text{m}$, respectively (Table 1). Centromeres were unambiguously identified as small and faintly DAPI fluorescing regions, flanked by brightly fluorescing heterochromatin on both sides. According to their centromere indexes (i), most chromosome pairs in *S. commersonii* and *S. chacoense* were median ($37.5 < i < 50$) and submedian ($25 < i < 37.5$), with only pairs 2 and 3 having a subterminal ($12.5 < i < 25$) centromere position. DAPI counterstaining provided clear distinction between the brightly fluorescing heterochromatin in the pericentromeres, NORs and distal heterochromatin blocks, and the weaker fluorescence of euchromatin. The size of the pericentromeres ranged from $16.2 \pm 3.7 \mu\text{m}$ (chr 8) to $31.3 \pm 6.7 \mu\text{m}$ (chr 2) in *S. commersonii* and from $17.0 \pm 5.5 \mu\text{m}$ (chr 3) to $29.3 \pm 12.8 \mu\text{m}$ (chr 1) in *S. chacoense*. Short stretches of euchromatin in the pericentromere heterochromatin were always noticeable on the long arms of chromosome pairs 1, 3 and 8 in *S. commersonii*, as well as interstitial heterochromatin knobs on the long arms of chromosome 6 and chromosome 7 for both species (Figure 3). The total area of heterochromatin comprises $45.8 \pm 10 \%$ of the genome of *S. commersonii* and $46 \pm 14 \%$

of the genome of *S. chacoense* (Table 1). Large BAC signals were observed on the pericentromere heterochromatin of chromosome pair 1 (hybridization from BAC RH158N18) pair 4 (hybridization from BAC RH097G17), pair 6 (hybridization from BAC RH069B12) and pair 12 (from BAC RH183I02) in both species, indicating the presence of repetitive sequences in those BACs complementary to the heterochromatin in those regions, whose hybridization was insufficiently suppressed by C_{0r} -100 DNA (Figure 2). Analysis of their sequence obtained from GenBank (<http://www.ncbi.nlm.nih.gov/nuccore/168804211>) or PGSC (http://solanaceae.plantbiology.msu.edu/data/Solanum_tuberosum.RH.bacs.zip) using Geneious R9 (<http://www.geneious.com/>, Kearse et al. 2012) revealed high content of repeats in these BACs. Distal heterochromatin blocks in both species were clearly distinguishable in all bivalents, showing brighter fluorescence and in most cases two separate dots, one for each of the homologs. The fluorescence was brighter in the distal end of short arms.

Comparative cytogenetic mapping

All BACs in this cross-species FISH showed clear signals in both species at the selected stringency level (see examples in Figures 1 and 2). We first checked that all BACs from one linkage group in potato hybridized to the same chromosome, meaning that each chromosome pair from *S. commersonii* or *S. chacoense* corresponded to one linkage group in potato and was equivalent to one of its chromosome pairs. We were also able to demonstrate that the order of BAC positions in both wild species corresponds to the genetic markers of the potato map (Table 2 and Figure 3). Chromosome pair 1 was found to harbour the 5S rDNA site on the short arm pericentromeric heterochromatin and chromosome pair 2 harbours the 45S rDNA cluster on the large satellite and NOR region in the short arm. Some BACs hybridized on the boundaries between euchromatin

and pericentromere heterochromatin, such as BAC RH092C10 on chromosome 1 of *S. commersonii* and BAC RH089A21 on chromosome 5 of *S. chacoense* (Figure 3), while BAC RH043B17 mapped on the boundary on the long arm of chromosome 12 for both species (see examples on Figures 1 and 2). BAC RH194M18 was located on the interstitial heterochromatic knob on the long arm of chromosome 6 in both species (Figure 3). It is worth noting that there is a clear double hybridization signal on chromosome pair 4 in *S. chacoense* – the blue signal from BAC RH097G17 is accompanied by a yellow signal from the distal BAC RH013K09 in all bivalents (Figure 2), pointing to the only rearrangement that may be observed in this species. The latter signal was not observed for *S. commersonii*.

In the pachytene complements of the two full-sib triploid CCT F₁ hybrids (*Solanum commersonii* x *S. tuberosum* Group Phureja) we observed that each BAC produced the expected hybridization signal on the same position of the *S. commersonii* homologues and potato homoeologues (Figure 4), although sometimes homoeologous pairing was broken and the two foci corresponding to the signals on the two homologs from *S. commersonii* were observed separately from the single focus on the *S. tuberosum* chromosome, assuming that it is the homoeologous chromosome that is failing to pair. More frequently, homoeologous pairing was discontinuous in between signals from contiguous BACs, although these pairing discontinuities were found all over the chromosomes (data not shown).

Physical distances and condensation patterns

Large variation was found in chromosome lengths across bivalents measured from different late pachytene complements (examples in Figure 2 and standard error bars in Figure 3). This variation was found both within and among individuals (from a variation

of 11.2 % of the total chromosome length in chr 10 to 22.3 % in chr 9 in *Solanum commersonii* and from 11.7 % of the total chromosome length in chr 3 to 34.1 % in chr 8 in *S. chacoense*, see Table 1). Variation was also found in the relative position of the different markers (from a lower variation of 0.4 % in the position of BAC RH013K09 on chr 4 to a higher variation of 8.3 % in the position of BAC RH122E19 on chr 8 in *S. commersonii* and from a lower variation of 0.6 % in the position of BAC RH049J10 on chr 10 to a higher variation of ± 16.8 % in the position of BAC RH092C10 on chr 1 in *S. chacoense*, see Table 2). Even though we expressed physical distances between BAC signals as relative distances, there were only slight differences among the species (Figure 3).

Discussion

Chromosome morphology is similar between potato and two diploid wild relatives

The overall chromosome morphology of both *S. commersonii* and *S. chacoense* wild species in late pachytene cells has been found highly similar to potato. Observed lengths varied considerably across observations in both species (see Table 1 and Figure 2). The extent of length variation of each chromosome in our study was similar to that reported in other studies working with late pachytene chromosomes in potato (Yeh and Peloquin 1965; Ramanna and Wagenvoort 1976; Iovene et al. 2008; Tang et al. 2009). The ordering of chromosomes according to average lengths in both species differed from that reported for potato (Tang et al. 2009), albeit the fact that the longest and shortest chromosomes in *S. commersonii* corresponded to the same chromosome pairs as in potato (chr 1 and chr 11, respectively). In *S. chacoense*, however, the longest and

shortest on average corresponded to chr 6 and chr 10, respectively (Table 1). Besides differences in condensation among replicates and studies, we expect that dissimilarities in total chromosome or chromosome arm length among closely related species result from changes in specific repetitive sequence distribution and abundance (Kubis et al. 1998; Sharma and Raina 2005).

The positions of the centromeres were very similar to those of potato (Yeh and Peloquin 1965; Ramanna and Wagenvoort 1976; Iovene et al. 2008; Tang et al. 2009) and the tuber-bearing *Solanum clarum* (Marks 1969). The characteristic pericentromere heterochromatin morphology in *S. commersonii* and *S. chacoense* (Figure 2 and Table 1) follows a similar pattern to that of cultivated potato (Tang et al. 2009), *S. clarum* (Marks 1969) and tomato (Chang et al. 2008). This brightly fluorescing pericentromere heterochromatin has been shown to match the distribution of hybridization with labelled C_{0t}-1000 in cultivated potato (Tang et al. 2014) and labelled C_{0t}-100 in tomato (Chang et al. 2008; Szinay 2010). Our measurements, however, indicate that there may be more heterochromatin in most chromosome pairs of both wild species (except for pairs 5 and 8), which is also seen in a higher relative heterochromatin percentage (Table 1) than in potato (Tang et al. 2009). Such differences in heterochromatin may evolve rapidly among related species and likely reflect large-scale variation in pericentromere-specific LTR retrotransposons (Rokka et al. 1998; Tek et al. 2005; Wang et al. 2006; Hemleben et al. 2007; Chang et al. 2008; Kejnovsky et al. 2012; He et al. 2013; Tang et al. 2014). Differences in the abundance of transposable elements causing intraspecific lack of collinearity in the pericentromere region, have also been directly observed for the heterochromatin haplotypes of RH (Group Tuberosum) and DM (Group Phureja) potato chromosome 5 in sequence based studies (de Boer et al. 2015).

Pericentromere heterochromatin blocks were sometimes interrupted by short stretches of euchromatin, as seen in the chromosomes 1, 3 and 8 for *S. commersonii*. Such uncondensed regions were previously described in potato (Yeh and Peloquin 1965; Ramanna and Wagenvoort 1976), *S. clarum* (Marks 1969), and in chromosome 6 of the tomato, which may have resulted from an inversion event between tomato and potato involving pericentromere heterochromatin and euchromatin (Iovene et al. 2008). A second, albeit different heterochromatin pattern was the long arm interstitial knob observed in chromosome 6 of both *S. commersonii* and *S. chacoense*. Comparable knobs in potato were previously mentioned (Ramanna and Wagenvoort 1976; Iovene et al. 2008), but were absent in the genotypes described in other studies (Yeh and Peloquin 1965; Tang et al. 2008), suggesting intraspecific variation between the cultivars. We also found such a knob in the long arm of chromosome 7 for both species, which so far was only reported for tuber-bearing *S. clarum* (Marks 1969). The nature of these interstitial knobs has been thoroughly described in tomato chromosome 7 as tandem arrays of the TGRI satellite repeat (Szinay 2010). Comparing the results of a BAC FISH and sequencing study, it was proposed that such interstitial islands of satellite repeats originated from other short arm telomere associated repeat arrays through transposition of extrachromosomal circular DNA during stages in which chromosomes are in the Rabl orientation (Szinay 2010).

The distal ends of the *S. commersonii* and *S. chacoense* chromosomes follows the typical morphology of compact telomere heterochromatin blocks as described for potato (Yeh and Peloquin 1965; Ramanna and Wagenvoort 1976; Tang et al. 2009), and are in general larger and more conspicuous at the short arms of the chromosomes. Sequence analysis of their repeats revealed the REP3 repeat, which is a member of the PGR1 family and 99% identical to the CL14 subtelomere repeat (Torres et al. 2011) and seems

specific to species of the potato clade (Tang et al. 2014). FISH of this REP3 probe on pachytene spreads from *S. commersonii* and the 3x hybrids demonstrated fluorescence foci on the distal ends of all short arms and a few of the long arms (data not shown), thus confirming the pattern in potato as described by Tang et al. (2014).

The BACs in our study were selected for their position in repeat-poor euchromatin regions in tomato and potato (Tang et al. 2009); yet some of them produced strong signals on the pericentromere heterochromatin in FISH experiments (Figure 1A, Figure 2). Such a repeat signal can be blocked in a FISH with excessive C_{0t}-100 but also results in overall hybridization and hence smaller / weaker fluorescence foci. It is likely that *gypsy*, *copia* and other LTR retrotransposable elements that are highly dispersed among stretches of single copy sequences.

***Solanum commersonii* and *S. chacoense* are highly collinear with potato**

Our study revealed a clear correspondence between the chromosomes of *Solanum commersonii* and *S. chacoense* to those in cultivated potato. Each chromosome pair from both wild species matches the corresponding chromosomes and linkage groups in potato and all BACs were in the same linear order (Figure 3). The distinct single foci with very strong signals obtained with most of the BAC probes in our cross-species BAC-FISH experiments, showed that genome differentiation among the three species at chromosomal scale is negligible if not absent at all. This is in line with the notion that diploid species in section Petota share a common genome A with only cryptic structural differences among species (Matsubayashi 1991). However, when comparing potato to species which are more distantly related such as tomato, eggplant or *S. tuberosum*, rearrangements are more prevalent (Tang et al. 2008; Iovene et al. 2008; Wu and Tanksley 2010; Lou et al. 2010; Szinay et al. 2012). In the tomato clade genome

differentiation seems to work differently, since many inversions have been described between cultivated tomato and its close relatives (Seah et al. 2004; Van Der Knaap et al. 2004; Anderson et al. 2010; Verlaan et al. 2011; Szinay et al. 2012; Aflitos et al. 2014) and shifts in centromere positions of homeologous bivalents have been observed in spread synaptonemal complexes of interspecific hybrids between tomato and wild relatives (Anderson et al. 2010).

Detection of smaller differences between homeologues are more difficult to demonstrate unequivocally. The small dissimilarities in neighbour BAC distances and the occurrence of a duplicated signal on chromosome pair 4 in *S. chacoense* might represent minor rearrangements or may point at local repeat dynamics across species in the euchromatic regions, where certain kinds of repetitive elements are more frequently located (Kidwell and Lisch 2001; Lamb et al. 2007; Kejnovsky et al. 2012 and references therein). Both explanations are congruent with the minor breaks in homeologous pairing observed in the triploid hybrids with potato. Fine-scale analysis of these regions requires a denser set of BAC probes (Tang et al. 2008; Iovene et al. 2008; Szinay et al. 2008; Achenbach et al. 2010; Verlaan et al. 2011), the use of smaller probes (Han et al. 2015; Aliyeva-Schnorr et al. 2016), advanced FISH with enhanced resolution and sensitivity (Kirov et al. 2015; Romanov et al. 2015; Khrustaleva et al. 2016) or comparative genomics including Mummer analyses (e.g., Peters et al. 2012).

Implications for breeding and genome evolution in Solanum section Petota

We have established cytogenetic maps for *Solanum commersonii* and *S. chacoense* using a reference set of five BACs per chromosome pair that had been selected and mapped on cultivated potato (Tang et al. 2009). No major rearrangements have been

found that would be expected to hamper recombination and introgression or that could cause linkage drag or missegregation of homoeologues during meiosis in hybrids. One possible exception may be the duplicated signal on chromosome 4 in *S. chacoense*. However, in general terms, our results support the interpretation that structural differences at the large-scale chromosomal level do not seem to underlie genome evolution in species belonging to section Petota, while they appear to be important across sections in the genus (Szinay et al. 2012) and within the tomato clade.

The correspondence found here supports the application of genetic tools like the UHD genetic map of potato to analyse these wild species. The cytogenetic maps and description of the chromosome morphology developed here are valuable tools to assist in improvement of the genome sequences of *Solanum commersonii* and *S. chacoense*, thanks to the identification of euchromatin and heterochromatin and other landmarks at the cytological level and the establishment of the relative positions and distances of BACs with known sequence.

The nature and extent of genome differentiation has not been an important barrier for hybridization among tuber-bearing *Solanum* species (Camadro et al 2004) making the vast potato secondary and tertiary genepool readily accessible for breeding. The possible effects of the minor differentiation between *S. commersonii* and *S. chacoense* suggested by our results will become evident when high-density genetic maps and genomic sequences become available for comparison in the near future.

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Figure captions:

Figure 1: Representative images of pachytene complements in *S. commersonii* (A) and *S. chacoense* (B) hybridized with BAC probes for chromosomes 5 and 12 (chr 5 and chr 12). The 45S rDNA and 5S rDNA probes were used as hybridization controls. Note the dispersed blue signal on the chromosomes in (A) indicating hybridization of repetitive sequences not blocked by C_{0r} -100. Scale bar = 10 μ m.

Figure 2: Examples of bivalents from chromosome 4 of *S. chacoense* and chromosome 12 of *S. commersonii*, with the colour scheme and the names of the five BAC clones used. Note the blue and yellow signals from BACs RH097G17 and RH013K09, respectively (yellow arrow), always map together in LG4 of *S. chacoense* and the blue and pink signals (blue and pink arrows) on the pericentromeric heterochromatin of chr 4 and chr 12 of *S. chacoense* and *S. commersonii* respectively, indicating that BACs RH097G17 and RH183I02 contained repeats that could not be blocked by C_{0r} -100 during the hybridization experiments. Scale bars = 10 μ m

Figure 3: Idiograms of the twelve pachytene bivalents of *S. tuberosum* (RH89-039-16), *S. commersonii* and *S. chacoense* hybridized with five BAC probes per linkage group in multicolour BAC-FISH experiments. The sizes and distances correspond to averages across cell complements measured and the bars represent the standard error for those measurements. The *S. tuberosum* idiogram was built based on the data presented by Tang et al. (2009). Scale bar = 10 μ m.

Figure 4: Examples of pachytene complements from two triploid interspecific hybrids (*S. commersonii* (2n) x *S. tuberosum* Group Phureja (n)) hybridized with five BAC probes belonging to a) chromosome 2 (chr 2), b) chr 5, c) chr 10, d) chr 11. The images

show breakpoints in homoeologous pairing (white arrows) which have been quantified and are not correlated with any specific physical location. Scale bar = 10 μm .

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Table 1. Absolute and relative length (with respect to total complement length), estimated DNA content, centromere index and heterochromatin content of the 12 chromosomes of *Solanum commersonii* (cmm) and *S. chacoense* (chc).

Chromosome ^a		1	2	3	4	5	6	7	8	9	10	11	12	Total
Absolute lenght (µm)	cmm	75.8±12.1	52.6±8.5	56.5±12.3	61.8±16.5	48.5±10.2	60.7±10.4	59.6±8.5	65.8±6.5	53.4±11.9	44.7±5.0	40.2±5.6	41.3±8.5	660.9±116.0
	chc	58.8±9.3	44.0±6.2	61.3±7.2	55.0±10.6	43.4±7.9	66.5±12.7	43.4±10.7	48.6±16.6	57.3±10.3	37.5±6.0	40.0±8.2	46.9±7.3	602.7±113.0
Relative lenght (%)	cmm	11.5±1.8	8.0±1.3	8.5±1.9	9.3±2.0	7.3±1.5	9.2±1.6	9.0±1.3	9.9±1.0	8.1±1.8	6.8±0.8	6.1±0.9	6.3±1.3	100
	chc	9.8±1.6	7.3±1.0	10.2±1.2	9.1±1.8	7.2±1.3	11.0±2.1	7.2±1.8	8.1±2.8	9.5±1.7	6.2±1.0	6.6±1.4	7.8±1.2	100
DNA content (Mb) ^b	cmm	95.2±15.2	66.1±10.6	71.0±15.5	77.6±20.8	60.9±12.8	76.3±13.0	74.9±10.6	82.6±8.2	66.9±15.0	56.1±6.3	50.5±7.0	51.9±10.6	830
	chc	60.1±9.6	45.1±6.3	62.7±7.4	56.3±10.9	44.5±8.1	68.1±13.0	44.4±11.0	49.8±17.0	58.7±10.6	38.4±6.2	40.9±8.4	48.0±7.5	617
Centromere index (%) ^c	cmm	23.0±4.9	13.7±3.6	18.9±2.6	37.8±9.8	41.0±7.0	21.1±5.0	31.9±5.6	33.3±4.6	37.4±4.5	32.1±7.6	49.6±6.9	44.3±10.2	
	chc	27.8±8.4	14.6±3.1	23.8±1.1	28.5±6.3	43.4±8.6	27.8±7.0	34.2±7.1	30.2±8.8	39.5±6.0	28.3±6.1	50.9±8.4	52.5±8.2	
Absolute pericentromere heterochromatin (µm)														
short arm	cmm	17.8±5.7	4.2±1.4	9.3±2.3	10.6±2.4	11.0±1.8	8.1±2.0	12.0±2.9	8.6±1.3	9.9±2.1	9.9±2.4	14.0±1.8	13.0±3.2	128.4±28.3
long arm		12.9±3.6	27.1±5.2	7.3±1.7	7.1±4.4	8.8±2.3	20.9±3.0	10.7±5.4	7.6±2.4	13.8±2.3	14.5±1.9	8.9±1.7	9.7±2.3	149.3±36.2
short arm	chc	12.6±5.8	3.7±0.8	9.5±2.1	4.4±0.9	7.7±2.0	12.0±4.1	9.1±1.6	7.7±3.4	8.3±2.8	7.3±0.3	11.9±2.9	15.2±2.9	109.4±29.6
long arm		16.7±7.0	22.9±2.5	7.6±3.4	19.6±2.8	11.7±2.3	15.9±6.7	9.5±2.2	13.1±4.2	17.2±3.8	13.5±3.1	8.3±2.5	8.0±1.8	164.0±42.3
Relative heterochromatin (%)	cmm	42.4±12.8	59.5±12.6	42.3±9.0	28.6±10.9	40.8±8.3	51.1±8.8	40.2±14.3	34.1±6.4	44.1±8.4	54.6±9.7	57.0±8.6	54.9±13.3	42.0±9.8
	chc	49.8±21.7	48.2±21.7	41.9±14.4	43.6±6.7	44.6±9.9	44.6±16.7	45.4±9.5	42.9±15.5	44.4±11.4	55.6±9.2	50.7±13.5	49.4±10.0	45.4±11.9

^aChromosomes were ordered and numbered according to their corresponding linkage groups (van Os et al. 2006).

^bEstimation of the DNA content of each chromosome was based on relative length and the assumption that (1) the haploid genome size for potato is 840 Mb, for *Solanum commersonii* is 0,85 pg = 830 Mb (Aversano et al., 2015) and for *S. chacoense* 0.63 pg = 617 Mb (Bennett et al., 1976); (2) the euchromatic and heterochromatic regions contain equal amounts of DNA (Mb/µm).

^cCentromere index was calculated as percentage of short arm/total chromosome length (Levan et al. 1964).

Table 2. Potato BACs used and their relative physical locations on *Solanum commersonii* (cmm) and *Solanum chacoense* (chc) chromosomes.

Chromosom e arm ^a	Selected BAC ^b	Relative physical location (%) ^c and number of measurements ^d	
		cmm	chc
1S	RH083J12	4.2±1.1 (4)	4.5±2.1 (5)
1S	RH078E17	8.0±3.7 (4)	8.7±3.4 (5)
1L	RH092C10	39.5±6.0 (4)	50.8±16.8 (5)
1L	RH158N18	55.2±4.7 (4)	69.6±14.3 (5)
1L	RH096H03	96.0±0.7 (4)	96.4±1.8 (5)
2L	RH075N07	25.2±1.5 (6)	28.3±9.1 (3)
2L	RH095G12	33.7±2.8 (6)	38.7±12.2 (3)
2L	RH174A15	44.0±3.3 (6)	54.8±8.0 (3)
2L	RH076J19	75.9±3.5 (6)	83.3±9.0 (3)
2L	RH055P13	80.6±3.4 (6)	89.7±8.0 (3)

3S	RH078O14	7.3±4.2 (4)	9.6±1.9 (4)
3S	RH159O01	10.8±4.5 (4)	12.8±1.9 (4)
3L	RH079E02	50.3±5.8 (4)	55.3±5.7 (4)
3L	RH074E07	66.0±6.1 (4)	64.8±6.9 (4)
3L	RH055M19	92.56±3.7 (4)	94.4±1.0 (4)
4S	RH013K09	4.9±0.4 (5)	5.6±2.7 (12)
4S	RH162B09	14.3±1.3 (5)	9.7±4.4 (12)
4S	RH097G17	26.6±2.7 (5)	17.9±4.7 (12)
4L	RH095G10	63.4±4.5 (5)	72.9±4.7 (12)
4L	RH191L17	88.7±6.3 (5)	90.9±3.3 (12)
5L	RH081B09	4.3±0.6 (7)	4.9±1.0 (7)
5L	RH095I08	18.0±2.3 (7)	17.9±3.1 (7)
5L	RH076O08	33.0±2.1 (7)	31.9±5.6 (7)
5S	RH089A21	80.0±4.7 (7)	76.4±3.4 (7)
5S	RH044A21	89.7±5.5 (7)	86.4±2.3 (7)

6S	RH026H24	3.3±1.0 (6)	3.0±0.7 (5)
6S	RH069B12	12.2±2.9 (6)	11.0±3.8 (5)
6L	RH075G13	49.6±4.5 (6)	53.4±6.8 (5)
6L	RH194M18	67.2±4.6 (6)	74.7±9.8 (5)
6L	RH054L23	82.2±6.9 (6)	88.1±5.3 (5)
7L	RH075O19	7.3±2.1 (5)	6.4±1.6 (7)
7L	RH078E15	17.1±2.6 (5)	14.4±1.4 (7)
7L	RH170M01	31.1±4.1 (5)	26.1±2.3 (7)
7L	RH093G10	34.7±4.8 (5)	29.5±2.3 (7)
7S	RH186I02	98.2±0.5 (5)	97.1±0.9 (7)
8S	RH122L16	3.7±1.2 (3)	6.5±1.3 (7)
8S	RH055L21	11.0±1.4 (3)	13.6±2.2 (7)
8L	RH184D07	59.6±8.2 (3)	47.0±1.9 (7)
8L	RH122E19	76.0±8.3 (3)	65.6±3.2 (7)
8L	RH127J02	92.5±2.9 (3)	86.6±2.7 (7)

9S	RH135I22	4.3±1.8 (4)	4.2±1.0 (2)
9S	RH061A13	22.1±3.8 (4)	28.6±1.6 (2)
9L	RH101N09	59.0±5.1 (4)	65.6±1.5 (2)
9L	RH168F09	84.1±0.7 (4)	76.8±2.0 (2)
9L	RH079O06	96.8±0.9 (4)	97.6±0.8 (2)
10L	RH049J10	5.2±1.3 (5)	4.8±0.6 (5)
10L	RH048F15	16.6±4.1 (5)	14.3±1.8 (5)
10L	RH184D02	23.2±4.9 (5)	21.9±1.8 (5)
10S	RH106M22	87.4±2.8 (5)	87.9±2.9 (5)
10S	RH178K07	94.7±1.9 (5)	95.2±1.0 (5)
11L	RH204G21	6.3±2.5 (6)	7.1±1.7 (11)
11L	RH097I18	20.0±4.0 (6)	21.6±3.5 (11)
11L	RH162O21	32.8±4.4 (6)	35.0±5.2 (11)
11S	RH058F17	82.6±4.9 (6)	79.6±2.4 (11)
11S	RH042C12	89.1±3.4 (6)	86.6±2.6 (11)

12L	RH106P06	2.5±1.0 (8)	2.8±1.1 (10)
12L	RH016H06	6.6±0.6 (8)	6.2±1.7 (10)
12L	RH183I02	10.5±1.0 (8)	10.7±1.7 (10)
12L	RH043B17	23.8±2.9 (8)	24.1±2.5 (10)
12S	RH084C24	97.8±1.0 (8)	97.8±1.0 (10)

^aArabic numerals, chromosome number; S and L, short and long arm of potato chromosomes. The numbering system was according to corresponding linkage groups (van Os et al. 2006).

^bSelected by Tang et al (2009) according to their genetic position in the ultradense RH genetic map (van Os et al. 2006).

^cRelative physical location was calculated as $(S/T) \times 100$, where S=the distance in μm from the FISH hybridization site to the end of the short chromosome arm, T=the total length of the chromosome in μm .

^dThe number of measurements.

Representative images of pachytene complements in *S. commersonii* (A) and *S. chacoense* (B) hybridized with BAC probes for chromosomes 5 and 12 (chr 5 and chr 12). The 45S rDNA and 5S rDNA probes were used as hybridization controls. Note the dispersed blue signal on the chromosomes in (A) indicating hybridization of repetitive sequences not blocked by C0t-100. Scale bar = 10 μ m.

79x120mm (300 x 300 DPI)

Examples of bivalents from chromosome 4 of *S. chacoense* and chromosome 12 of *S. commersonii*, with the colour scheme and the names of the five BAC clones used. Note the blue and yellow signals from BACs RH097G17 and RH013K09, respectively (yellow arrow), always map together in LG4 of *S. chacoense* and the blue and pink signals (blue and pink arrows) on the pericentromeric heterochromatin of chr 4 and chr 12 of *S. chacoense* and *S. commersonii* respectively, indicating that BACs RH097G17 and RH183I02 contained repeats that could not be blocked by C0t-100 during the hybridization experiments. Scale bars = 10 μ m.

161x96mm (300 x 300 DPI)

Idiograms of the twelve pachytene bivalents of *S. tuberosum* (RH89-039-16), *S. commersonii* and *S. chacoense* hybridized with five BAC probes per linkage group in multicolour BAC-FISH experiments. The sizes and distances correspond to averages across cell complements measured and the bars represent the standard error for those measurements. The *S. tuberosum* idiogram was built based on the data presented by Tang et al. (2009). Scale bar = 10 μ m.

86x237mm (300 x 300 DPI)

Examples of pachytene complements from two triploid interspecific hybrids (*S. commersonii* (2n) x *S. tuberosum* Group Phureja (n)) hybridized with five BAC probes belonging to a) chromosome 2 (chr 2), b) chr 5, c) chr 10, d) chr 11. The images show breakpoints in homoeologous pairing (white arrows) which have been quantified and are not correlated with any specific physical location. Scale bar = 10 μ m.

160x120mm (300 x 300 DPI)