Genomic in situ hybridization reveals both auto- and allopolyploid origins of different North and Central American hexaploid potato (*Solanum* sect. *Petota*) species

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Abstract: Wild potato (*Solanum* L. sect. *Petota* Dumort.) species contain diploids ($2n = 2x = 24$) to hexaploids ($2n = 6x = 72$). J.G. Hawkes classified all hexaploid Mexican species in series *Demissa* Bukasov and, according to a classic five-genome hypothesis of M. Matsubayashi in 1991, all members of series *Demissa* are allopolyploids. We investigated the genome composition of members of Hawkes's series *Demissa* with genomic in situ hybridization (GISH), using labeled DNA of their putative progenitors having diploid AA, BB, or PP genome species or with DNA of tetraploid species having AABB or AAA^aA^a genomes. GISH analyses support *S. hougasii* Correll as an allopolyploid with one AA component genome and another BB component genome. Our results also indicate that the third genome of *S. hougasii* is more closely related to P or a P genome-related species. *Solanum demissum* Lindl., in contrast, has all three chromosome sets related to the basic A genome, similar to the GISH results of polyploid species of series *Acaulia* Juz. Our results support a more recent taxonomic division of the Mexican hexaploid species into two groups: the allopolyploid Iopetala group containing *S. hougasii*, and an autopolyploid Acaulia group containing *S. demissum* with South American species *S. acaule* Bitter and *S. albicans* (Ochoa) Ochoa.

Key words: GISH, polyploids, potato, *Solanum* section *Petota*.

Résumé : Les espèces sauvages de la pomme de terre (*Solanum* L. sect. *Petota* Dumort.) comptent des espèces diploïdes ($2n = 2x = 24$) jusqu'à des espèces hexaploïdes ($2n = 6x = 72$). J.G. Hawkes a classifié toutes les espèces hexaploïdes mexicaines au sein de la série *Demissa* Bukasov et, selon l'hypothèse classique des cinq génomes de M. Matsubayashi en 1991, tous les membres de la série *Demissa* seraient allopolyploïdes. Les auteurs ont investigué la composition génomique de membres de la série *Demissa* de Hawkes à l'aide de l'hybridation génomique in situ (GISH) avec des sondes d'ADN génomique provenant des espèces ancestrales putatives à génomes AA, BB ou PP, ou encore provenant des espèces tétraploïdes à génome AABB ou AAA^aA^a. Les analyses GISH suggèrent que le *S. hougasii* Correll serait un allopolyploïde possédant un génome AA et un génome BB. Les résultats suggèrent également que le troisième génome du *S. hougasii* serait plus proche d'un génome P ou apparenté au génome P. Le *S. demissum* Lindl., au contraire, possède trois jeux chromosomiques apparentés au génome A de base comme le suggèrent les analyses GISH pour les espèces polyploïdes de la série *Acaulia* Juz. Ces résultats viennent appuyer une récente division taxonomique des espèces hexaploïdes mexicaines en deux groupes : le groupe Iopetala d'allopolyploïdes comprenant le *S. hougasii* et le groupe Acaulia d'autopolyploïdes comprenant le *S. demissum* avec les espèces sud-américaines *S. acaule* Bitter et *S. albicans* (Ochoa) Ochoa.

Mots-clés : GISH, polyploïdes, pomme de terre, *Solanum* section *Petota*.

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Introduction

Wild and cultivated potatoes, *Solanum* L. sect. *Petota* Dumort., contain over 230 tuber-bearing species according to the latest comprehensive taxonomic treatment by Hawkes (1990), but recent estimates propose about 100–110 species (Spooner 2009). Section *Petota* is part of a larger clade (the potato clade) containing 160 species as outlined on the Sola-

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naceae Web site: http://www.nhm.ac.uk/research-curation/research/projects/solanaceaesource/. All of these have a basic chromosome number of 12 ($x = 12$), with over 60% of the species exclusively diploid ($2n = 2x = 24$), with the rest tetraploid ($2n = 4x = 48$), hexaploid ($2n = 6x = 72$), and rare triploids and pentaploids (Hijmans et al. 2007; Gavrilenko 2011). Determination of the type of polyploidy and the development of the genome concept for members of section *Petota* traditionally has been based on classical genome analysis of chromosome pairing in interspecific hybrids and in polyploid species (Gavrilenko 2007, 2011). According to an hypothesis of Matsubayashi (1991), based on traditional genomic analysis, five genomes (A, B, C, D, and P) were recognized in potato species. Numerous genomic variants of the major genomic group A were predicted for diploid potato species, but no diploid species have ever been identified in classic genome analysis with the B, C, D, and P genomes that were considered by Matsubayashi (1991) as component genomes of allopolyploid species in series *Longipedicellata* Bukasov, *Conicibaccata* Bitter, *Demissa* Bukasov, and *Piurana* Hawkes, respectively (Table 1). For continuity with classic literature, we use the system of Hawkes (1990) for species and series designations and that of Matsubayashi (1991) for genome designations, with reference to a more recent classification into the Acaulia, Iopetala, and Piurana groups as described below (Spooner et al. 2004; Rodríguez and Spooner 2009).

Using genomic in situ hybridization (GISH), Pendinen et al. (2008) demonstrated significant genome differentiation among the diploid North and Central American species *S. verrucosum* Schltdl. (genome AA) and species of series *Pinnatisecta* (Rydb.) Hawkes (genome BB). These results provided the first GISH confirmation that the North and Central American tetraploid species of series *Longipedicellata* are allotetraploids (AABB) (Pendinen et al. 2008) (Table 1). These GISH results showing clear discrimination of two divergent parental genomes (A and B) are concordant with recent DNA sequence data (Spooner et al. 2008; Rodríguez and Spooner 2009).

In the taxonomic treatment of Hawkes (1990), all North and Central American hexaploid species ($2n = 6x = 72$) belong to series *Demissa*, although they have been placed in different series (Bukasov 1955; Correll 1962) or groups (Spooner et al. 2004). According to the five-genome hypothesis of Matsubayashi (1991), all members of series *Demissa* are allopolyploids with genome formulae AADDD'D' (Table 1), although other cytogeneticists gave different genome symbols to these Mexican hexaploids as $ABB^{(1-4)}$ (Marks 1955), A_1A_4B (Hawkes 1958), or AB_sB_d (Irikura 1976). Classic genome concepts did not provide the experimental evidence for the donor of the D or B component genomes of members of series *Demissa*. Marks (1965) suggested that hexaploid species of series *Demissa* (*S. demissum* Lindl. and *S. hougasii* Correll) evolved through hybridization of Mexican allotetraploids of series *Longipedicellata* with diploid species *S. verrucosum*, followed by amphidiploidization.

DNA sequence data provided the first molecular evidence of allopolyploidy in wild potatoes and revealed their putative diploid progenitors (Spooner et al. 2008; Rodríguez and Spooner 2009) (Table 1). They supported a division of the hexaploid species of series *Demissa* into two groups that dif-

Table 1. Taxonomy and genome formulae of Mexican and Central American hexaploid ($2n = 6x = 72$) species *Solanum demissum*, *S. hougasii*, and related species that were mentioned in this study.

Species	Series*	Group†	Geographical origin	Ploidy	Genome formula by classical genome analysis‡	Genome formula by DNA sequence data§	Genome formula by GISH‖
Solanum ehrenbergii	*Pinnatisecta*	Pinnatisecta	NA	2*x*	$A^{p'}A^{p'}$	BB	BB
Solanum jamesii	*Pinnatisecta*	Pinnatisecta	NA	2*x*	$A^{pj}A^{pj}$	BB	BB
Solanum stoloniferum	*Longipedicellata*	Longipedicellata	NCA	4*x*	AABB	AABB	AABB
Solanum hougasii	*Demissa*	Iopetala	NCA	6*x*	AADDD'D'	AAPPPP (Spooner et al. 2008; Rodríguez and Spooner 2009); AABB (A^P, P, or C)	*S. hougasii* AABB (P)
Solanum iopetalum	*Demissa*	Iopetala	NCA	6*x*	AADDD'D'		
Solanum schenckii	*Demissa*	Iopetala	NCA	6*x*	AADDD'D'		
Solanum demissum	*Demissa*	Acaulia	NCA	6*x*	$AADDD^dD^d$	AAAAAA (two types of slightly different A genomes)	A genome hexaploid
Solanum acaule	*Acaulia*	Acaulia	SA	4*x*	AAA^aA^a	AAAA	A genome tetraploid
Solanum albicans	*Acaulia*	Acaulia	SA	6*x*	AAA^aA^aXX	AAAAAA	A genome hexaploid
Solanum verrucosum	*Tuberosa*	Verrucosa	NCA	2*x*	AA	AA	AA
Solanum andreanum	*Tuberosa*	Piurana	SA	2*x*	—	PP or A^PA^P	PP
Solanum chomatophilum	*Piurana*	Piurana	SA	2*x*	A^PA^P	PP or A^PA^P	PP
Solanum piurae	*Piurana*	Piurana	SA	2*x*	A^PA^P	PP or A^PA^P	PP

Note: NCA, North and Central America; SA, South America. Genome symbol X (Matsubayashi 1991) used for an unknown component genome in *S. albicans*.

*According to Hawkes (1990).

†According to Spooner et al. (2004).

‡From Matsubayashi (1991).

§From Spooner et al. (2008) and Rodríguez and Spooner (2009).

‖From Pendinen et al. 2008 and the present study.

fer by their genomes. The Iopetala group includes only the allohexaploids, with *S. hougasii* and *S. iopetalum* (Bitter) Hawkes containing two component genomes A and P and *S. schenckii* Bitter with three component genomes A, B, and P. The Acaulia group contains *S. demissum*, possessing two minor variants of genome A (Spooner et al. 2008; Rodríguez and Spooner 2009).

Solanum verrucosum (or its progenitor) is the sole A genome species in North and Central America, and DNA sequence results support *S. verrucosum* as an A genome contributor of all of the Mexican and Central American hexaploids. DNA sequence results suggest the possible genome contribution of species from South American series *Piurana* (P genome) into the formation of allohexaploids of the Iopetala group. These results also support North and Central American diploid species of series *Pinnatisecta* and (or) *Bulbocastana* (Rydb.) Hawkes as B genome contributors of the hexaploid species *S. schenckii* of the Iopetala group (Spooner et al. 2008; Rodríguez and Spooner 2009) (Table 1).

The distinctiveness of *S. demissum* from other members of series *Demissa* also is supported by phenetic analysis of morphological data (Spooner et al. 1995; Kardolus 1999) that demonstrates its similarity to the South American species of series *Acaulia* Juz. (*S. acaule* Bitter and *S. albicans* (Ochoa) Ochoa) rather than to other species of series *Demissa*. This relationship also is supported by nuclear and plastid restriction fragment length polymorphisms (Debener et al. 1990; Kardolus et al. 1998; Nakagawa and Hosaka 2002) and by DNA sequence data (Rodríguez and Spooner 2009), providing strong support for the close relationship among *S. demissum* and species of series *Acaulia* (*S. acaule* and *S. albicans*).

The purpose of our study is to examine the following with GISH analysis (*i*) the genome composition of North and Central American hexaploid species belonging to series *Demissa*, namely *S. demissum* (the Acaulia group) and *S. hougasii* (the Iopetala group); (*ii*) whether A, B, and P genomes are present in these hexaploid species of series *Demissa*; and (*iii*) the evolutionary relationships of these species relative to North and Central American diploids and tetraploids and to some wild South American diploid and polyploid species.

Materials and methods

Plant materials

Eleven potato species belonging to six series of section *Petota* (see Table 1 for series and genome designations) were included in the present study. Three accessions of *S. hougasii* (PI 161726, 239424, 558402) and four accessions of *S. demissum* (PI 225711, 275206, 545763, 604049) were used in GISH analysis. For each species, we selected accessions from different geographic areas. Root tips and anthers of greenhouse-grown hexaploid plants were used for preparing slides of mitotic and meiotic chromosomes.

We chose the diploid species ($2n = 2x = 24$) putative progenitors for hexaploid species of the above members of series *Demissa* based on prior hypotheses of classic genome analyses and the nuclear DNA sequencing data as described above. The diploid species *S. verrucosum* (PI 545745) was used as a putative A genome diploid progenitor. Putative B genome diploid progenitors included North American diploid species of series *Pinnatisecta*, namely *S. ehrenbergii* Bitter (PI 275216) and *S. jamesii* Torrey (PI 458424). Putative P genome diploid progenitors included South American species of the Piurana group, namely *S. andreanum* Baker (PI 320345), *S. chomatophilum* Bitter (PI 365339), and *S. piurae* Bitter (PI 310997). *Solanum andreanum* is a taxonomically difficult species that was variously placed into a different series. Association of *S. andreanum* (series *Tuberosa* (Rydb.) Hawkes in system of Hawkes (1990)) with species of series *Piurana* is strongly supported by nuclear DNA sequence results (Spooner et al. 2008; Ames and Spooner 2010) and by GISH (Pendinen et al. 2008).

In addition to these diploid species, we used DNA of the putative tetraploid progenitor species *S. stoloniferum* Schltdl. (AABB genome, $2n = 4x = 48$; PI 251740) based on prior hypotheses of classic genome analysis. We also included members of series *Acaulia* (or Acaulia group), namely *S. acaule* (AAA^aA^a genome according to Matsubayashi 1991) ($2n = 4x = 48$; PI 473439, 473485, 473486) and *S. albicans* (AAA^aA^aXX genome according to Matsubayashi 1991) ($2n = 6x = 72$; PI 230494, 365310, 568915, 590888) as putative sister species of *S. demissum*.

GISH procedures

Chromosomal preparations and the GISH procedure were used the same as that described by Pendinen et al. (2008). Roots were collected from greenhouse-grown plants and pretreated in 0.002 mol/L 8-hydroxyquinoline at 20 °C for 3 h. Root tips and flower buds were fixed in a 3:1 (100% ethanol: glacial acetic acid) solution and stored in a freezer (–20 °C) until use. Root tips or anthers were digested by 4% cellulase and 1% pectinase at 37 °C for 80 min or 95 min, respectively. The macerated root tips were suspended by forceps in a drop of 45% acetic acid and squashed. Slides were pretreated by pepsin solution (final concentration 0.1 mg/mL) for 45 min at 37 °C and subsequently incubated in an RNase A solution (6 μL stock solution – 10 μg/μL + 24 μL 2× saline-sodium citrate (SSC) per slide; 40 min at 37 °C) and then in formaldehyde solution (4% for 10 min). After each treatment slides were washed in 2× SSC buffer for 5 min for three times at room temperature. Slides were then incubated in a 70%, 90%, 100% ethanol series for 3 min each at room temperature. Genomic DNA was isolated from the putative diploid A, B, and P genome progenitor species using young leaves of greenhouse-grown plants. The GISH technique followed standard protocols (Leitch et al. 1994; Dong et al. 2001) with minor modifications. DNA was either labeled with DIG-UTP or Biotin-UTP by nick-translation (DIG and Biotin-Nick Translation Mix, Roche, cat. nos. 11745816910, 11745824910, Indianapolis, Ind.).

The hybridization mix (40 μL per slide) for GISH was prepared with differentially labeled DNA from the putative parental species (species No. 1 and No. 2) and included sheared fish sperm DNA (20 μg), probe DNA of species No. 1 (100 ng), probe DNA of species No. 2 (100 ng), 10% dextran sulfate, and deionized formamide (50%). In some experiments the blocking DNA (2000 ng per slide of blocking DNA: 100 ng of labeling probe DNA; ratio 1:20) of a third species (species No. 3) was added to the hybridization mix. The blocking DNA was prepared by boiling. Hybridization was performed overnight at 37 °C. Denaturation of probes and slides was performed according to Schrader et al.

(2000). Stringent washing was performed using subsequent washing of slides: three times in 2× SSC for 5 min each at 37 °C, three times in 0.1× SSC for 5 min each at 42 °C, and two times in 2× SSC for 5 min each at room temperature.

DIG-labeled DNA was detected with rhodamine anti-DIG conjugate, and biotin-labeled probes were detected with FITC-conjugated avidin (Roche, cat. Nos. 11207750910, 11975595910). Twenty-nine microlitres of blocking reagent (30 mg bovine serum albumin (BSA) solution in 999 μL 4× SSC) was added to the slides, followed by incubation for 30 min at room temperature. The antibody solution, composed of 1 μL anti-DIG-rhodamine stock solution + 1 μL avidin-fluorescein stock solution + 28 μL detection buffer (0.1 g BSA dissolved in 9.9 mL 4× SSC, pH 7.4) was added to each slide; followed by incubation for 45 min at 37 °C. Slides were washed three times in 4× SSC (pH 7.4) for 5 min each at 42 °C. Chromosomes were counterstained by 4′,6-diamidino-2-phenylindole (DAPI) in vectashield antifade solution (Vector Laboratories, Berlingame, Calif.).

Most images were captured digitally using a SenSys CCD (charge-coupled device) camera (Roper Scientific, Tucson, Ariz.) attached to an Olympus (Center Valley, Pa.) BX60 epifluorescence microscope. The CCD camera was controlled using IPLab Spectrum v3.1 software (Signal Analytics, Vienna, Va.) on a Macintosh computer. In addition, some replications for images of *S. acaule* and *S. demissum* were prepared using an AxioCam MRm camera attached to an Imager.M2 epifluorescence microscope and AxioVision Rel 4.8 Imaging System (Carl Zeiss, Göttingen Germany).

GISH combinations

We probed mitotic and meiotic chromosomes of the hexaploid species of series *Demissa* (*S. demissum* and *S. hougasii*) with differentially labeled DNA of their putative diploid or tetraploid progenitors in three series of GISH experiments (see Supplementary data,[1] Tables S1 and S2) based on the prior hypothesis of their genome composition:

1. Genome composition of species of series *Demissa* (*S. demissum* and *S. hougasii*) including the A and the B component genomes: (1a) A genome putative diploid progenitor *S. verrucosum* (DIG) and B genome putative diploid progenitor *S. ehrenbergii* or *S. jamesii* (BIO); (1b) Reciprocal GISH: *S. verrucosum* (BIO) and *S. ehrenbergii* or *S. jamesii* (DIG).
2. Genome composition of species of series *Demissa* including the P component genome: (2a) A genome putative diploid progenitor *S. verrucosum* (DIG) and P genome putative diploid progenitor *S. andreanum*, *S. chomatophilum,* or *S. piurae* (BIO); (2b) Reciprocal GISH: *S. verrucosum* (BIO) and *S. andreanum*, *S. chomatophilum*, or *S. piurae* (DIG); (2c) *S. verrucosum* (DIG) and *S. andreanum*, *S. chomatophilum*, or *S. piurae* (BIO) + blocking DNA of the B genome diploid progenitor species *S. ehrenbergii* or *S. jamesii*; (2d) AABB genome putative tetraploid progenitor *S. stoloniferum* (DIG) and *S. andreanum, S. chomatophilum*, or *S. piurae* (BIO); (2e) Reciprocal GISH: *S. stoloniferum* (BIO) and *S. andreanum*, *S. chomatophilum*, or *S. piurae* (DIG); (2f) *S. verrucosum* (DIG) and *S. andreanum*, *S. chomatophilum*, or *S. piurae* (BIO) + blocking DNA of the B genome diploid progenitor, *S. ehrenbergii* or *S. jamesii*. In addition, in GISH with *S. demissum* chromosomes, we included labeling DNA of *S. acaule* as a putative tetraploid progenitor species combined with labeling DNA of *S. jamesii* or *S. andreanum*.
3. Genome compositions of South American species of series *Acaulia* (tetraploid *S. acaule* and hexaploid *S. albicans*) include different variants of the basic A genome (Table S3): (3a) A genome putative diploid progenitor *S. verrucosum* (DIG) and B genome putative diploid progenitors *S. jamesii* (BIO); (3b) Reciprocal GISH: *S. verrucosum* (BIO) and *S. jamesii* (DIG): (3c) A genome putative diploid progenitor *S. verrucosum* (DIG) and P genome putative diploid progenitor *S. andreanum* or *S. chomatophilum* (BIO); (3d) Reciprocal GISH: *S. verrucosum* (BIO) and *S. andreanum* or *S. chomatophilum* (DIG); (3e) *S. jamesii* (DIG) and *S. andreanum* + blocking DNA of the A genome diploid progenitor *S. verrucosum*; (3f) *S. stoloniferum* (DIG) and *S. andreanum* (BIO); (3g) *S. acaule* (DIG) and *S. jamesii* (BIO); (3h) *S. acaule* (DIG) and *S. andreanum* (BIO).

Results

GISH analysis of the genome composition of North and Central American hexaploid species of series *Demissa* was performed using DNA of putative diploid progenitors of the A genome (*S. verrucosum*), B genome (*S. ehrenbergii* or *S. jamesii*), P genome (*S. andreanum*, *S. chomatophilum*, or *S. piurae*), or DNA of putative tetraploid progenitors (*S. acaule* AAA^aA^a or *S. stoloniferum* AABB) in three series of GISH experiments including 29 species/DNA probe combinations (Tables S1–S3). The results show the following.

1. GISH of Mexican hexaploid species *Solanum hougasii* ($2n = 6x = 72$) of series *Demissa* (or Iopetala group)

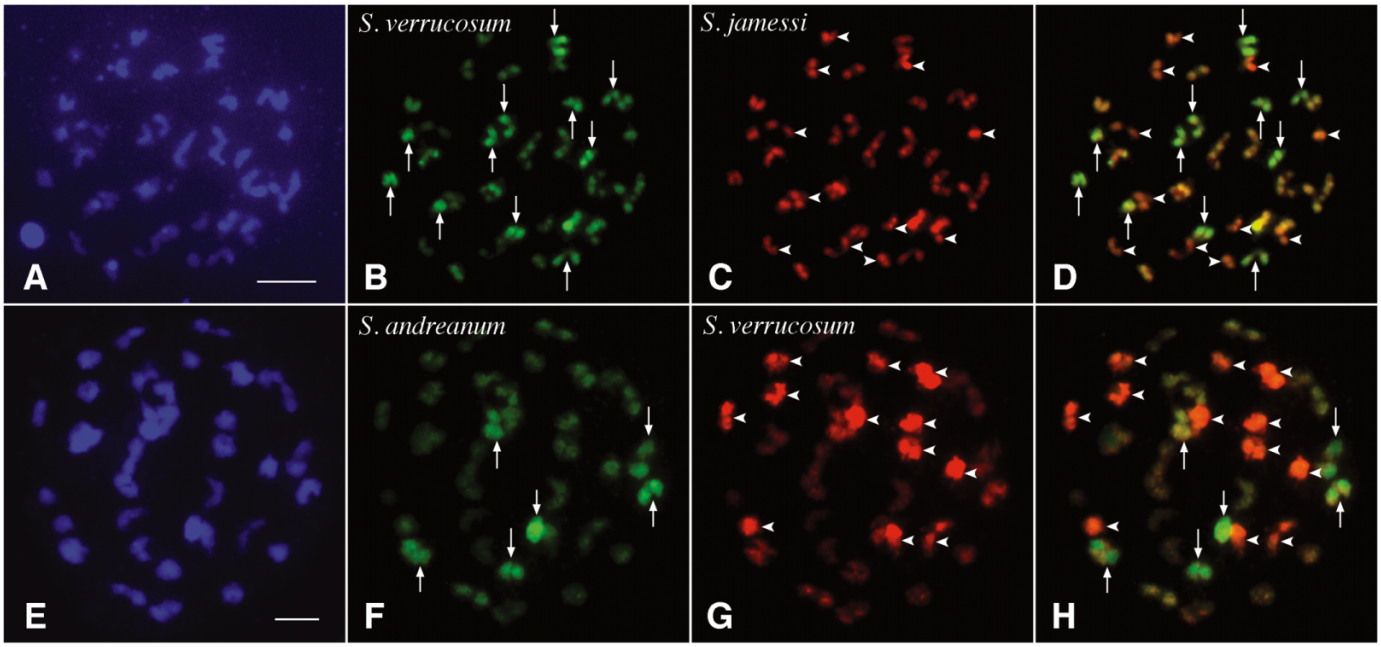
As summarized in Fig. 1 and Table S1, GISH analysis supports the hexaploid Mexican species *S. hougasii* as an allopolyploid.

Contribution of the A and B genomes to S. hougasii.

The first series of GISH (A-B genome probe combinations) revealed differentiation of the two component genomes on chromosome preparations of all three accessions of *S. hougasii*, with differentially labeled DNA of *S. verrucosum* (AA genome) and *S. ehrenbergii* or *S. jamesii* (BB genome) (Fig. 1; Table S1). Differentiation of two component genomes was observed as approximately one complete set (12 pairs of chromosomes) showing enhanced hybridization to the genomic DNA probe derived from *S. verrucosum*. The numbers of unambiguously A genome hybridizing chromosomes varied from 11 to 12 chromosome pairs in different cells. This could be caused by an uneven cytoplasm background in each cell. However, the majority of the cells showed 12 pairs of chromosomes with enhanced hybridization signals. Similarly, approximately one complete set of chromosomes showed an enhanced hybridization to the genomic DNA probe derived from B genome species *S. jamesii* (or *S. ehrenbergii*) (Figs. 1A–1D). When both *S. verrucosum*

[1]Supplementary data are available with the article through the journal Web site (http://nrcresearchpress.com/doi/suppl/10.1139/g2012-027).

Fig. 1. GISH analysis of *Solanum hougasii*. (A–D) Chromosomes of a meiotic cell (at diakinesis) of *S. hougasii* were hybridized with equal amounts of labeled DNA of *S. verrucosum* (green) and *S. jamesii* (red). (A) DAPI-stained chromosomes, scale bar = 5 μm; (B) GISH signals from *S. verrucosum* DNA probe; (C) GISH signals from *S. jamesii* DNA probe; (D) Merged GISH signals from both probes. Arrows and arrowheads in (D) point to bivalent chromosomes with enhanced green and red GISH signals, respectively. The same arrows and arrowheads are also displayed in (B) and (C), respectively. (E–H) Chromosomes of a meiotic cell of *S. hougasii* were hybridized with equal amounts of labeled DNA of *S. andreanum* (green) and *S. verrucosum* (red); DNA of *S. jamesii* was used as block. (E) DAPI-stained chromosomes, scale bar = 5 μm; (F) GISH signals from *S. andreanum* DNA probe; (G) GISH signals from *S. verrucosum* DNA probe; (H) Merged GISH signals from both probes. Arrows and arrowheads in (H) point to bivalent chromosomes with enhanced green and red GISH signals, respectively. The same arrows and arrowheads are also displayed in (F) and (G), respectively.



and *S. jamesii* (or *S. ehrenbergii*) were labeled as probes in the GISH experiments, approximately one complete set of chromosomes did not preferentially hybridize to either probe, indicating that the remaining chromosomes belong to neither the A genome nor the B genome. Thus, the first series of GISH (A-B genome probe combinations) indicates *S. hougasii* as an allopolyploid involving A and B genomes.

Contribution of the P genome to S. hougasii.

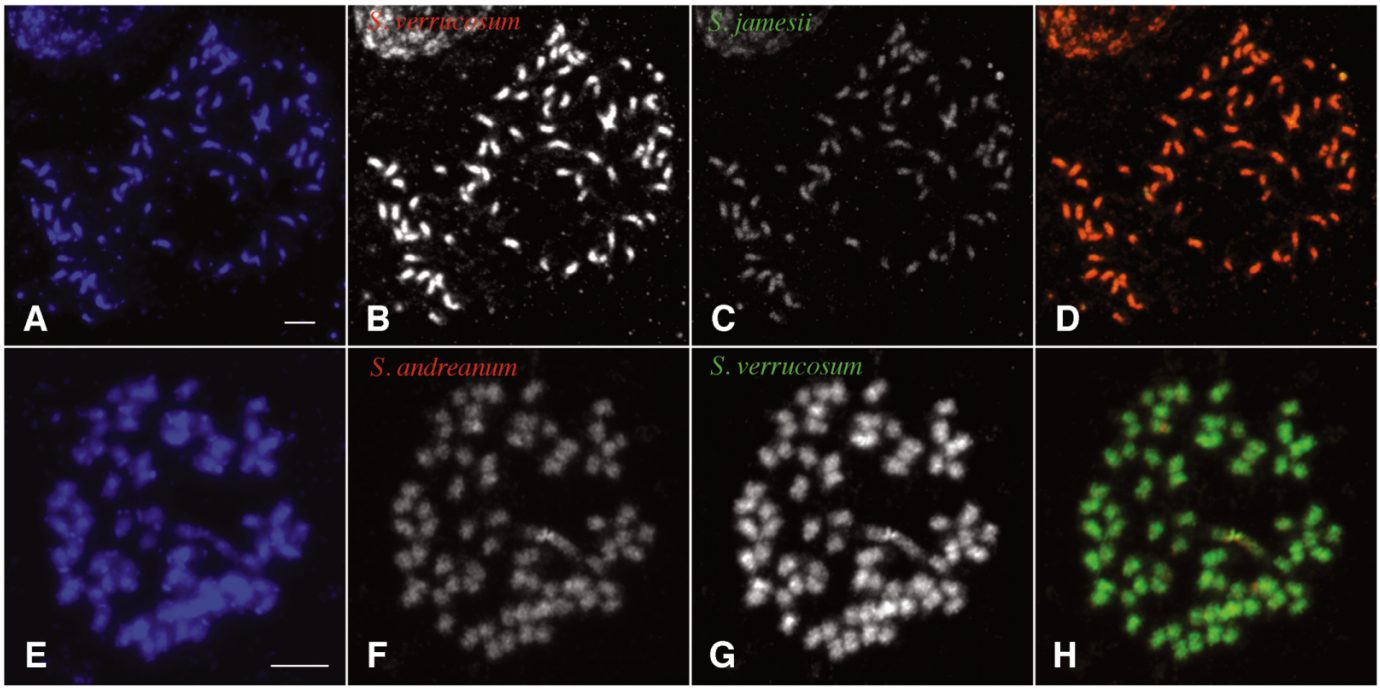
To investigate whether diploid P genome species contributed to *S. hougasii*, we probed separately with genomic DNA from P genome species *S. andreanum*, *S. chomatophilum*, or *S. piurae* in combination with genomic DNA from A-B genome species. When DNA from a P genome species, such as *S. andreanum*, was labeled in green, DNA from an A genome species, such as *S. verrucosum*, was labeled in red, and DNA of a B genome species, such as *S. jamesii*, was used as block—and these DNA mixtures hybridized to *S. hougasii* chromosomes—strong and uniform hybridization red signals from the A genome probe were observed on one complete set of chromosomes of *S. hougasii* (Figs. 1E–1H), whereas the second incomplete set of *S. hougasii* chromosomes showed an enhanced hybridization to the P genome probe. The numbers of chromosome pairs with unambiguous strong hybridization with the P genome probe varied in different cells from five to six; however, the majority of the cells showed six such chromosome pairs (Table S1). The rest of the chromosome pairs did not preferentially hybridize to either probe.

2. GISH of North and Central American hexaploid species *Solanum demissum* ($2n = 6x = 72$) of series *Demissa* (or Acaulia group)

GISH of another North and Central American hexaploid species *S. demissum* was performed in the same series of experiments and with the same DNA probes as for allohexaploid species *S. hougasii*. We probed chromosomes of *S. demissum* with differentially labeled DNA of A genome (*S. verrucosum* or *S. acaule*), B genome (*S. ehrenbergii* or *S. jamesii*), or P genome (*S. andreanum*, *S. chomatophilum*, or *S. piurae*) species. When DNA from an A genome species was labeled in red, DNA from a B genome species in green, and the two probes were hybridized to *S. demissum* chromosomes, strong and uniform hybridization signals were generated from the A genome probe. In contrast, the B genome probe generated weak and uniform hybridization signals (Figs. 2A–2D; Table S2). When we swapped the BIO-green/DIG-red labeling for DNA from the A-B genome species, strong and uniform hybridization to *S. demissum* chromosomes was always associated with the A genome probe regardless of its red or green labeling, confirming that the strong hybridization was not caused by a different labeling or detection system.

When total genomic DNA from A genome species and DNA from P genome species were hybridized to chromosome preparations of *S. demissum*, all its chromosomes were strongly hybridized with labeling signals corresponding to the A genome probe, whereas the P genome probe generated a weak signal, implying that there is no P genome in *S. de-*

Fig. 2. GISH analysis of *Solanum demissum*. (A–D) Chromosomes of a somatic metaphase cell of *S. demissum* were hybridized with equal amounts of labeled DNA of *S. verrucosum* (red) and *S. jamesii* (green). (A) DAPI-stained chromosomes, scale bar = 5 μm; (B) GISH signals from *S. verrucosum* DNA probe; (C) GISH signals from *S. jamesii* DNA probe; (D) Merged GISH signals from both probes. Note: the original grayscale images are shown in (B) and (C), so the relative fluorescence intensities from the two probes are better compared. (E–H) Chromosomes of a somatic metaphase cell of *S. demissum* were hybridized with an equal amount of labeled DNA of *S. andreanum* (red) and *S. verrucosum* (green); (E) DAPI-stained chromosomes, scale bar = 5 μm; (F) GISH signals from *S. andreanum* DNA probe; (G) GISH signals from *S. verrucosum* DNA probe; (H) Merged GISH signals from both probes. Note: the original grayscale images are shown in (F) and (G), so the relative fluorescence intensities from the two probes are better compared.



missum (Figs. 3E–3H, Table S2). When both B and P genome species were labeled as probes and hybridized together with an A genome block, no labeled chromosomes were observed in GISH (Table S2). These results suggest that *S. demissum* contains three sets of highly homologous chromosomes that may be derived from the same ancestral species. The GISH results also suggest that the progenitor species of *S. demissum* is closer to an A genome species than to a B or P genome species.

3. GISH of South American tetraploid *Solanum acaule* and hexaploid *S. albicans* species of series *Acaulia* (or Acaulia group)

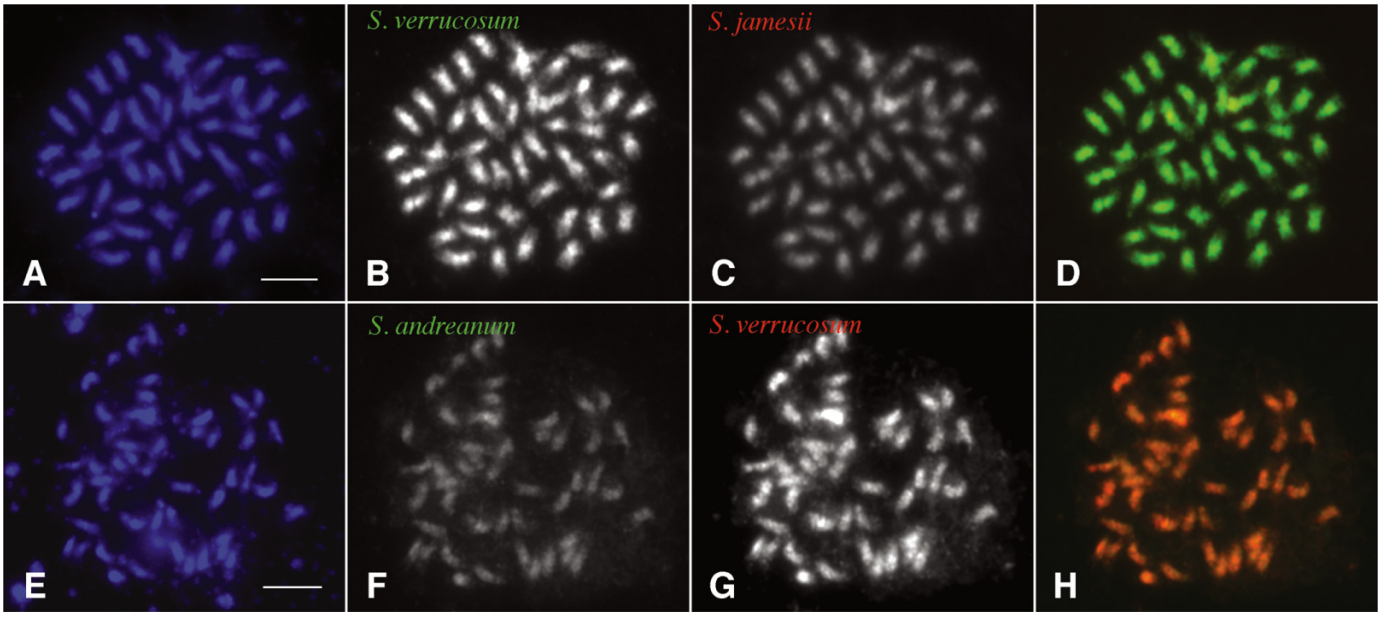
Meiotic and mitotic chromosomes of two polyploid South American species of series *Acaulia* (Acaulia group), *S. acaule* and *S. albicans*, were probed with differentially labeled DNA of diploid species in the same series of GISH experiments and with the same DNA probes as for North and Central American hexaploid species (Table S3). When DNA from A genome species *S. verrucosum* was labeled in green, DNA from a B genome species, such as *S. jamesii*, was labeled in red, and the two probes were hybridized to *S. acaule* chromosomes, strong and uniform BIO green hybridization signals were generated from the A genome probe. In contrast, the B genome probe generated weak and uniform DIG red hybridization signals (Figs. 3A–D). The same results were obtained in reciprocal GISH experiments, that is, strong and uniform hybridization to *S. acaule* chromosomes was always associated with the A genome probe (Table S3).

When the DNA probe of A genome species *S. verrucosum* was labeled in red, DNA from a P genome species, such as *S. piurae*, *S. chomatophilum*, or *S. andreanum*, was labeled in green, and these two probes hybridized to *S. acaule* chromosomes; all chromosomes of *S. acaule* showed a red uniform signal, whereas only weak green signals on all chromosomes of *S. acaule* were observed (Figs. 3E–3H). The same results as for *S. acaule* were obtained for *S. albicans* (Table S3). GISH results of polyploid species of series *Acaulia* are similar to GISH of *S. demissum*, that is, their chromosomal sets are highly homologous to the A genome species.

Discussion

A recent taxonomic treatment by Spooner et al. (2004) divided members of series *Demissa*, as recognized by Hawkes (1990), into two groups by genome composition: the Iopetala group containing allohexaploids *S. hougasii* and *S. iopetalum* with two (A and P) component genomes, and allohexaploid *S. schenckii* with three (A, B, and P) component genomes from diploid North (A and B) and South (P) American species; and the Acaulia group with North and Central American species *S. demissum* and South American species *S. acaule* and *S. albicans*, containing two slightly diverged A genomes (Spooner et al. 2004, 2008; Rodríguez and Spooner 2009). Here, we provide the first demonstration of genome compositions of hexaploid species *S. hougasii*, *S. demissum*, *S. albicans*, and tetraploid species *S. acaule* with GISH.

Fig. 3. GISH analysis of *Solanum acaule*. (A–D) Chromosomes of a somatic metaphase cell of *S. acaule* were hybridized with equal amounts of labeled DNA of *S. verrucosum* (green) and *S. jamesii* (red). (A) DAPI-stained chromosomes, scale bar = 5 μm; (B) GISH signals from *S. verrucosum* DNA probe; (C) GISH signals from *S. jamesii* DNA probe; (D) Merged GISH signals from both probes. Note: the original grayscale images are shown in (B) and (C), so the relative fluorescence intensities from the two probes are better compared. (E–H) Chromosomes of a somatic metaphase cell of *S. acaule* were hybridized with an equal amount of labeled DNA of *S. andreanum* (green) and *S. verrucosum* (red); (E) DAPI-stained chromosomes, scale bar = 5 μm; (F) GISH signals from *S. andreanum* DNA probe; (G) GISH signals from *S. verrucosum* DNA probe; (H) Merged GISH signals from both probes. Note: the original grayscale images are shown in (F) and (G), so the relative fluorescence intensities from the two probes are better compared.



Based on the GISH results, hexaploid species of series *Demissa* could be divided into (*i*) allohexaploid species *S. hougasii* with a complex genome, and (*ii*) *S. demissum* with all three chromosomal sets related to the basic A genome. The first component genome (A) of *S. hougasii* is homologous to the A genome of *S. verrucosum*, and its second component genome (B) is homologous to the B genome of Mexican diploid species of series *Pinnatisecta* (*S. ehrenbergii* and *S. jamesii*) or a diploid species closely related to them or to their ancestor species. In the case of the B genome contribution, GISH results do not support the results of Spooner et al. (2008) regarding the A and P genome composition of *S. hougasii* based on the granule-bound starch synthase (GBSSI) gene sequencing data.

Our results supporting the contribution of the P genome species in *S. hougasii* are in agreement with DNA sequence data (Spooner et al. 2008; Rodríguez and Spooner 2009), which show this species to have alleles falling on a clade with series *Piurana*. Our GISH data revealed six pairs of *S. hougasii* chromosomes with an enhanced strong hybridization to the P genome probe. We suggest that the remaining six chromosomes of the P genome ancestral species showing the weaker signal were rearranged during formation of the allohexaploid genome of *S. hougasii* or substituted in ancestral species. Genomes of allopolyploids are dynamic and can undergo genomic rearrangements and (or) partial elimination (Wendel 2000; Levy and Feldman 2004; Adams and Wendel 2005; Chen and Ni 2006; Soltis et al. 2010). If so, this may be why DNA sequencing data failed to detect the B component genome in *S. hougasii* (Spooner et al. 2008). In the case of the P genome contribution, GISH results do not support the suggestion of Spooner et al. (2008) concerning the presence of the complete PP component genome in allohexaploid species *S. hougasii*. This suggests that DNA sequence analysis using limited numbers of probes restricted to only a small part of the genome is able to differentiate an auto- or allopolyploid, but it cannot reveal the events in allopolyploid formation.

GISH data indicate that *S. hougasii* has a complex genome derived through hybridization of North and Central American diploid species having an AA genome (as *S. verrucosum*) and a BB genome (as *S. ehrenbergii*, *S. jamesii*, or related B genome species). Our results do not contradict the hypothesis of Marks (1965) that allohexaploid *S. hougasii* may have been derived from Mexican allotetraploid species of series *Longipedicellata* (*S. stoloniferum*, genome AABB). We suppose that *S. hougasii* could have originated through hybridization of Mexican AABB allotetraploids (as *S. stoloniferum*) with natural hybrids having genetic material (introgression) of the P genome of South American species of series *Piurana* and of some unknown species. It is also possible that *S. hougasii* progressively lost or gained the P component genome, depending on its ancestral state.

Our preliminary GISH data also support the other members of series *Demissa* (or Iopetala group), *S. schenckii* and *S. iopetalum*, as allopolyploids suggesting the involvement of A, B, and P genome species in their speciation. Our continuing studies are exploring genome compositions of these Mexican hexaploid species.

In contrast, GISH data clearly indicate that *S. demissum* has another genome composition than the allohexaploid species *S. hougasii*, with *S. demissum* containing identical or

very similar AA genomes, but not B and P component genomes. This contradicts Marks (1955) who postulated a genome formula for *S. demissum* as AABBB′B′. GISH results are concordant with the DNA sequence data, indicating that *S. demissum* contains two types of genome A, which are only slightly different, having alleles falling on close clades 4a and 4b with the other A genome species (Spooner et al. 2008; Rodríguez and Spooner 2009). However, identification of the similar variants of the A genomes and corresponding species contributing to the speciation of *S. demissum* is not possible by GISH.

GISH results also provide support for including in the Acaulia group Mexican and Central American hexaploid species *S. demissum* together with South American species *S. acaule* and *S. albicans* as proposed by Spooner et al. (2004), a taxonomic division also in agreement with AFLP results from Kardolus et al. (1998). Our GISH data indicate that all species of the Acaulia group (*S. acaule*, *S. albicans*, *S. demissum*) are A-genome polyploids. GISH data do not contradict hypothesis of Nakagawa and Hosaka (2002) that South American autotetraploid species *S. acaule* could be directly involved in the origin of Central and North American A genome hexaploid species *S. demissum*.

In classical genome analysis, *S. demissum* as well as *S. acaule* and *S. albicans* were considered as allopolyploids based on their bivalent pairing. We confirmed the bivalent meiosis for these species and suppose that they may be disomic polyploids. During evolution of A-genome polyploids (*S. acaule*, *S. albicans*, and *S. demissum*), selection pressure for high fertility could select mutations in pairing control gene(s) that resulted in regular bivalent pairing. In the future, testing for disomic modes of genetic inheritance using nuclear SSR markers (Catalán et al. 2006) could be applied to shed some light on this issue.

Our conclusions provide data useful for understanding the effectiveness of breeding programs using these hexaploid species. The North and Central American hexaploid species *S. demissum* and *S. hougasii* have been the subject of numerous applied studies, because they possess multiple resistances to diseases and pests. Because these species all have an endosperm balance number (EBN) of 4 (Hawkes 1990), they are able to cross with the common potato, *S. tuberosum* L. ($2n = 4x = 48$, EBN = 4). According to EBN hypothesis, seed development in hybrid crosses is successful when crossing partners had identical EBN values (Johnston et al. 1980). Thus, *S. demissum* is an important source of late blight resistance and 11 race-specific late blight *R* genes have been identified in A genome hexaploid *S. demissum* and were transferred into numerous commercial potato cultivars (AAAA or AAA^tA^t genome as proposed by classical genome analysis) by relatively easy crossing and backcrossing (Umaerus and Umaerus 1994). *Solanum hougasii* is a source of durable resistance to late blight (Inglis et al. 2007), to potato virus Y (Cockerham 1970), to Columbia root-knot nematode (Brown et al. 1999), and to bruising (Culley et al. 2002). However, the breeding efforts with allohexaploid species *S. hougasii* were not so effective in comparison with A genome hexaploid *S. demissum*, which could be due to the low level (or absence) of pairing and crossing over between homeological B and P component genomes of *S. hougasii*, and A genomes of common potato. Knowledge about the genome composition of the Mexican hexaploid species is important for developing effective breeding strategies based on introgressive hybridization.

In conclusion, our results contradict classical hypotheses on genome composition of the North and Central American hexaploids, support recent sequence results of these species, provide new data on the extent of presence of parental genomes in allohexaploid species of *S. hougasii*, and support a more recent taxonomic division of the species of series *Demissa* into two groups: the allopolyploid Iopetala group containing *S. hougasii*, and an autopolyploid Acaulia group containing *S. demissum* with South American species *S. acaule* and *S. albicans*.

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