Genet Resour Crop Evol (2013) 60:2391–2404
DOI 10.1007/s10722-013-0007-z

RESEARCH ARTICLE

A recombination-derived mitochondrial genome retained stoichiometrically only among *Solanum verrucosum* Schltdl. and Mexican polyploid wild potato species

Rena Sanetomo · Kazuyoshi Hosaka

Received: 7 February 2013 / Accepted: 22 May 2013 / Published online: 15 August 2013

Abstract We previously found a specific DNA fragment, designated Band 1, in the cytoplasm of a Mexican hexaploid wild potato species, *Solanum demissum*, and varieties with the *S. demissum* cytoplasm. In this study, we show that Band 1 also occurs substoichiometrically in various species and varieties. The *S. demissum* DNA sequence harboring Band 1 was extended by genome walking to 7,040 bp, and whole-genome sequencing of the *S. verrucosum* genome generated a 10,794-bp contig with the corresponding sequence. Their 3′-terminal end sequences had 100 % homology with segment 2 of the *S. tuberosum* mitochondrial genome, proving that Band 1 originated from a recombination-derived mitochondrial genome. Both normal and recombination-derived mitochondrial genomes co-existed in Band 1 carriers. The presence or absence of Band 1 was surveyed for four accessions of tomato and wild relatives and 172 accessions of 38 *Solanum* species or potato and tuber-bearing wild relatives, mostly from Mexican species. Together, with the results of our previous survey (mostly for South American species), we conclude that Band 1 is retained stoichiometrically only among *S. verrucosum* and Mexican polyploid species, supporting *S. verrucosum* as a maternal ancestor for all Mexican polyploid species. The presence or absence of Band 1 was not uniform within these species and was not associated with ploidy, geographical distribution, or latitude. Several evolutionary hypotheses to explain intraspecific variation were discussed.

Keywords Potato · Recombination-derived mitochondrial genome · *Solanum demissum* · *Solanum verrucosum* · Substoichiometric shifting · Tuber-bearing *Solanum* species

Introduction

Potato (*Solanum tuberosum* L.) and its tuber-bearing wild relatives (collectively classified in *Solanum* section *Petota* Dumort.) form a rich gene pool that is useful in breeding. Hawkes (1990) recognized seven cultivated and 226 wild species, which were divided into 21 taxonomic series. However, many species are highly polymorphic and their species boundaries are often obscure. Spooner et al. conducted molecular studies on species boundaries and revealed many synonyms, which significantly reduced the species number to less than half of the 233 species described by Hawkes (Spooner et al. 2009; Solanaceae Source—http://www.nhm.ac.uk/research-curation/research/projects/solanaceaesource/). Chloroplast DNA phylogeny revealed four clades: (1) Mexican (including

R. Sanetomo · K. Hosaka (✉)
Potato Germplasm Enhancement Laboratory,
Obihiro University of Agriculture and Veterinary
Medicine, Obihiro, Hokkaido 080-8555, Japan
e-mail: spudman@obihiro.ac.jp

the southern US and Central America) diploid species, exclusive of *S. bulbocastanum* Dunal, *S. cardiophyllum* Lindl., and *S. verrucosum* Schltdl.; (2) *S. bulbocastanum* and *S. cardiophyllum*; (3) all examined members of the South American series *Piurana* and some South American species classified to other series, such as *S. andreanum* Baker; and (4) all remaining South American species, Mexican polyploid species, and *S. verrucosum* (Spooner and Castillo 1997).

Almost all diploids in the tuber-bearing *Solanum* species are self-incompatible (SI), since they are controlled by the gametophytic SI locus (Pushkarnath 1942; Pandey 1962; Cipar et al. 1964; Thompson and Kirch 1992). An exception is *S. verrucosum* that can self-pollinate (Abdalla and Hermsen 1973). *Solanum verrucosum* can be crossed as a female, but not as a male, with SI species (Abdalla and Hermsen 1973; Jansky and Hamernik 2009). The Mexican polyploid species, classified into a tetraploid species group (series *Longipedicellata*) and a hexaploid species group (series *Demissa*), are highly self-fertile, but are reproductively isolated from the Mexican diploid species, except for *S. verrucosum*. These polyploid species and *S. verrucosum* can be crossed with the South American species, including cultivated potatoes, and these species have the A genome in common (Matsubayashi 1991). *Solanum stoloniferum* Schltdl. is a highly polymorphic tetraploid that belongs to the series *Longipedicellata*. Cultivars carrying *S. stoloniferum*-derived cytoplasm exhibit complete male sterility, called "tetrad sterility" (Abdalla and Hermsen 1971) or "lobed sterility" (Grun et al. 1962) because anthers shed small quantities of pollen that mostly cluster in tetrads and have a four-lobed appearance. *Solanum demissum* Lindl. is a hexaploid species that belongs to the series *Demissa* and is widely used in potato breeding as a source of resistance to the most serious disease, late blight [*Phytophthora infestans* (Montague) de Bary] (Ross 1986; Plaisted and Hoopes 1989). A pentaploid hybrid from *S. demissum* × *S. tuberosum* is readily obtained when *S. demissum* is used as the female parent. The resulting pentaploid F_1 hybrids produce abundant normal-looking pollen, but are non-functional as males and usually produce seeds only if backcrossed with the pollen of *S. tuberosum* (Dionne 1961). Thus, male gametophytes from *S. verrucosum*, *S. stoloniferum*, and *S. demissum* or genotypes that have the cytoplasm of these species exhibit peculiar behaviors during crossing. The molecular basis for their male sterility remains unknown.

Previously, we found that one amplified fragment length polymorphism band was different between reciprocal hybrids of *S. tuberosum* × *S. demissum*. This band was 170 bp in size, derived from *S. demissum*, maternally transmitted to subsequent progeny, and designated Band 1 (Sanetomo and Hosaka 2011a). Although its sequenced region was extended to 1,032 bp, it did not show any homology to known sequences. We surveyed for the presence or absence of Band 1 for 164 accessions of 36 cultivated and closely related wild species belonging to the South American taxonomic series *Yungasensa*, *Megistacroloba*, *Conicibaccata*, *Piurana*, *Tuberosa*, and *Acaulia*, two accessions of *S. pinnatisectum* Dunal (series *Pinnatisecta*), two accessions of *S. stoloniferum* (series *Longipedicellata*), and three accessions of *S. demissum* (series *Demissa*). Band 1 was detected only in three accessions of *S. demissum* (Sanetomo and Hosaka 2011b). Due to its specificity, we used Band 1 as an indicator of *S. demissum* cytoplasm when we developed a rapid identification method for potato cytoplasm types (Hosaka and Sanetomo 2012). Using this method, we evaluated the cytoplasmic diversity in varieties and breeding lines of different countries (Hosaka et al. unpublished). On the basis of our analysis, we noted that Band 1 was frequently amplified very weakly by polymerase chain reaction (PCR) from many genotypes that should not have *S. demissum* cytoplasm.

In this study, we further sequenced Band 1 containing regions to reveal its cellular origin. In addition, we conducted a comprehensive survey for the presence or absence of Band 1 in the tuber-bearing *Solanum* species, and discuss on its evolutionary implications.

Materials and methods

Plant material

A *S. tuberosum* breeding line Saikai 35 and *S. demissum* PI 186551 (referred to as T and D, respectively), F_1 plants of D × T and T × D, and BC_1 plants (D × T) × T and (T × D) × T were used to detect Band 1. The presence or absence of Band 1 was surveyed for four accessions of tomato group and

172 of 38 *Solanum* species, including non-tuber-bearing species (series *Etuberosa*), Mexican diploid series (series *Morelliformia*, *Bulbocastana*, *Pinnatisecta*, and *Polyadenia*), South American series not previously examined (series *Circaeifolia* and *Lignicaulia*), Mexican polyploid series (series *Longipedicellata* and *Demissa*), series *Piurana* and *Conicibaccata*, and *S. verrucosum* (Table 1). *Solanum* species seeds were obtained from the US Potato Genebank (NRSP-6; Sturgeon Bay, Wisconsin). The classification system of Hawkes (1990) was used throughout this text for continuity with our previous publications, although Spooner's most updated taxonomy is also given in Table 1.

Band 1 detection by PCR

Total DNA was extracted from fresh leaves using the method of Hosaka and Hanneman (1998). Overlapping sequences of Regions 1, 2, and 3 (Fig. 1) were used to detect Band 1. PCR was performed using 5 µl of the PCR mixture containing 1 µl of template DNA (~5 ng/µl), 2.5 µl of Ampdirect® Plus (Shimadzu Co., Japan), 0.125 U *Taq* DNA polymerase (BIOTAQ™ HS DNA Polymerase, Bioline Ltd., UK), and 0.3 µM primers (Table 2). For a wide survey, a granule-bound starch synthase I gene (GBSS) marker (Table 2) was included at 0.3 µM in the reaction as a positive control to check that PCR was conducted accurately. PCR consisted of one cycle of 10 min at 95 °C; 35 cycles of 30 s at 94 °C, 30 s at 60 °C, and 1 min at 72 °C; and finally, one cycle of 5 min at 72 °C on the Veriti® 96-well thermal cycler (Applied Biosystems). Gel electrophoresis and staining procedures were as described previously (Sanetomo and Hosaka 2011b).

Genome walking to extend the Band 1 sequence

The sequenced region containing Band 1 (1,032 bp; Sanetomo and Hosaka 2011b) was extended from both ends using the LA PCR™ in vitro Cloning Kit (Takara Bio Inc., Japan) and DNA Walking *SpeedUp*™ Premix Kit II (Seegene, Inc.) according to the manufacturers' instructions and using inverse PCR (Green and Sambrook 2012). PCR products were purified using the LaboPass™ PCR kit (Hokkaido System Science Co., Ltd., Sapporo, Japan) and directly sequenced.

Illumina sequencing

A genomic DNA library of a monoploid clone of *S. verrucosum* (11H23, PI 160228) was sequenced by a commercial service provider (Hokkaido System Science Co., Ltd., Sapporo, Japan). A 100-base paired-end run was performed on the Illumina Hiseq 2000 platform. All 100 bases in each read were filtered to ensure sequence quality and complexity, and generated high quality sequences with a total of 47 gigabases. After adapter-trimming, read sequences were assembled using the Velvet program (Zerbino and Birney 2008) for de novo assembly. The hash length was optimized to 75-mer, with which the Velvet assembly performed the highest contig contiguity (longer contig length) and specificity (fewer spurious overlaps). *Solanum verrucosum* contigs were BLAST-searched for homology with the extended *S. demissum* Band 1 sequence.

Distribution maps

To visualize the geographic distribution of presence or absence of Band 1, wild species accessions, the locality data obtained from the NRSP-6 database were localized on maps using DIVA-GIS software (Hijmans et al. 2001).

Results

Amplification variation of Band 1

A PCR-amplified band from Region 1 of Band 1 has been used as an indicator of *S. demissum*-derived cytoplasm among potato varieties (D marker in Hosaka and Sanetomo 2012). We frequently observed weak bands of the expected size from various species and varieties that did not carry *S. demissum*-derived cytoplasm (Fig. 2). The possibility of accidental contamination was excluded by examining all solutions, enzymes, and plastic ware used in our procedures. We performed experiments also in China and Peru. Our colleagues also examined using the same experimental protocols in their laboratories. However, this weak band was amplified randomly among samples, repeated experiments, people, and laboratories in different countries. For example in Fig. 3a, D, D × T, and (D × T) × T, all of which had *S. demissum* cytoplasm, showed clear amplified bands from Regions 1 to 3 of

Table 1 Materials used in this study

Hawkes' taxonomy (Hawkes 1990)		Spooner's taxonomy[a]		2*n*	Accession[b]
Taxonomic series	Species	Series, Section or Group	Species		
Subgenus *Lycopersicon*	*Lycopersicon esculentum* Miller	*Solanum* section Lycopersicon (Miller) Wettstein	*Solanum lycopersicum* L.	24	cv. Rutger, cv. Sugar Lump, cv. Micro-Tom
Subgenus *Eriopersicon*	*Lycopersicon peruvianum* (L.) Miller	*Solanum* section Lycopersicon (Miller) Wettstein	*Solanum peruvianum* L.	24	11H19
Series *Etuberosa* Juz.	*S. brevidens* Phil.	Section *Etuberosum* (Bukasov et Kameraz) A. Child	*S. palustre* Schltdl.	24	PI 218228
Series *Morelliformia* Hawkes	*S. morelliforme* Bitt. et Muench	Morelliforme Group	*S. morelliforme* Bitter et Muench	24	PI 275223, PI 545775
Series *Bulbocastana* (Rydb.) Hawkes	*S. bulbocastanum* Dunal	Bulbocastana Group	*S. bulbocastanum* Dunal	24	PI 498011, PI 498223, PI 243512, PI 243507
Series *Pinnatisecta* (Rydb.) Hawkes	*S. cardiophyllum* Lindl.	Bulbocastana Group	*S. cardiophyllum* Lindl.	24	PI 283063, PI 347759
	S. pinnatisectum Dunal	Pinnatisecta Group	*S. pinnatisectum* Dunal	24	PI 184764, PI 275230, PI 230489, PI 275232
Series *Polyadenia* Buk. ex Correll	*S. polyadenium* Greenm.	Polyadenia Group	*S. polyadenium* Greenm.	24	PI 347768
Series *Circaeifolia* Hawkes	*S. circaeifolium* Bitter	–	*S. circaeifolium* Bitter	24	PI 498120
Series *Lignicaulia* Hawkes	*S. lignicaule* Vargas	–	*S. lignicaule* Vargas	24	PI 473351
Series *Conicibaccata* Bitter	*S. agrimonifolium* Ryd.	Conicibaccata Group	*S. agrimonifolium* Rydb.	48	PI 243349, PI 275175, PI 558370
	S. colombianum Dunal	Conicibaccata Group	*S. colombianum* Dunal	48	PI 247322, PI 473462, PI 498150, PI 567822, PI 583312
	S. flahaultii Bitter	Conicibaccata Group	*S. flahaultii* Bitter	48	PI 498166, PI 498167, PI 558111, PI 570620, PI 583306, PI 597674
	S. longiconicum Bitter	Conicibaccata Group	*S. longiconicum* Bitter	48	PI 186568, PI 208780
	S. moscopanum Hawkes	Conicibaccata Group	*S. colombianum* Dunal	72	PI 567844, PI 567845, PI 570628
	S. oxycarpum Schiede	Conicibaccata Group	*S. oxycarpum* Schiede	48	PI 275224, PI 498272, PI 545721, PI 607852
	S. chomatophilum Bitter	Series *Piurana* Hawkes	*S. chomatophilum* Bitter	24	PI 243340, PI 266387, PI 310990, PI 365327
	S. irosinum Ochoa	Series *Piurana* Hawkes	*S. burkartii* Ochoa	24	PI 568985
	S. paucijugum Bitter	Series *Piurana* Hawkes	*S. andreanum* Baker	48	PI 561644, PI 561645, PI 561650, PI 561651, PI 583303

Table 1 continued

Hawkes' taxonomy (Hawkes 1990)		Spooner's taxonomy[a]		2*n*	Accession[b]
Taxonomic series	Species	Series, Section or Group	Species		
Series *Piurana* Hawkes	*S. acroglossum* Juz.	Series *Piurana* Hawkes	*S. acroglossum* Juz.	24	PI 498204
	S. blanco-galdosii Ochoa	Series *Piurana* Hawkes	*S. blanco-galdosii* Ochoa	24	PI 442701, PI 442702, PI 498214
	S. pascoense Ochoa	Series *Piurana* Hawkes	*S. chomatophilum* Bitter	24	PI 365339
	S. paucissectum Ochoa	Series *Piurana* Hawkes	*S. paucissectum* Ochoa	24	PI 365340, PI 473489
	S. piurae Bitter	Series *Piurana* Hawkes	*S. piurae* Bitter	24	PI 310997, PI 365365, PI 473501
	S. solisii Hawkes	Series *Piurana* Hawkes	*S. andreanum* Baker	48	PI 473472
	S. tuquerrense Hawkes	Series *Piurana* Hawkes	*S. andreanum* Baker	24	PI 246961, PI 498177, PI 583300
Series *Tuberosa* (Rydb.) Hawkes	*S. verrucosum* Schltdl.	Verrucosa Group	*S. verrucosum* Schltdl.	24	PI 160228*, PI 195170*, PI 275260*, PI 498061*, PI 545745, PI 545746, PI 545747*, PI 545810*, PI 558488*
	S. leptosepalum Correll	Longipedicellata Group	*S. stoloniferum* Schltdl.	48	PI 607843*
Series *Longipedicellata* Buk.	*S. fendleri* Asa Gray	Longipedicellata Group	*S. stoloniferum* Schltdl.	48	PI 251062*, PI 275162*, PI 283102*, PI 458418*, PI 497994*, PI 498000*, PI 498238, PI 558395*, PI 558449, PI 558484*
	S. hjertingii Hawkes	Longipedicellata Group	*S. hjertingii* Hawkes	48	PI 186559*, PI 251063*, PI 251067*, PI 545715*, PI 545768*, PI 570625*
	S. matehualae Hjerting et Tarn	Longipedicellata Group	*S. hjertingii* Hawkes	48	PI 498050*
	S. papita Rydb.	Longipedicellata Group	*S. stoloniferum* Schltdl.	48	PI 249929, PI 251740*, PI 283101, PI 498027*, PI 498030*, PI 498035, PI 545723, PI 545726*
	S. polytrichon Rydb.	Longipedicellata Group	*S. stoloniferum* Schltdl.	48	PI 184770*, PI 255547*, PI 498038*, PI 545780, PI 545788*, PI 545789*, PI 558446, PI 558447*, PI 558451*
	S. stoloniferum Schltdl.	Longipedicellata Group	*S. stoloniferum* Schltdl.	48	PI 161178, PI 186544, PI 186555*, PI 195167, PI 275248*, PI 283108, PI 310964, PI 338621*, PI 558450*, PI 558455

Table 1 continued

Hawkes' taxonomy (Hawkes 1990)		Spooner's taxonomy[a]		2*n*	Accession[b]
Taxonomic series	Species	Series, Section or Group	Species		
Series *Demissa* Buk.	*S. brachycarpum* Correll	Iopetala Group	*S. iopetalum* (Bitter) Hawkes	72	PI 230459, PI 255514, PI 275180*, PI 498020*, PI 498251, PI 545716*, PI 545772, PI 558426, PI 558430, PI 607859
	S. guerreroense Correll	Iopetala Group	*S. guerreroense* Correll	72	PI 161730*, PI 653828*
	S. hougasii Correll	Iopetala Group	*S. hougasii* Correll	72	PI 161174*, PI 161727*, PI 161726*, PI 239423*, PI 283107*, PI 558402*, PI 558422*
	S. iopetalum (Bitter) Hawkes	Iopetala Group	*S. iopetalum* (Bitter) Hawkes	72	PI 275182, PI 498249*, PI 558410, PI 570611*, PI 604098*
	S. schenckii Bitter	Iopetala Group	*S. schenckii* Bitter	72	PI 275261*, PI 498041*, PI 498049*, PI 498250*, PI 498280, PI 545733*, PI 545790*
	S. demissum Lindl.	Acaulia Group	*S. demissum* Lindl.	72	PI 160208*, PI 160220*, PI 160222*, PI 161149*, PI 161151*, PI 161155*, PI 161164*, PI 161169*, PI 161365*, PI 161366*, PI 161719*, PI 161729*, PI 175408*, PI 175423*, PI 186551*, PI 186556*, PI 201850*, PI 201853*, PI 205518*, PI 230488*, PI 230579*, PI 338619*, PI 365381*, PI 365382*

[a] Taxonomic treatment by Spooner and Hijmans (2001), Spooner et al. (2004), Peralta et al. (2008), Ames and Spooner (2010), Fajardo and Spooner (2011), and by Spooner DM (Solanaceae Source—http://www.nhm.ac.uk/research-curation/research/projects/solanaceaesource/)

[b] Band 1 carriers indicated by asterisks

Band 1, whereas T, T × D, and (T × D) × T, all of which had *S. tuberosum* cytoplasm, showed very weak and similarly sized bands randomly among samples with different Regions. These weak bands were sequenced, which demonstrated sequences that were completely identical to those of the corresponding clear bands (data not shown). When PCR amplification was extended from the usual 35 to 40 cycles, all samples produced a clear Band 1 (Fig. 3b).

Band 1 originated from the mitochondrial genome

The *S. demissum* DNA sequence harboring Band 1 (1,032 bp; Sanetomo and Hosaka 2011b) were extended from both terminal ends by genome walking to 7,040 bp (Fig. 1). Whole-genome sequencing of the *S. verrucosum* genome generated 174,235 contigs with an average length of 3,633 bases. The extended *S. demissum* sequence of Band 1 was BLAST-searched against these *S. verrucosum* contigs. One 10,794-bp contig was found to encompass the 7,040-bp *S. demissum* sequence with only 3 base substitutions in the corresponding regions (Fig. 1). A further search indicated that the 1,034-bp 3′-terminal end sequence of *S. demissum* or 1,063-bp 3′-terminal end sequence of *S. verrucosum* had 100 % homology with segment 2 of the *S. tuberosum* mitochondrial genome (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml;

Fig. 1 Schematic representation of sequences harboring Band 1. *Broken lines* indicate recombination-derived sequences

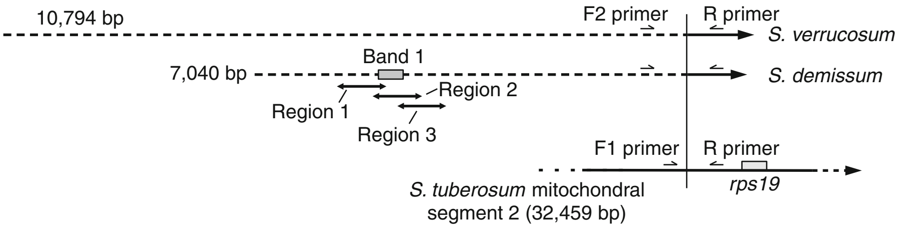


Table 2 Primer sequences used in this study

Target	Primer (5′–3′ sequence)	Size (bp)
Region 1	Band 1-F11 (CGGGAGGTGGTGTACTTTCT)	527
	Band 1-R6 (ACGGCTGACTGTGTGTTTGA)	
Region 2	Band 1-F8 (AACTTGGAAGCGAAAGCTCA)	434
	Band 1-R9 (ATTGCCGATGTCCAAGTAGG)	
Region 3	Band 1-F9 (CCCTTTGTTTGAGCCCTTGT)	446
	Band 1-R3 (GCTCCCGTTTCCCACTATTT)	
Recombination point	F1 primer (GAAAGATTGCCTTTGCCAAC)	660
	F2 primer (AGTCATTCGATTCAAACCCTTG)	749
	R primer (CCCATCACAGAGATGCACAG)	
GBSS[a]	GBSS-01 (ATGGCAAGCATCACAG)	981
	GBSS-02 (CAAAACTTTAGGTGCCTC)	

[a] Primer sequences for amplification of GBSS were obtained from Takeuchi et al. (2009)

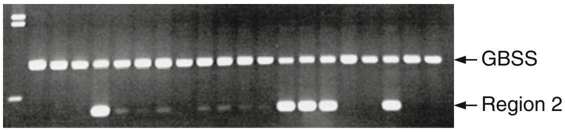


Fig. 2 Representative gel photograph for evaluating the presence of Band 1 among varieties and breeding lines. Five genotypes clearly showed the presence of Band 1, whereas weak Band 1-like bands were observed in other genotypes. The left-most lane contains λDNA *Hin*dIII digests

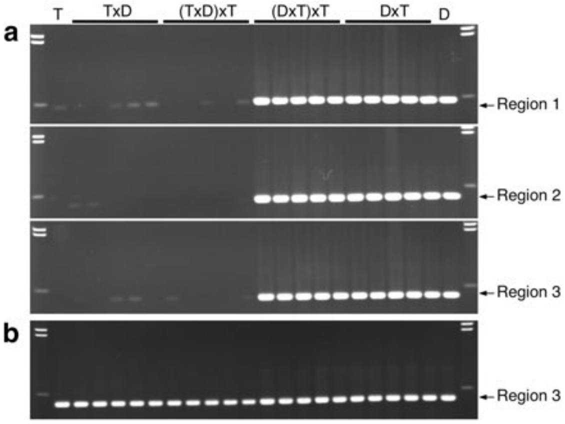


Fig. 3 Detecting Band 1 using Region 1–3 markers in *S. tuberosum* (T), *S. demissum* (D), five each of F_1 (T × D) and F_1 (D × T), and five each of BC_1 [(T × D) × T] and BC_1 [(D × T) × T]. **a** Even in T, F_1 (T × D) and BC_1 [(T × D) × T], weak bands were detected randomly among samples and regions. **b** Longer PCR amplification cycles generated the Region 3 marker band from all samples, which made it impossible to discriminate Band 1 carriers from non-carriers. The marginal lanes contain λDNA *Hin*dIII digests

Potato Genome Sequencing Consortium 2011). The 3′-terminal ends (54 bp of *S. demissum* and 83 bp of *S. verrucosum* sequences) were included in the *rps19* gene coding region (Fig. 1).

An F2 and R primer pair (Fig. 1; Table 2) amplified a 749-bp band from both *S. demissum* and *S. verrucosum*, but not from *S. tuberosum* (Fig. 4). An F1 and R primer pair (Fig. 1; Table 2) amplified a 660-bp band from not only *S. tuberosum* but also *S. demissum* and *S. verrucosum*. When PCR was subsequently run with three primers together, *S. demissum* and *S. verrucosum* amplified both 749- and 660-bp bands with approximately the same intensities (Fig. 4). The 660-bp band of *S. demissum* was sequenced and compared with the sequences of *S. tuberosum* segment 2, *S. verrucosum* contig, and extended Band 1 (Fig. 5). The 660-bp band of *S. demissum* had a sequence completely identical to that of *S. tuberosum* segment 2. However, the *S. verrucosum* contig and extended Band 1 sequences proximal beyond the point shown by the arrow in

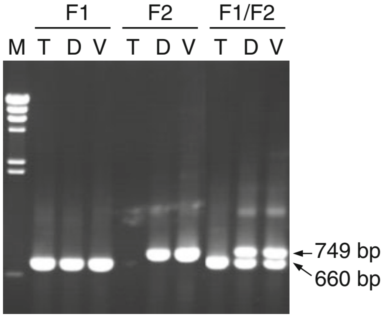


Fig. 4 PCR amplification using a *S. tuberosum* breeding line Saikai 35 (T), *S. demissum* PI 186551 (D), and *S. verrucosum* PI 160228 (V) and a pair of F1 and R1 primers (F1), a pair of F2 and R1 primers (F2), or a set of F1, F2 and R1 primers (F1/F2). M denotes λDNA *Hin*dIII digests

Fig. 5 were completely different from those of the 660-bp band of *S. demissum* and *S. tuberosum* segment 2; these showed no homology to known sequences.

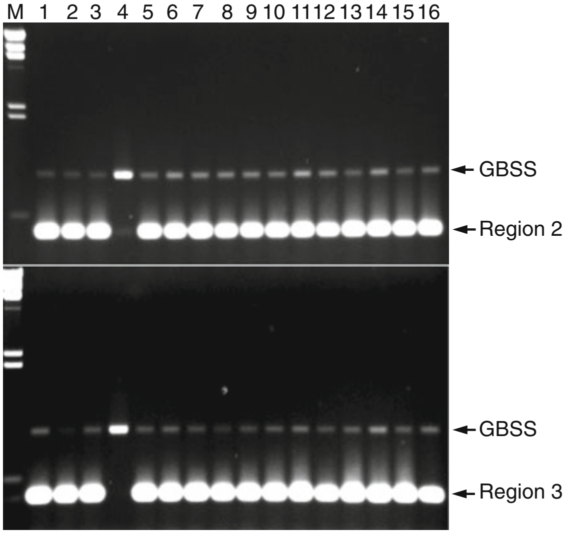
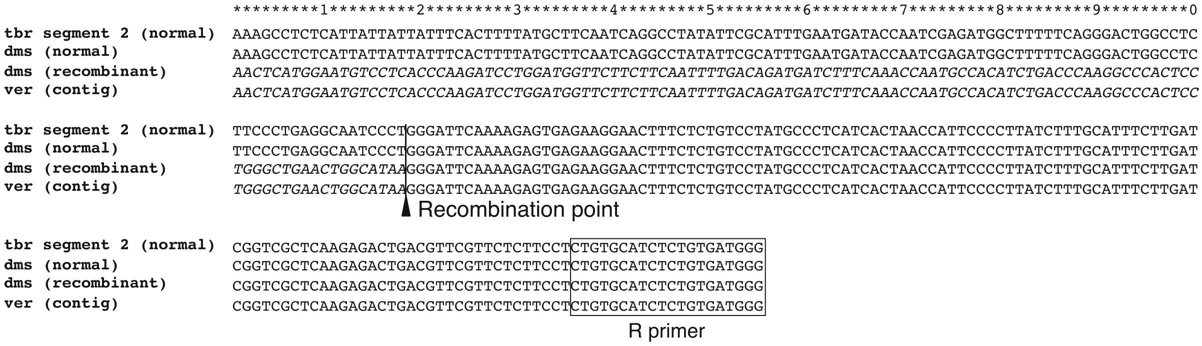


Fig. 6 Representative photographs to evaluate Mexican polyploid species using Region 2 (*upper*) and Region 3 (*lower*) markers. GBSS marker was included in the PCR run as a positive control. Different accessions of *S. schenckii* (*lanes* 1–3), *S. iopetalum* (*lanes* 4–7), and *S. demissum* (*lanes* 8–16). M denotes λDNA *Hin*dIII digests

Distribution of Band 1 among species

The presence or absence of Band 1 was surveyed for 176 accessions, among which 10 accessions of seven species were previously examined for Region 2 of Band 1 (Sanetomo and Hosaka 2011b). In this study, three primer sets (Regions 1–3) were used for all samples. However, these three primer sets gave the same results; thus, these results are shown simply as the presence or absence of Band 1 in Table 1. Band 1 was present as a clearly amplified band in 83 accessions of *S. verrucosum* and Mexican polyploid species (Table 1; Fig. 6). Neither tomato, South American potato species, nor Mexican diploid species had Band 1 (Table 1). The species of series *Conicibaccata* are distributed from Central America to South America, but Band 1 was not detected among these.

Band 1 was detected in all 24 accessions of *S. demissum*. When multiple accessions per species were examined, all accessions of *S. hjertingii* Hawkes (6 accessions), *S. guerreroense* Correll (2 accessions), and *S. hougasii* (7 accessions) exhibited Band 1. In *S. verrucosum*, however, 7 of 9 accessions exhibited Band 1. Similarly, in other Mexican polyploid species, the presence or absence of Band 1 was segregated within



```
                        *********1*********2*********3*********4*********5*********6*********7*********8*********9*********0
tbr segment 2 (normal)  AAAGCCTCTCATTATTATTATTTCACTTTTATGCTTCAATCAGGCCTATATTCGCATTTGAATGATACCAATCGAGATGGCTTTTTCAGGGACTGGCCTC
dms (normal)            AAAGCCTCTCATTATTATTATTTCACTTTTATGCTTCAATCAGGCCTATATTCGCATTTGAATGATACCAATCGAGATGGCTTTTTCAGGGACTGGCCTC
dms (recombinant)       AACTCATGGAATGTCCTCACCCAAGATCCTGGATGGTTCTTCTTCAATTTTGACAGATGATCTTTCAAACCAATGCCACATCTGACCCAAGGCCCACTCC
ver (contig)            AACTCATGGAATGTCCTCACCCAAGATCCTGGATGGTTCTTCTTCAATTTTGACAGATGATCTTTCAAACCAATGCCACATCTGACCCAAGGCCCACTCC

tbr segment 2 (normal)  TTCCCTGAGGCAATCCCTGGGATTCAAAAGAGTGAGAAGGAACTTTCTCTGTCCTATGCCCTCATCACTAACCATTCCCCTTATCTTTGCATTTCTTGAT
dms (normal)            TTCCCTGAGGCAATCCCTGGGATTCAAAAGAGTGAGAAGGAACTTTCTCTGTCCTATGCCCTCATCACTAACCATTCCCCTTATCTTTGCATTTCTTGAT
dms (recombinant)       TGGGCTGAACTGGCATAAGGGATTCAAAAGAGTGAGAAGGAACTTTCTCTGTCCTATGCCCTCATCACTAACCATTCCCCTTATCTTTGCATTTCTTGAT
ver (contig)            TGGGCTGAACTGGCATAAGGGATTCAAAAGAGTGAGAAGGAACTTTCTCTGTCCTATGCCCTCATCACTAACCATTCCCCTTATCTTTGCATTTCTTGAT
                                          Recombination point

tbr segment 2 (normal)  CGGTCGCTCAAGAGACTGACGTTCGTTCTCTTCCTCTGTGCATCTCTGTGATGGG
dms (normal)            CGGTCGCTCAAGAGACTGACGTTCGTTCTCTTCCTCTGTGCATCTCTGTGATGGG
dms (recombinant)       CGGTCGCTCAAGAGACTGACGTTCGTTCTCTTCCTCTGTGCATCTCTGTGATGGG
ver (contig)            CGGTCGCTCAAGAGACTGACGTTCGTTCTCTTCCTCTGTGCATCTCTGTGATGGG
                                                                R primer
```

Fig. 5 Comparisons of PCR product sequences from *S. demissum* using a pair of F1 and R1 primers (normal type) with the corresponding regions of extended Band 1 (recombination-derived type), *S. verrucosum* contig, and publicly available segment 2 sequences of *S. tuberosum* mitochondrial genome

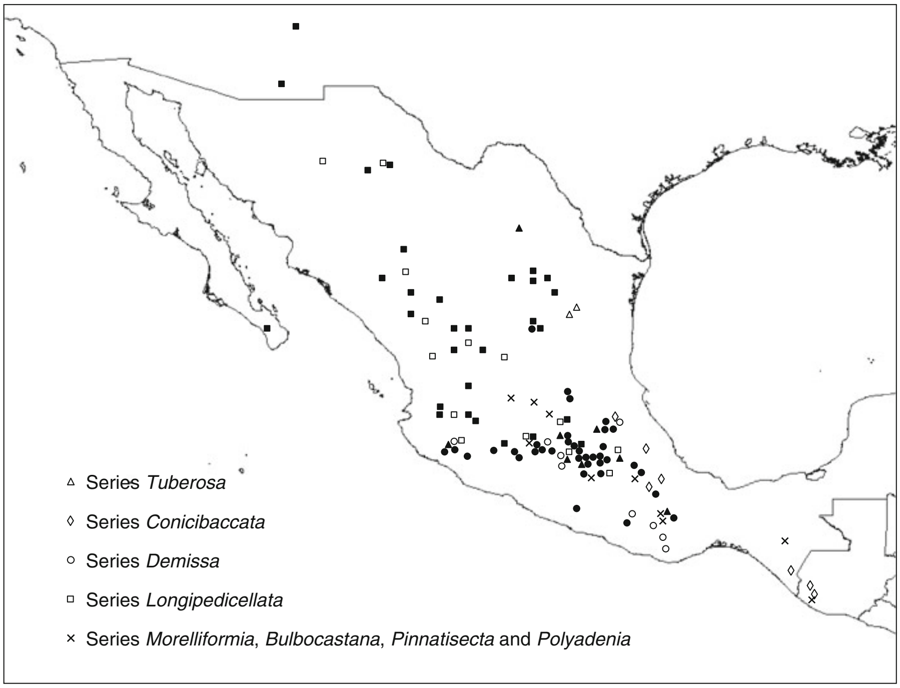


Fig. 7 Distribution of North and Central American accessions used in this study. Accessions shown by *solid triangles*, *circles*, and *squares* denote Band 1 carriers, while those shown by empty marks and *crosses* denote non-carriers

these species, although at least one accession in each species exhibited Band 1. Thus, the presence of Band 1 was not specific to *S. demissum* cytoplasm, but was widely distributed among *S. verrucosum* and Mexican polyploid species. Also, the presence or absence of Band 1 did not correlate with species or groups defined by Spooner's updated taxonomy (Table 1).

Geographical distribution of Band 1

Of 176 accessions surveyed in this study, 129 represent species that grow in North and Central America, and among them, 116 accessions of 20 species had detailed passport data; thus, their collection sites could be localized on a map (Fig. 7). Band 1 carriers (shown by solid marks) were scattered widely and randomly on this map. Most accessions were collected in the Mexican highlands. Thus, the presence or absence of Band 1 did not correlate with the collection site altitudes (data not shown).

Species distributions of series *Tuberosa* and *Longipedicellata* (Fig. 8) and *Demissa* (Fig. 9) were further investigated. The accessions of *S. verrucosum* were collected in a northern region (2 accessions) and a southern region (7 accessions); only the latter exhibited Band 1. All accessions of *S. demissum*, *S. hjertingii*, and *S. hougasii* exhibited Band 1, partly because all accessions of each species were collected in nearby vicinities. For all the other species, accessions with Band 1 were widely distributed within the species habitats and even accessions with and without Band 1 were localized close to each other.

Discussion

The present results indicated that the weak bands of Band 1 were amplified from extremely low DNA copy number (substoichiometric), while the clear bands of Band 1 were amplified from high DNA copy number (stoichiometric) in the samples. High-frequency inter- and intramolecular recombination is detected within the mitochondrial genomes of higher plants. We found that Band 1 originated in a recombination-derived mitochondrial subgenome (sometimes referred to as sublimon), and that Band 1 non-carriers also contained this subgenome with extremely low DNA copy number. A dramatic and rapid change in the relative copy number of portions of the mitochondrial genome over time of one generation is known as substoichiometric shifting

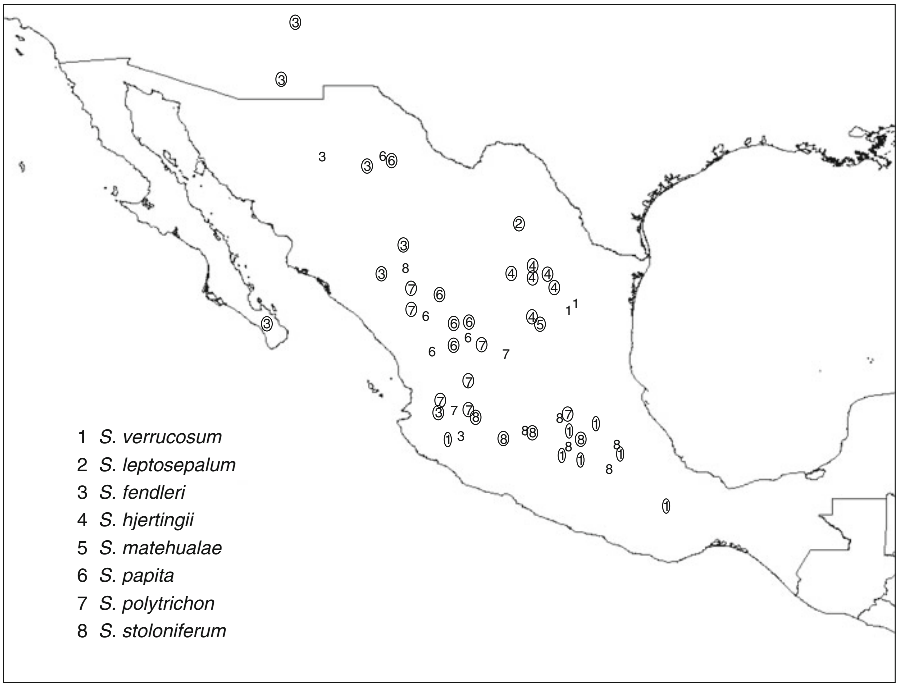


Fig. 8 Distribution of *S. verrucosum* and tetraploid species accessions. *Circled* accessions carry Band 1

(Janska et al. 1998). This often accompanies ectopic recombination, which derives chimeric sequences of mitochondrial DNA molecules (Kanazawa et al. 1994; Janska et al. 1998; Kim et al. 2007). Substoichiometric forms have been estimated at levels as low as one copy per every 100–200 cells (Arrieta-Montiel et al. 2001; Feng et al. 2009). Thus, it is likely that the Band 1-containing subgenomic mitochondrial DNA molecules are retained in the manner of substoichiometric shifting in tuber-bearing *Solanum* species.

Since the first report in maize (Small et al. 1987) of stable subgenomic mitochondrial DNA molecules within the genome at nearly undetectable levels, a recombination-derived subgenome has been found to be associated with cytoplasmic male sterility (CMS) in many plants, such as common bean (Janska et al. 1998; Arrieta-Montiel et al. 2001), *Brassica napus* (L'Homme and Brown 1993; Chen et al. 2011), *Arabidopsis* (Abdelnoor et al. 2003), *Solanum* (Scotti et al. 2004), and pearl millet (Feng et al. 2009). Chen et al. (2011) reported that in every cultivar of *Brassica napus*, both Polima (pol) and nap mitochondrial genomes were present. CMS plants primarily had a pol mitochondrial genome, and the nap mitochondrial genome was the primary genome of normal (fertile) plants. Large variations in the copy number ratios of pol and nap mitochondrial genomes were found even among cultivars sharing the same type of cytoplasm, and these ratios appeared to be maintained from generation to generation, except under conditions of CMS when the substoichiometric form may be amplified (Janska et al. 1998; Feng et al. 2009; Chen et al. 2011). *Solanum verrucosum*, *S. stoloniferum*, and *S. demissum* are self-fertile, and contradictorily, their cytoplasms are known to aberrantly affect male gametogenesis, resulting in non-functional pollen during interspecific hybridization as well as in hybrids (Dionne 1961; Grun et al. 1962; Abdalla and Hermsen 1971, 1973; Jansky and Hamernik 2009; Sanetomo et al. 2011). Mitochondrial ectopic recombination events have often been reported in a region associated with the ATPase subunit 6 (*atp6*) gene as well as with CMS in *Brassica napus* (Singh and Brown 1991; L'Homme and Brown 1993), pearl millet (Feng et al. 2009), and pepper (Kim and Kim 2006). The 5′-terminal sequence of the *S. verrucosum* contig harboring Band 1 appeared to be associated with *atp6* gene (data not shown). Thus, Band 1 or the present recombination-derived mitochondrial subgenome may be associated with male gametogenesis.

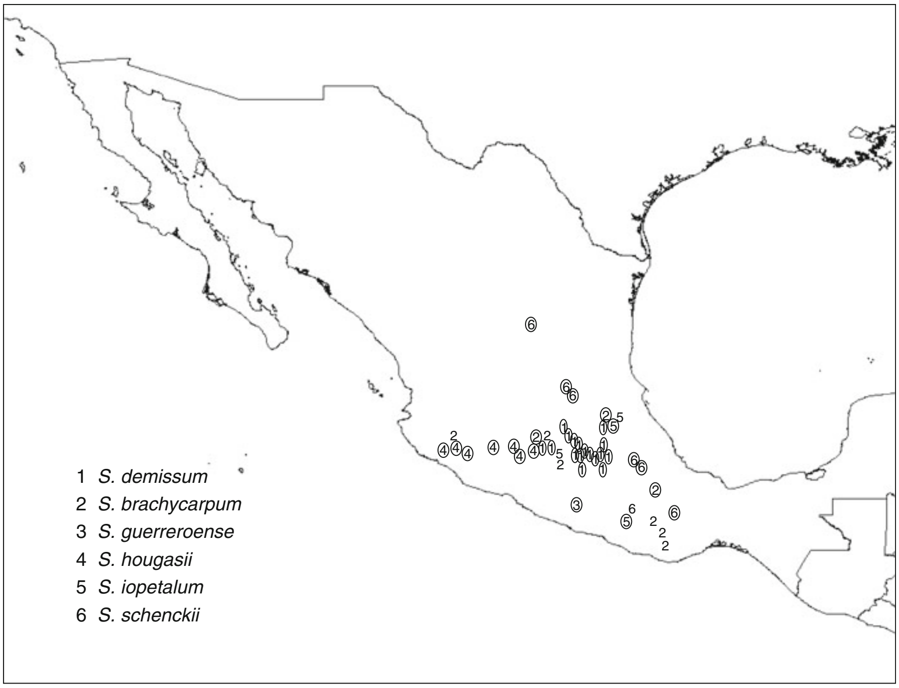


Fig. 9 Distribution of hexaploid species accessions. *Circled* accessions carry Band 1

In this study, we used all *Longipedicellata* and *Demissa* species, except for odd-ploidy species [*S. vallis-mexici* (3*x*), *S. edinense* (5*x*), and *S. semidemissum* (5*x*)]. The Band 1 sequence was found in all of the species examined, although its presence or absence was segregated within these species. Together with the results of our previous survey for cultivated and wild species in South America (Sanetomo and Hosaka 2011b), we conclude that Band 1 is shared only among *S. verrucosum* and Mexican polyploid species. Based on classical genome analysis of chromosome paring in interspecific hybrids and polyploid species, *Longipedicellata* and *Demissa* species are strict allotetraploid and allohexaploid species, respectively, and they share the A genome with *S. verrucosum* and many South American species (Matsubayashi 1991). Recently, the allotetraploid nature of *S. stoloniferum* (AABB genome) was confirmed by DNA sequence data for the GBSS gene (Spooner et al. 2008) and the nitrate reductase gene (Rodríguez and Spooner 2009) and by genomic *in situ* hybridization (GISH) analysis (Pendinen et al. 2008). DNA sequence data (Spooner et al. 2008; Rodríguez and Spooner 2009; Cai et al. 2012) and GISH analysis (Pendinen et al. 2012) also supported a taxonomic division of series *Demissa* into an allopolyploid Iopetala group (*S. guerreroense*, *S. hougasii*, *S. iopetalum* (Bitter) Hawkes, and *S. schenckii* Bitter, with component genomes A, B, and P) and an autopolyploid Acaulia group (*S. demissum*, possessing two minor variants of the A genome). *Solanum verrucosum* is the sole A genome species in North and Central America, and DNA sequence results support that *S. verrucosum* is an A genome contributor to all Mexican hexaploids (Spooner et al. 2008; Rodríguez and Spooner 2009). Chloroplast DNA analysis indicated that *S. verrucosum* and Mexican polyploids were in the same maternal lineage (Hosaka et al. 1984; Spooner and Castillo 1997). *Solanum verrucosum* was also proposed as a maternal ancestor of all Mexican polyploid species (Spooner et al. 2008; Rodríguez and Spooner 2009). The species distribution data for Band 1 also supports *S. verrucosum* as a maternal ancestor, from which all Mexican polyploid species were derived.

The Mexican polyploid species *S. demissum* is now classified into the Acaulia group together with the South American tetraploid species *S. acaule* Bitter and a hexaploid species *S. albicans* (Ochoa) Ochoa (Spooner et al. 2004). Their close relationships are well documented, based on data from morphology, AFLPs, nuclear RFLPs, and flavonoids (Spooner et al. 1995, 2004; Kardolus et al. 1998; Nakagawa and

Hosaka 2002). However, Band 1 does not support the inclusion of *S. demissum* in the Acaulia group, because at least all examined accessions of *S. acaule* (two accessions) and *S. albicans* (two accessions) did not have Band 1 (Sanetomo and Hosaka 2011b).

All Mexican polyploid species and *S. verrucosum* possessed Band 1. However, species, ploidy, geographical distance, and altitude were not associated with the presence of Band 1. Moreover, the presence or absence of Band 1 was not uniform within these species, except for *S. demissum*, *S. hjertingii*, and *S. hougasii* in which all accessions had Band 1. We have sometimes observed the non-association of specific DNA segments with evolutionary events. For example, a 241-bp deletion of chloroplast DNA was found in some accessions of unrelated species *S. berthaultii* Hawkes (=*S. tarijense* Hawkes) and *S. neorossii* Hawkes et Hjerting (Hosaka 2002). Ames et al. (2007) discovered a 41-bp deletion of chloroplast DNA among some accessions of the unrelated species *S. chiquidenum* Ochoa, *S. chomatophilum* Bitter, and *S. jalcae* Ochoa. Multivariate morphological analyses showed no morphological associations to this deletion. They suggested that there was extensive interspecific gene flow among these three species, or that there was a common evolutionary history among these species that had never been suggested to be interrelated (Ames et al. 2007). In the case of Band 1, a common maternal history can be proposed. However, this does not completely explain the intraspecific variation of Band 1 because assuming all Mexican polyploid species originated from a Band 1 carrier of *S. verrucosum*, all polyploids would probably become Band 1 carriers. To explain this intraspecific variation at least three evolutionary hypotheses can be proposed: (1) tetraploids and hexaploids were all derived from Band 1 carriers of *S. verrucosum*, and later, they lost the Band 1 subgenome (intraspecific evolution from presence to absence); (2) tetraploids and hexaploids were all derived from Band 1 non-carriers of *S. verrucosum*, and later, they acquired the Band 1 subgenome (intraspecific evolution from absence to presence); or (3) tetraploids were derived multiple times from both Band 1 carriers and non-carriers of *S. verrucosum*, and hexaploids were also derived multiple times from Band 1 carriers and non-carriers of *S. verrucosum* or tetraploids (multiple origin).

Substoichiometric shifting is known to be controlled by nuclear genes, at least in *Arabidopsis* (Martinez-Zapater et al. 1992; Abdelnoor et al. 2003; Zaegel et al. 2006; Shedge et al. 2007) and *Phaseolus vulgaris* (Mackenzie and Chase 1990). The co-existence of nuclear genes that control substoichiometric shifting and stably retain a recombination-derived subgenome appears to be adaptive in plants (Feng et al. 2009; Chen et al. 2011). Therefore, we propose a fourth, alternative hypothesis. The most ancestral diploid species, *S. verrucosum*, evolved the nuclear genes that could trigger predominant amplification of a recombination-derived subgenome to retain both Band 1 carriers and non-carriers with a balanced ratio toward an adaptive peak of these species, and these nuclear genes were preferentially transmitted to the polyploid species. Thus, the intraspecific frequency of Band 1 carriers has neither decreased nor increased; rather, it has stabilized. To examine these hypotheses for the mechanism that retains the Band 1 subgenome, it will be necessary to investigate functional differences, particularly the reproductive capabilities between Band 1 carriers and non-carriers of the same species or those with the same genetic background. It will also be necessary to determine the molecular functions of these Band 1 sequences.

Acknowledgments We thank the US Potato Genebank (NRSP-6), Sturgeon Bay, Wisconsin, for providing the *Solanum* seeds used in this study, and Dr. David M. Spooner for kindly checking taxonomic identities with his updated taxonomy and reviewing the manuscript. We also thank Dr. Itsuro Sugimura and Naoyuki Sugimoto, Hokkaido System Science Co. Ltd., for initial handling of Illumina data, and Shizuka Souma for technical assistance. This study was supported by Calbee, Inc. and Calbee Potato, Inc.

References

Abdalla MMF, Hermsen JGTh (1971) The plasmon-genic basis of pollen lobedness and tetrad sterility in *Solanum verrucosum* hybrids and duplicate linkage groups. Genetica 42:261–270

Abdalla MMF, Hermsen JGTh (1973) An evaluation of *Solanum verrucosum* Schlechtd. for its possible use in potato breeding. Euphytica 22:19–27

Abdelnoor RV, Yule R, Elo A, Christensen AC, Meyer-Gauen G, Mackenzie SA (2003) Substoichiometric shifting in the plant mitochondrial genome is influenced by a gene homologous to MutS. Proc Natl Acad Sci USA 100: 5968–5973

Ames M, Spooner DM (2010) Phylogeny of *Solanum* series *Piurana* and related species in *Solanum* section *Petota* based on five conserved ortholog sequences. Taxon 59: 1091–1104

Ames M, Salas A, Spooner DM (2007) The discovery and phylogenetic implications of a novel 41 bp plastid DNA deletion in wild potatoes. Plant Syst Evol 268:159–175

Arrieta-Montiel M, Lyznik A, Woloszynska M, Janska H, Tohme J, Mackenzie S (2001) Tracing evolutionary and developmental implications of mitochondrial stoichiometric shifting in the common bean. Genetics 158:851–864

Cai D, Rodríguez F, Teng Y, Ané C, Bonierbale M, Mueller LA, Spooner DM (2012) Single copy nuclear gene analysis of polyploidy in wild potatoes (*Solanum* section *Petota*). BMC Evol Biol 12:70

Chen J, Guan R, Chang S, Du T, Zhang H, Xing H (2011) Substoichiometrically different mitotypes coexist in mitochondrial genomes of *Brassica napus* L. PLoS One 6:e17662

Cipar MS, Peloquin SJ, Hougas RW (1964) Inheritance of incompatibility in hybrids between *Solanum tuberosum* haploids and diploid species. Euphytica 13:163–172

Dionne LA (1961) Cytoplasmic sterility in derivatives of *Solanum demissum*. Am Potato J 38:117–120

Fajardo D, Spooner DM (2011) Phylogenetic relationships of *Solanum* series *Conicibaccata* and related species in *Solanum* section *Petota* inferred from five conserved ortholog sequences. Syst Bot 36:163–170

Feng X, Kaur AP, Mackenzie SA, Dweikat IM (2009) Substoichiometric shifting in the fertility reversion of cytoplasmic male sterile pearl millet. Theor Appl Genet 118:1361–1370

Green MR, Sambrook J (2012) Molecular cloning: a laboratory manual, 4th edn: three-volume set. Cold Spring Harbor Laboratory Press, New York

Grun P, Aubertin M, Radlow A (1962) Multiple differentiation of plasmons of diploid species of *Solanum*. Genetics 47:1321–1333

Hawkes JG (1990) The potato—evolution, biodiversity and genetic resources. Belhaven Press, London

Hijmans RJ, Guarino L, Cruz M, Rojas E (2001) Computer tools for spatial analysis of plant genetic resources data: 1 DIVA-GIS. Plant Genet Resour Newsl 127:15–19

Hosaka K (2002) Distribution of the 241 bp deletion of chloroplast DNA in wild potato species. Am J Potato Res 79:119–123

Hosaka K, Hanneman RE Jr (1998) Genetics of self-compatibility in a self-incompatible wild diploid potato species *Solanum chacoense*. 2. Localization of an S locus inhibitor (*Sli*) gene on the potato genome using DNA markers. Euphytica 103:265–271

Hosaka K, Sanetomo R (2012) Development of a rapid identification method for potato cytoplasm and its use for evaluating Japanese collections. Theor Appl Genet 125: 1237–1251

Hosaka K, Ogihara Y, Matsubayashi M, Tsunewaki K (1984) Phylogenetic relationship between the tuberous *Solanum* species as revealed by restriction endonuclease analysis of chloroplast DNA. Jpn J Genet 59:349–369

Janska H, Sarria R, Woloszynska M, Arrieta-Montiel M, Mackenzie S (1998) Stoichiometric shifts in the common bean mitochondrial genome leading to male sterility and spontaneous reversion to fertility. Plant Cell 10:1163–1180

Jansky SH, Hamernik AJ (2009) The use of *Solanum verrucosum* as a bridge species. Genet Resour Crop Evol 56: 1107–1115

Kanazawa A, Tsutsumi N, Hirai A (1994) Reversible changes in the composition of the population of mtDNAs during dedifferentiation and regeneration in tobacco. Genetics 138:865–870

Kardolus JP, van Eck HJ, van den Berg RG (1998) The potential of AFLPs in biosystematics: a first application in *Solanum* taxonomy (*Solanaceae*). Plant Syst Evol 210:87–103

Kim DH, Kim BD (2006) The organization of mitochondrial *atp6* gene region in male fertile and CMS lines of pepper (*Capsicum annuum* L.). Curr Genet 49:59–67

Kim S, Lim H, Park S, Cho KH, Sung SK, Oh DG, Kim KT (2007) Identification of a novel mitochondrial genome type and development of molecular markers for cytoplasm classification in radish (*Raphanus sativus* L.). Theor Appl Genet 115:1137–1145

L'Homme Y, Brown GG (1993) Organizational differences between cytoplasmic male sterile and male fertile *Brassica* mitochondrial genomes are confined to a single transposed locus. Nucleic Acids Res 21:1903–1909

Mackenzie SA, Chase CD (1990) Fertility restoration is associated with loss of a portion of the mitochondrial genome in cytoplasmic male-sterile common bean. Plant Cell 2:905–912

Martínez-Zapater JM, Gil P, Capel J, Somerville CR (1992) Mutations at the *Arabidopsis* CHM locus promote rearrangements of the mitochondrial genome. Plant Cell 4: 889–899

Matsubayashi M (1991) Phylogenetic relationships in the potato and its related species. In: Tsuchiya T, Gupta PK (eds) Chromosome engineering in plants: genetics, breeding, evolution Part B. Elsevier, Amsterdam, pp 93–118

Nakagawa K, Hosaka K (2002) Species relationships between a wild tetraploid potato species, *Solanum acaule* Bitter, and its related species as revealed by RFLPs of chloroplast and nuclear DNA. Am J Potato Res 79:85–98

Pandey KK (1962) Interspecific incompatibility in *Solanum* species. Am J Bot 49:874–882

Pendinen G, Gavrilenko T, Jiang J, Spooner DM (2008) Allopolyploid speciation of the tetraploid Mexican potato species *S. stoloniferum* and *S. hjertingii* revealed by genomic in situ hybridization. Genome 51:714–720

Pendinen G, Spooner DM, Jiang J, Gavrilenko T (2012) Genomic in situ hybridization (GISH) reveals both autopolyploid and allopolyploid origins of different North and Central American hexaploid potato (*Solanum* section *Petota*) species. Genome 55:407–415

Peralta IE, Spooner DM, Knapp S (2008) The taxonomy of tomatoes: a revision of wild tomatoes (*Solanum* section *Lycopersicon*) and their outgroup relatives in sections *Juglandifolium* and *Lycopersicoides*. Syst Bot Monogr 84:1–186

Plaisted RL, Hoopes RW (1989) The past record and future prospects for the use of exotic potato germplasm. Am Potato J 66:603–627

Pushkarnath (1942) Studies on sterility in potatoes. 1. The genetics of self- and cross-incompatibilities. Indian J Genet Plant Breed 2:11–36

Rodríguez F, Spooner DM (2009) Nitrate reductase phylogeny of potato (*Solanum* sect. *Petota*) genomes with emphasis on the origins of the polyploid species. Syst Bot 34:207–219

Ross H (1986) Potato breeding—problems and perspectives. Verlag Paul Parey, Berlin and Hamburg

Sanetomo R, Hosaka K (2011a) Reciprocal differences in DNA sequence and methylation status of the pollen DNA between F_1 hybrids of *Solanum tuberosum* × *S. demissum*. Euphytica 182:219–229

Sanetomo R, Hosaka K (2011b) A maternally inherited DNA marker, descended from *Solanum demissum* ($2n = 6x = 72$) to *S. tuberosum* ($2n = 4x = 48$). Breed Sci 61:426–434

Sanetomo R, Ono S, Hosaka K (2011) Characterization of crossability in the crosses between *Solanum demissum* and *S. tuberosum*, and the F_1 and BC_1 progenies. Am J Potato Res 88:500–510

Scotti N, Maréchal-Drouard L, Cardi T (2004) The *rpl5-rps14* mitochondrial region: a hot spot for DNA rearrangements in *Solanum* spp. somatic hybrids. Curr Genet 45:378–382

Shedge V, Arrieta-Montiel M, Christensen AC, Mackenzie SA (2007) Plant mitochondrial recombination surveillance requires unusual RecA and MutS homologs. Plant Cell 19:1251–1264

Singh M, Brown GG (1991) Suppression of cytoplasmic male sterility by nuclear genes alters expression of a novel mitochondrial gene region. Plant Cell 3:1349–1362

Small ID, Isaac PG, Leaver CJ (1987) Stoichiometric differences in DNA molecules containing the *atpA* gene suggest mechanisms for the generation of mitochondrial genome diversity in maize. EMBO J 6:865–869

Spooner DM, Castillo RT (1997) Reexamination of series relationships of South American wild potatoes (Solanaceae: *Solanum* sect. *Petota*): evidence from chloroplast DNA restriction site variation. Am J Bot 84:671–685

Spooner DM, Hijmans RJ (2001) Potato systematics and germplasm collecting, 1989–2000. Am J Potato Res 78:237–268, 395

Spooner DM, van den Berg RG, Bamberg JB (1995) Examination of species boundaries of *Solanum* series *Demissa* and potentially related species in series *Acaulia* and series *Tuberosa* (sect. *Petota*). Syst Bot 20:295–314

Spooner DM, van den Berg RG, Rodríguez A, Bamberg J, Hijmans RJ, Lara-Cabrera SI (2004) Wild potatoes (*Solanum* section *Petota*) of North and Central America. Syst Bot Monogr 68:1–209

Spooner DM, Rodríguez F, Polgár Z, Ballard HE Jr, Jansky SH (2008) Genomic origins of potato polyploids: GBSSI gene sequencing data. Plant Genome Suppl Crop Sci 48(S1): S27–S36

Spooner DM, Ames M, Fajardo D, Rodríguez F (2009) Species boundaries and interrelationships of *Solanum* sect. Petota (wild and cultivated potatoes) are drastically altered as a result of PBI-funded research, ASA-CSSA-SSSA Annual Meeting Abstracts. Paper No 54478

Takeuchi T, Sasaki J, Suzuki T, Horita H, Hiura S, Iketani S, Fujita R, Senda K (2009) DNA markers for efficient selection of disease and pests resistance genes in potato. Hokkaido Nogyo-Shiken-Kaigi-Shiryo 2008:1–26

The Potato Genome Sequencing Consortium (2011) Genome sequence and analysis of the tuber crop potato. Nature 475:189–195

Thompson RD, Kirch HH (1992) The *S* locus of flowering plants: when self-rejection is self-interest. Trends Genet 8:381–387

Zaegel V, Guermann B, Le Ret M, Andrés C, Meyer D, Erhardt M, Canaday J, Gualberto JM, Imbault P (2006) The plant-specific ssDNA binding protein OSB1 is involved in the stoichiometric transmission of mitochondrial DNA in *Arabidopsis*. Plant Cell 18:3548–3563

Zerbino DR, Birney E (2008) Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18:821–829