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Sukhotu, Thitaporn

Hosaka, Kazuyoshi

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Origin and evolution of Andigena potatoes revealed by chloroplast and nuclear DNA markers

Thitaporn Sukhotu and Kazuyoshi Hosaka

Abstract: Andigena potatoes (*Solanum tuberosum* L. subsp. *andigena* Hawkes) ($2n = 4x = 48$) are important, native-farmer-selected cultivars in the Andes, which form a primary gene pool for improving a worldwide grown potato (*S. tuberosum* subsp. *tuberosum*). To elucidate the origin of Andigena, 196 Andigena accessions were compared with 301 accessions of 33 closely related cultivated and wild species using several types of chloroplast DNA (ctDNA) markers and nuclear DNA (nDNA) restriction fragment length polymorphism (RFLP) markers. Fourteen ctDNA types (haplotypes) and 115 RFLP bands were detected in Andigena, of which the main haplotypes and frequent RFLP bands were mostly shared with a cultivated diploid species, *S. stenotomum* Juz. et Buk. Principal component analysis of nDNA polymorphisms revealed a progressive and continuous variation from Peruvian wild species with C-type ctDNA to a group of wild species having S-type ctDNA in its variation range (*S. bukasovii*, *S. canasense*, *S. candolleanum*, and *S. multidissectum*), to cultivated diploid potatoes (*S. phureja* and *S. stenotomum*), and to cultivated tetraploid potatoes (Andigena and Chilean *S. tuberosum* subsp. *tuberosum*). These results suggest that the initial Andigena population arose with multiple origins exclusively from *S. stenotomum*. The overall evolutionary process toward the present-day Andigena was discussed.

Key words: Andigena, chloroplast DNA, nuclear DNA RFLPs, origin, sexual tetraploidization.

Résumé : Les pommes de terre Andigena (*Solanum tuberosum* subsp. *andigena* Hawkes) ($2n = 4x = 48$) constituent d'importants cultivars dans les Andes et ont été sélectionnées par les cultivateurs autochtones. Ces cultivars forment un pool génique primaire pour l'amélioration de la pomme de terre cultivée mondialement (*S. tuberosum* subsp. *tuberosum*). Afin d'élucider l'origine d'Andigena, 196 accessions Andigena ont été comparées à 301 accessions appartenant à 33 espèces cultivées très apparentées ou sauvages à l'aide de divers marqueurs RFLP au sein de l'ADN chloroplastique (ADNct) ou nucléaire (ADNn). Quatorze types d'ADNct (haplotypes) et 115 bandes RFLP ont été détectées chez Andigena. Les haplotypes prédominants et les bandes RFLP les plus fréquentes étaient habituellement partagés avec l'espèce diploïde cultivée *S. stenotomum* Juz. et Buk. Une analyse en composantes principales des polymorphismes nucléaires a révélé une relation progressive et continue entre les espèces péruviennes à ADNct de type C, un groupe d'espèces sauvages dont la variation incluait l'ADNct de type S (*S. bukasovii*, *S. candolleanum* et *S. multidissectum*), les pommes de terre cultivées diploïdes (*S. phureja* et *S. stenotomum*), jusqu'aux pommes de terre cultivées tétraploïdes (Andigena et les *S. tuberosum* subsp. *tuberosum* chiléennes). Ces résultats suggèrent que la population Andigena initiale a connu de multiples origines au sein du seul *S. stenotomum*. Le parcours évolutif ayant mené à l'Andigena actuel est discuté.

Mots clés : Andigena, ADN chloroplastique, ADN nucléaire, RFLP, origine, tétraploïdisation sexuelle.

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T. Sukhotu.[1] Graduate School of Science and Technology, Kobe University, Rokkodai, Nada, Kobe 657-8501, Japan.
K. Hosaka.[2] Food Resources Education and Research Center, Kobe University, 1348 Uzurano, Kasai, Hyogo 675-2103, Japan.

[1]Present address: Plant Genetics Conservation Project Under The Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, Chitralada Palace Dusit, Bangkok 10303, Thailand.
[2]Corresponding author (e-mail: hosaka@kobe-u.ac.jp).

Introduction

Andigena (*Solanum tuberosum* L. subspecies *andigena* Hawkes) ($2n = 4x = 48$) (the classification system of Hawkes (1990) is tentatively adopted throughout the text) is the most important tetraploid potato in the Andean highlands (2000–4000 m). It is most closely related to, and a likely ancestor of, the worldwide grown potato *S. tuberosum* L. subspecies *tuberosum* (Hawkes 1956, 1990; Brücher 1963; Hosaka and Hanneman 1988*a*; Hosaka 2004) and is thus important as a primary gene pool in potato breeding, conferring resistance to late blight, *potato virus X*, *potato virus Y*, nematodes, tuber moth, etc. (Huamán 1983; Ross 1986; Hawkes 1990). Andigena is also a good source to broaden genetic diversity and induce superior heterosis in potato (Glendinning 1969; Cubillos and Plaisted 1976; Tarn and Tai 1977; Maris 1989).

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Andigena is widely grown by native farmers from Mexico, Guatemala, Venezuela, and southwards along the Andes as far as northwestern Argentina. The North and Central American cultivars, however, were most likely introduced from South America, whereas the cultivars from Colombia to Argentina are likely native (Hawkes 1990). Andigena forms tubers only under short-day conditions, but *S. tuberosum* subsp. *tuberosum*, grown in the coastal regions in Chile (hereinafter, Chilean tuberosum) and worldwide, can form tubers under long-day conditions. According to Hawkes (1990), 6 other cultivated species are known in the Andes: diploid (*S. stenotomum* Juz. et Buk., *S. phureja* Juz. et Buk., and *S. ajanhuiri* Juz. et Buk.), triploid (*S. chaucha* Juz. et Buk. and *S. juzepczukii* Buk.), and pentaploid (*S. curtilobum* Juz. et Buk.) species. *S. stenotomum* is believed to be the most primitive diploid species of these and is still cultivated on a large scale in southern Peru and northern Bolivia (Hawkes 1990). As for ancestral species of *S. stenotomum*, various wild diploid species, most of which are closely related to each other and are controversial in their taxonomy (Spooner et al. 2005), have been proposed: *S. brevicaule* Bitt., *S. bukasovii* Juz., *S. canasense* Hawkes, *S. candolleanum* Berth., *S. coelestipetalum* Vargas, *S. gourlayi* Hawkes, S. *leptophyes* Bitt., *S. multidissectum* Hawkes, *S. multiinterruptum* Bitt., *S. sparsipilum* (Bitt.) Juz. et Buk., and *S. spegazzinii* Bitt. (Hawkes 1958, 1988, 1990; Bukasov 1966, 1978; Ugent 1970; Hawkes and Hjerting 1989; Ochoa 1990).

Very wide morphological and physiological variability has been recognized in Andigena (Salaman 1946; Hawkes 1956; Simmonds 1964; Glendinning 1968; Ugent 1970; Oliver and Martínez-Zapater 1984; Ochoa 1990). Extreme overlapping character states in Andigena form a large variable complex, which makes even separation from *S. tuberosum* subsp. *tuberosum* difficult (Simmonds 1964; Hawkes 1990; Huamán and Spooner 2002). Possibly as a result of its wide variability, the origin of Andigena has long been argued. The genetic variability in *S. stenotomum* is tremendous and is quite comparable to that of Andigena (Hawkes 1956). Consequently, Swaminathan and Magoon (1961) suggested that Andigena arose directly from *S. stenotomum* through chromosome doubling. They further mentioned that this process might have occurred several times, the parent diploid involved being of different strains or intervarietal hybrids of *S. stenotomum* or hybrids between this species and its close relatives (Swaminathan and Magoon 1961).

On the basis of the morphological similarity, Hawkes (1956, 1990) proposed that Andigena (regular calyx with relatively short sepals) originated from a hybrid between *S. stenotomum* (typically irregular calyx with relatively long sepals) and a wild diploid weed species, *S. sparsipilum* (regular calyx with small sepals). Howard (1973) supported this hypothesis because dihaploids from *S. tuberosum* showed both calyx types, although calyx types might not be good evidence because of this species' considerable variation (Woodcock and Howard 1975). The hypothesis was later strengthened by isozyme variability findings, showing that most of the isozyme electromorphs of Andigena were a combination of those found in *S. stenotomum* and *S. sparsipilum* (Oliver and Martínez-Zapater 1984), and by the fact that artificially resynthesized tetraploid hybrids from *S. stenotomum* and *S. sparsipilum*, which were completely indistinguishable from Andigena (Cribb and Hawkes 1986). On the basis of crossability and meiotic chromosome pairing, Matsubayashi (1991) suggested *S. phureja* as 1 of the ancestral species, with which *S. stenotomum* was naturally crossed, this being followed by chromosome doubling to form Andigena. As well, Brücher (1964) suggested that an Argentine wild diploid species, *S. vernei* Bitt. et Wittm., was a direct ancestor or at least 1 of the parents of Andigena.

Five chloroplast DNA (ctDNA) types (W, T, C, S, and A), distinguished by the restriction fragment patterns of ctDNA, were disclosed among Andigena accessions (Hosaka and Hanneman 1988*a*). All but T-type ctDNA were shared with *S. stenotomum* and partly with various presumed ancestral wild species (Hosaka and Hanneman 1988*b*; Hosaka 1995; Sukhotu et al. 2004). From this maternal relation, Hosaka (1995) proposed the successive domestication hypothesis: potatoes were successively domesticated, and in parallel several wild species were differentiated from time to time and place to place from a common "primitive ancestral species". Subsequent sexual polyploidization from various combinations of cultivated diploid potatoes formed wide ctDNA diversity in Andigena (Hosaka 1995). Recently, potato ctDNA was reinvestigated using high-resolution ctDNA markers (mostly microsatellite markers), which distinguished many different ctDNA types within each of W- and C-type ctDNA (Sukhotu et al. 2004, 2006). Compared with other putative ancestral wild species, *S. stenotomum* showed somewhat limited ctDNA diversity, which threw the successive domestication hypothesis into the shade (Sukhotu et al. 2006).

Polymorphisms in nuclear DNA (nDNA), as detected by restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), or microsatellite markers, are efficient at revealing both interspecific and intraspecific differences (Powell et al. 1996). Close relations among Andigena, *S. stenotomum*, and presumed wild ancestral species have been revealed using RFLP or AFLP markers (Bonierbale et al. 1990; Debener et al. 1990; Kardolus et al. 1998; Sukhotu et al. 2004). Recently, Spooner et al. (2005) investigated 264 wild species accessions (mostly members of ≈20 morphologically very similar wild species, known as the *S. brevicaule* complex) and 98 landraces (Andigena, *S. stenotomum*, *S. phureja*, and Chilean tuberosum) using AFLP markers. They showed that all landrace populations form a monophyletic clade, derived from the northern members of the *S. brevicaule* complex in Peru. None of these studies, however, tested hypotheses on the origin of Andigena.

To move toward a full understanding of the origin of Andigena, we first investigated the genetic diversity in both ctDNA and nDNA among 185 accessions of Andigena and 6 accessions of Chilean tuberosum (Sukhotu et al. 2005). Most of the nDNA RFLP bands were randomly distributed throughout the distribution area and proved that the same gene pool was shared among these widely collected accessions. Nevertheless, the geographic trend of the nDNA differentiation from north to south along the Andes could also be seen (Sukhotu et al. 2005). Finally, in the current study, the same set of ctDNA and nDNA markers were

applied to 100 accessions of *S. stenotomum* and 194 accessions of cultivated and wild species, including most members of the *S. brevicaule* complex and some others for comparison. The origin of Andigena is the focus of discussion, and we combine the results of this study with our previously obtained knowledge in an attempt to reveal the whole evolutionary history of this important Andean potato.

Materials and methods

Plant material and DNA isolation

In this study, 196 accessions of Andigena, 7 accessions of Chilean tuberosum, 100 accessions of *S. stenotomum*, 7 accessions of *S. phureja*, and 187 accessions of 31 wild species, including distantly related *S. pinnatisectum* Dun. and *S. stoloniferum* Schlechtd. et Bché, were used (Table 1). Most Andigena and Chilean tuberosum accessions were those analyzed in Sukhotu et al. (2005), and most of the others were newly prepared for this study. The wild species were chosen primarily to cover the species suggested as involved in the origin of Andigena (Brücher 1964; Hawkes and Hjerting 1989; Ochoa 1990; Matsubayashi 1991; Spooner et al. 2005). The other species were chosen on the basis of the morphological similarity, geographic features, and seed availability. Since *S. stenotomum* was likely domesticated in Peru (Sukhotu et al. 2006), Peruvian wild species were preferentially chosen. Although Andigena and Chilean tuberosum are classified as the same species (Hawkes 1990), these were treated as different taxa for convenience. Accession identity numbers are available upon request.

Most of the DNA samples of *S. stenotomum* were extracted by 1 of us (K.H.) from in-vitro-propagated plants at the International Potato Center (CIP), Lima, Peru, in November 2002. For some of the cultivated materials, DNA samples were obtained from CIP; for all the others, seeds were obtained from the Potato Introduction Station (National Research Support Program-6), Sturgeon Bay, Wis., U.S.A. Since each accession corresponded to 1 genotype or cultivar in all of *S. stenotomum*, *S. phureja*, Chilean tuberosum, and 37 accessions of Andigena, for the other seed accessions 1 seedling per accession was used. Although genetic segregation among seedlings within an accession is expected because of the heterozygous nature, a sampling strategy of 1 genotype per accession has been clarified in DNA analysis for biosystematic interpretation (Kardolus et al. 1998; Sukhotu et al. 2005). Total DNA was isolated from fresh leaves by the method described in Hosaka and Hanneman (1998).

DNA analysis

For ctDNA analysis, total DNA was digested by *Bam*HI, *Hin*dIII, or *Pvu*II and probed with potato ctDNA. Seven ctDNA microsatellite markers (NTCP 6–9, 12, 14, and 18, all developed by Provan et al. 1999) and H3 marker (developed by Hosaka 2003) were used to further reveal ctDNA polymorphisms. For nDNA RFLP analysis, 26 probes were used: TG14, TG22, TG28, TG46, TG61, TG63, TG115, TG123, TG134, TG152, TG413, TG560, P41, P101, P116, P215, P251, P357, P368, P537, P695, P697, P769, P808, P894, and P948. The TG probes were single-copy tomato probes obtained from Dr. S.D. Tanksley, Cornell University, Ithaca, N.Y. (Tanksley et al. 1992). The P probes were random genomic clones from *S. phureja* clone 1.22 (Hosaka and Spooner 1992). Detection procedures have all been described previously (Sukhotu et al. 2004).

Data analysis

ctDNA restriction fragment patterns were compared, as described by Hosaka and Hanneman (1988*b*). High-resolution ctDNA markers were scored as previously described (Sukhotu et al. 2004, 2005).

For the analysis of nDNA, only visibly reliable and polymorphic (presence versus absence) bands were used as RFLPs. Pairwise distances between species were calculated as Euclidean distances using intrataxon frequencies of respective nDNA RFLP bands, from which a dendrogram based on the unweighted pair-group method with arithmetic means (UPGMA) was constructed using PAST v. 1.14 (Hammer et al. 2001). nDNA RFLPs were also analyzed by means of a principal component analysis (PCA) using PAST, in which the presence or absence of respective RFLP bands was used as the variable for each accession.

Results

Chloroplast DNA polymorphisms

ctDNA was analyzed for all 497 accessions. Restriction fragment patterns of ctDNA obtained in this study were all similar to those reported previously (Hosaka and Hanneman 1988*a*, 1988*b*); thus, known ctDNA types (A, S, C, W, W2, and T) could be assigned to all accessions used (Table 1).

All ctDNA microsatellites generated single-fragment patterns in all accessions, and a total of 63 different fragments were detected: 11 with NTCP6, 5 with NTCP7, 6 with NTCP8, 20 with NTCP9, 7 with NTCP12, 10 with NTCP14, and 4 with NTCP18 primers.

The H3 marker generated 5 different restriction fragment patterns (types 1–5). One of the *S. ambosinum* accessions, 2 of the *S. bukasovii* accessions, 1 of the *S. dolichocremastrum* accessions, and 1 *S. orophilum* repeatedly failed to amplify for this marker. Thus, 6 phenotypes were detected with the H3 marker. Type 2 predominated in the cultivated materials except for those with W- or T-type ctDNA, which had a type-1 fragment pattern. Most wild species had either type 1 or type 2. Types 3–5 were very rare (1 of 2 *S. raphanifolium* accessions, 1 *S. acroglossum*, and 1 each of *S. acroscopicum* and *S. ambosinum* accessions, respectively).

To sum up, 75 phenotypes were distinguished, of which 37 were shared between cultivated and wild species, and 38 occurred only in wild species. The combination of 75 phenotypes discriminated 137 different ctDNA types (hereinafter referred to as haplotypes). Types A, C, S, W, and W2 ctDNA were separated into 6, 60, 5, 60, and 5 different haplotypes, respectively. T-type ctDNA was not separated further by these ctDNA microsatellites or H3 marker. Four haplotypes were shared between cultivated and wild species, and 13 and 120 were specifically found in cultivated and wild species, respectively. Ninety-four of 187 wild species accessions (50.3%) were distinguished by unique haplotypes, whereas 7 of 310 cultivated species accessions had unique haplotypes.

Table 1. *Solanum* species used in this study.

Series and species[a]	ctDNA analysis		nDNA analysis			
	ctDNA types[b]	No. of haplotypes	No. of accessions	Average no. of bands (SD)	Total no. of bands	No. of[c] shared bands
Series *Pinnatisecta* (Rydb.) Hawkes						
S. pinnatisectum Dun. (pnt) [1]	2W	2	2	29.0 (1.41)	31	20
Series *Yungasensa* Corr.						
S. tarijense Hawkes (tar) [8]	5T, 1W	2	5	21.8 (1.30)	29	22
Series *Megistacroloba* Cár d. et Hawkes						
S. boliviense Dun. (blv) [7]	1W, 1W2	2	2	23.5 (0.71)	26	23
S. megistacrolobum Bitt. (mga) [7]	4C	4	2	24.0 (4.24)	30	23
S. raphanifolium Cár d. et Hawkes (rap) [6]	2C	2	2	22.5 (3.54)	35	30
S. sogarandinum Ochoa (sgr) [6]	1W	1	1	26	26	10
Series *Conicibaccata* Bitt.						
S. chomatophilum Bitt. (chm) [6]	2W	2	2	25.0 (0.00)	32	17
S. irosinum Ochoa (irs) [6]	1W2	1	1	25	25	13
Series *Piurana* Hawkes						
S. acroglossum Juz. (acg) [6]	1W	1	1	21	21	11
S. blanco-galdosii Ochoa (blg) [6]	1W	1	1	22	22	14
Series *Tuberosa* (Rydb.) Hawkes						
S. acroscopicum Ochoa (acs) [6]	1C, 1W	2	2	23.5 (0.71)	38	27
S. ambosinum Ochoa (amb) [6]	2C	2	2	24.0 (1.41)	32	29
S. brevicaule Bitt. (brc) [7]	15W	8	15	24.0 (1.93)	50	38
S. bukasovii Juz. (buk) [6, 7]	4A, 30C, 3S, 1W	32	33	25.2 (2.76)	100	75
S. canasense Hawkes (can) [6]	15C, 6S	11	14	23.7 (2.73)	53	41
S. candolleanum Berth. (cnd) [7]	4C, 1S	4	3	22.7 (2.08)	29	28
S. coelestipetalum Vargas (cop) [6]	2C	2	2	26.0 (5.66)	38	33
S. dolichocremastrum Bitt. (dcm) [6]	2C	2	2	24.5 (2.12)	28	15
S. immite Dun. (imt) [6]	2W	2	2	22.0 (2.83)	27	14
S. leptophyes Bitt. (lph) [6, 7]	4C, 20W, 2W2	19	22	24.6 (2.94)	89	57
S. marinasense Vargas (mrn) [6]	2C	2	2	21.5 (0.71)	26	23
S. medians Bitt. (med) [6]	3C	3	3	26.7 (0.58)	42	26
S. multidissectum Hawkes (mlt) [6]	4C, 5S	7	8	25.3 (2.12)	56	43
S. multiinterruptum Bitt. (mtp) [6]	1C, 1W	2	2	22.5 (0.71)	37	25
S. oplocense Hawkes (opl) [7, 8]	6W, 1W2	6	7	29.4 (3.46)	58	43
S. orophilum Corr. (orp) [6]	1C	1	1	27	27	25
S. pampasense Hawkes (pam) [6]	2W	1	2	23.0 (0.00)	26	22
S. sparsipilum (Bitt.) Juz. et Buk. (spl) [6, 7]	9W	5	8	24.1 (1.64)	46	40
S. × *sucrense* Hawkes (scr) [7]	1A, 1W	2	2	29.5 (4.95)	40	40
S. vernei Bitt. et Wittm. (vrn) [7, 8]	11W	11	8	22.8 (2.19)	46	28
S. phureja Juz. et Buk. (phu) [4, 6, 7]	1A, 6S	2	7	28.0 (2.16)	45	45
S. stenotomum Juz. et Buk. (stn) [6, 7]	24A, 1C, 73S, 2W	7	70	30.4 (3.59)	84	74

Table 1 (*concluded*).

Series and species[a]	ctDNA analysis		nDNA analysis			
	ctDNA types[b]	No. of haplotypes	No. of accessions	Average no. of bands (SD)	Total no. of bands	No. of[c] shared bands
S. tuberosum L.						
subsp. *andigena* Hawkes (adg) [1–8]	117A, 43C, 18S, 2T, 16W	14	184	35.8 (3.84)	113	—
subsp. *tuberosum* (tbr) [9]	1A, 6T	2	6	35.7 (4.80)	63	61
Series *Longipedicellata* Buk.						
S. stoloniferum Schlechtd. et Bché (sto) [1]	2W	2	2	42.0 (1.41)	46	32

[a]Species abbreviation and locality for the accessions used given in parentheses and square brackets, respectively. 1, Mexico; 2, Guatemala; 3, Venezuela; 4, Colombia; 5, Ecuador; 6, Peru; 7, Bolivia; 8, Argentina; 9, Chile.
[b]The number of accessions prefixed to the ctDNA type (A, S, C, W, W2, or T type).
[c]Shared bands with Andigena.

Table 2. ctDNA haplotypes detected in Andigena, and the number of accessions having these haplotypes in Andigena, *S. stenotomum*, and other species (the number of accessions in parentheses).

ctDNA haplotype	Andigena	*S. stenotomum*	Others[a]
A1	115	22	buk (1), scr (1), phu (1), tbr (1)
A2	3	0	0
C1	36	1	0
C2	5	0	0
C3	1	0	0
C4	1	0	0
S	18	72	buk (1), can (6), cnd (1), mlt (1), phu (6)
T	2	0	tar (5), tbr (5)
W1	9	1	0
W2	3	0	0
W3	1	0	0
W4	1	0	brc (1), lph (2), spl (2)
W5	1	0	0
W6	1	0	0

[a]See Table 1 for species abbreviations.

Chloroplast DNA relation between Andigena and other species

Fourteen ctDNA haplotypes (A1, A2, C1, C2, C3, C4, S, T, W1, W2, W3, W4, W5, and W6) have been found in Andigena (Sukhotu et al. 2005). The haplotype A1 (previously named haplotype 1 in Sukhotu et al. 2006) was the most prevalent haplotype in Andigena (58.7%) and also 1 of the main haplotypes among accessions of *S. stenotomum* (22.0%) (Table 2), which, however, has never been found in any wild species in previous reports (Sukhotu et al. 2004, 2005). This haplotype, A1, was found in 1 accession each of *S. bukasovii* (PI 498222, from the Department of Junin, Peru) and *S.* × *sucrense* (PI 442691, from the Department of Potosi, Bolivia) (Table 2). The second major haplotype, C1 (18.4%), was found only in 1 of the *S. stenotomum* accessions (CIP 703088). The third major haplotype, S (9.2%), was the most prevalent haplotype in *S. stenotomum* (72.0%) and was found in 1 of *S. bukasovii* (PI 283084), 6 of *S. canasense* (PI 265863, PI 310956, PI 442695, PI 458375, PI 458376, and PI 473345), 1 of *S. candolleanum* (PI 498226), and 1 of *S. multidissectum* (PI 310955) accessions. Haplotype T was found only in *S. tarijense* and Chilean tuberosum, as expected, because *S. tarijense* is a hypothesized maternal ancestor conferring T ctDNA haplotype to Andigena and Chilean tuberosum (Hosaka 2004). W1 was found in 1 accession of *S. stenotomum* (CIP 702583). W4 was not found in any *S. stenotomum* accession but was found in *S. brevicaule* (PI 545971), *S. leptophyes* (PI 545986 and PI 545989), and *S. sparsipilum* (PI 498136 and PI 498137). The other 8 ctDNA haplotypes were not found in any species other than Andigena.

Fig. 1. UPGMA dendrogram showing nDNA similarity between species. ctDNA types detected in each species are also shown on the right of species abbreviations (see Table 1).

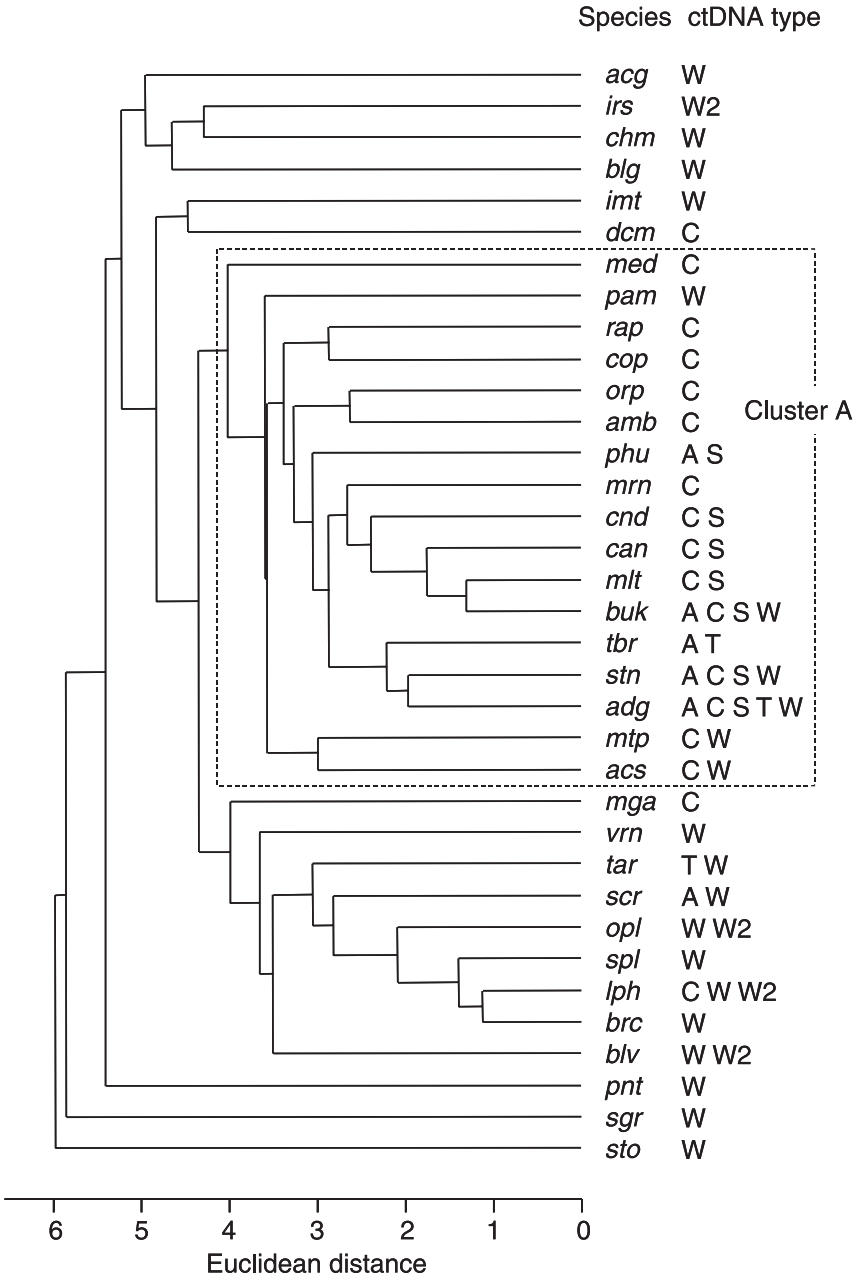


Table 3. Characterization of RFLP bands scored for Andigena.

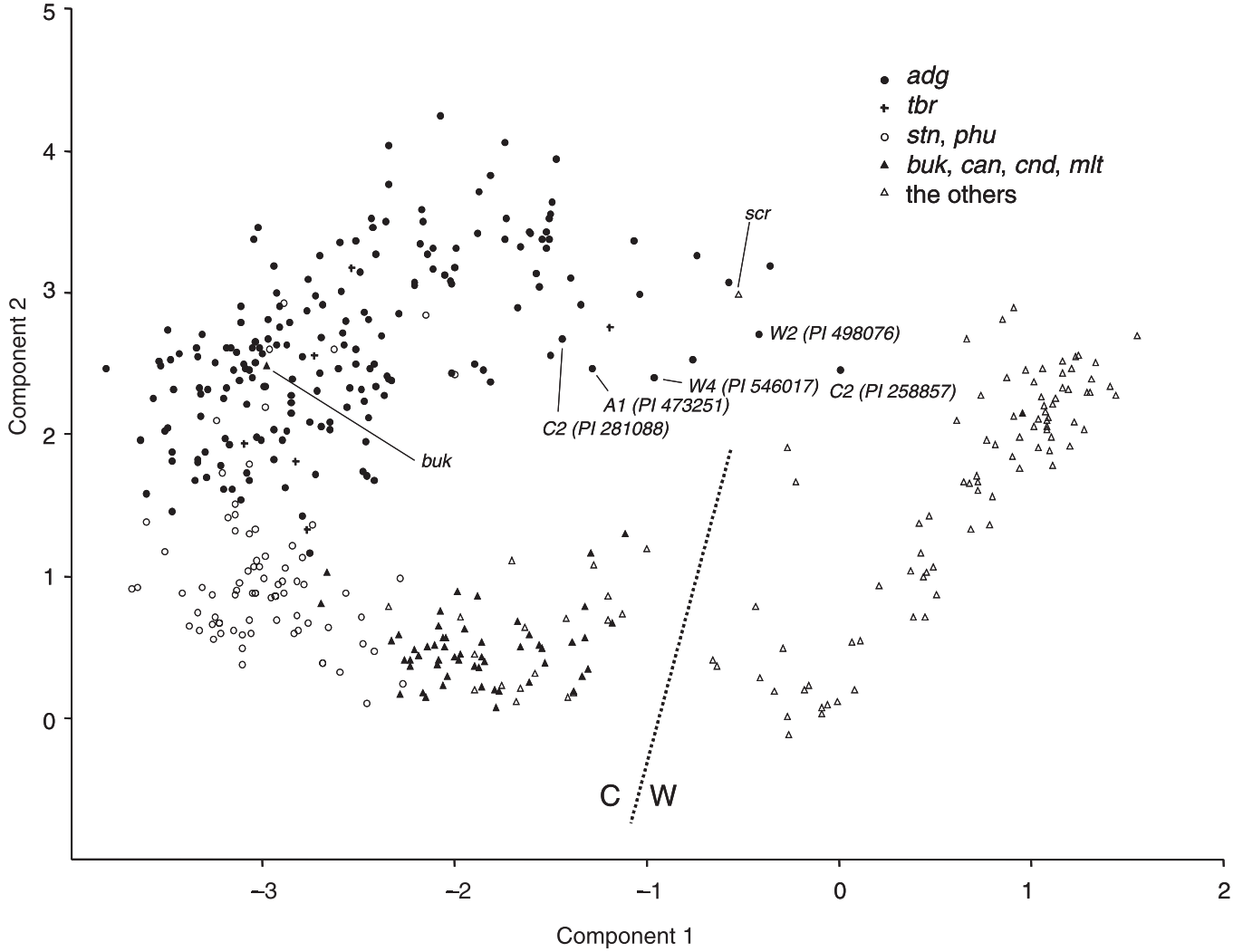
Frequency	No.	Endemic[a]	Shared with *S. stenotomum*
<20%	58	11	21
20% to 40%	23	0	21
40% to 60%	9	0	9
60% to 80%	5	0	5
80% to 100%	18	0	18
Total	113	11	74

[a]Appeared only in Andigena.

Nuclear DNA polymorphisms

Because of the poor quality or small quantity of some DNA samples, the population size used for nDNA RFLP analysis was reduced to 428 accessions (86.1%). A total of 278 polymorphic bands with an average of 30.6 bands per accession were scored, of which 65 bands were unique to single accessions. The average band number in each species ranged from 21 (*S. acroglossum*, $n = 1$) to 42.0 (*S. stoloniferum*) (Table 1). Both Andigena and Chilean tuberosum had significantly more bands than *S. stenotomum* ($t = 10.116$ and 3.356, respectively) or all the other species except *S. stoloniferum*. All 40 bands in *S.* × *sucrense* were shared with Andigena, and the others shared their bands with Andigena

Fig. 2. PCA plot by first and second principal components based on nDNA RFLPs showing relations among all accessions used (for species abbreviations, see Table 1).



with frequencies from 38.5% (*S. sogarandinum*) to 96.7% (Chilean tuberosum).

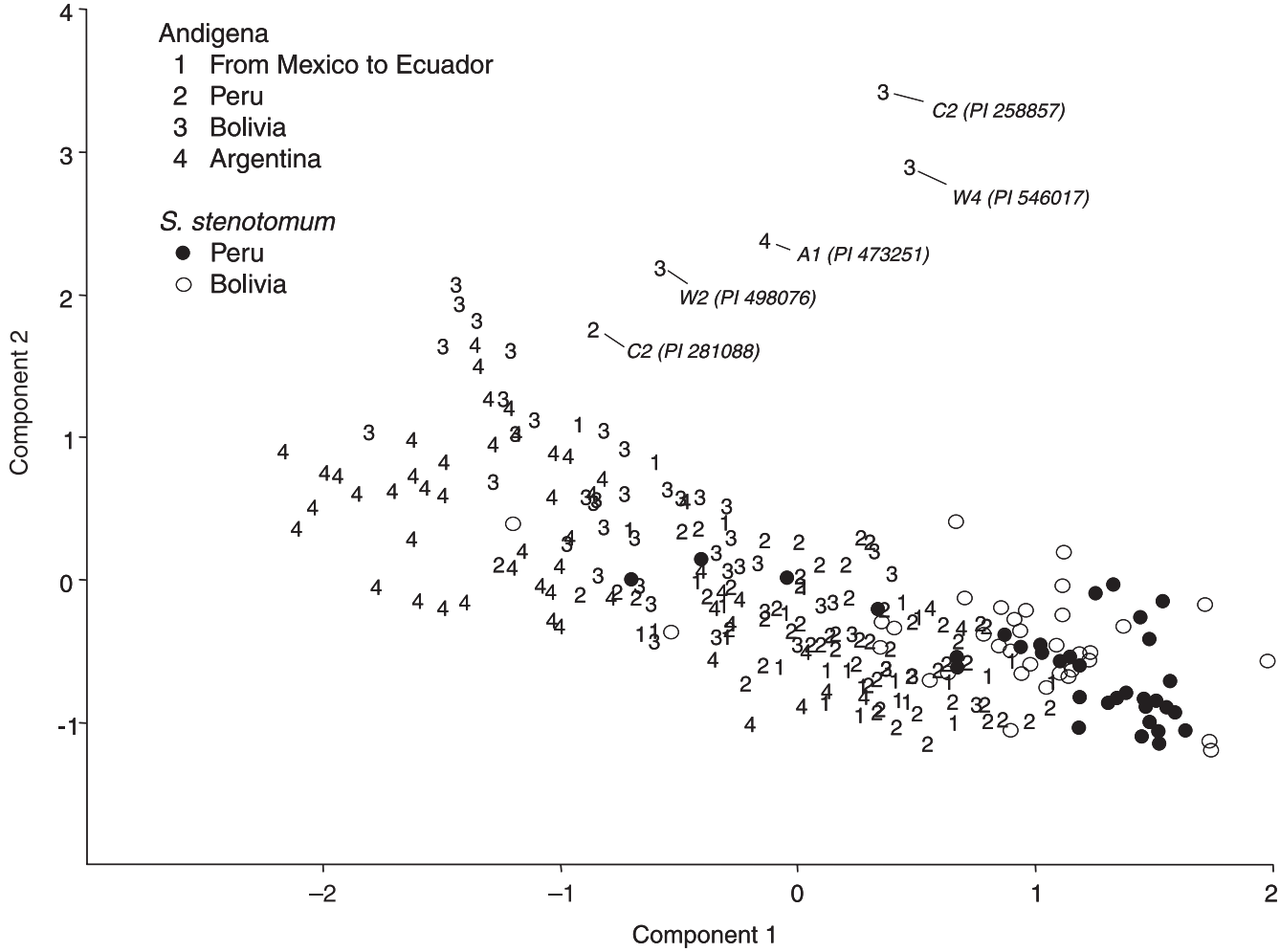
Out of 113 bands detected in Andigena, 74 bands were shared with *S. stenotomum*, and 11 bands were uniquely detected at low frequencies (<20%) (Table 3). All high-frequency (>20%) bands in Andigena were shared with *S. stenotomum* except for 2 bands (frequencies of 20.1% and 29.9%, respectively), which, as was the case with the 26 low-frequency bands, were found in some wild species.

Nuclear DNA relation between Andigena and other species

Interspecific nDNA relations were measured as Euclidean distances and shown by a UPGMA dendrogram (Fig. 1). Andigena was most closely related to *S. stenotomum* and then to Chilean tuberosum. Since the raw data used were band frequencies, no alternative dendrogram was drawn even though the sample input order was altered. Neighbor-joining methods also placed Andigena, *S. stenotomum*, and Chilean tuberosum in 1 cluster (not shown). These 3 taxa, then, formed 1 cluster with *S. bukasovii*, *S. multidissectum*, *S. canasense*, *S. candolleanum*, *S. marinasense*, and *S. phureja* (Fig. 1). This cluster contained all cultivated species and was characterized by possession of A- or S-type ctDNA within a species, except *S. marinasense* (C type alone).

The nDNA similarity among all 428 samples was analyzed by PCA. The PCA plot in Fig. 2 shows that the first and second principal components accounted for 24.1% of the total variance. A clear sample distribution gap was found, dividing accessions into 2 groups (denoted as W and C). The W group included wild species accessions having W- or W2-type ctDNA, into which *S. tarijense* (T- and W-type ctDNA), S. *megistacrolobum* (C-type ctDNA), and *S. dolichocremastrum* (C-type ctDNA) were included as exceptions. The C group consisted of all cultivated species accessions and the wild species accessions having C-, S-, or A-type ctDNA. These wild species accessions were all, except *S. candolleanum* accessions, from Peru. The only exception was *S. pampasense*, which had W-type ctDNA but was included in the C group. Thus, the C group distinguished by PCA perfectly corresponded to cluster A depicted in Fig. 1. Within the C group a continuum in variation was found from (1) Andigena and Chilean tuberosum, (2) *S. stenotomum* and *S. phureja*, (3) *S. bukasovii*, *S. canasense*, *S. candolleanum*,

Fig. 3. PCA plot by first and second principal components based on nDNA RFLPs showing genetic differentiation in Andigena and *S. stenotomum* (for species abbreviations, see Table 1).



and *S. multidissectum* (wild species having S-type ctDNA in its variation range), and (4) the other species having C-type ctDNA. Andigena accessions were distributed more frequently on the leftmost side of the plot and partially overlapped with *S. stenotomum* accessions. Toward the right direction on the plot they were sparsely and broadly distributed. One of *S. bukasovii* and 1 of *S.* × *sucrense* accessions, both having A1 ctDNA haplotype, were found within the frequently distributed area and sparsely distributed areas of Andigena, respectively (Fig. 2).

Geographic differentiation in Andigena and *S. stenotomum*

To elucidate whether the geographic differentiation in Andigena occurred independently of that of *S. stenotomum*, nDNA variations in Andigena and *S. stenotomum* accessions (296 accessions) were compared by PCA (Fig. 3). As expected, the cumulative contribution percentage for the first and second principal components was very low (12.7%), and the accessions of the 2 taxa broadly overlapped with each other. Even so, Andigena accessions from north to south along the Andes were distributed from right to left along the first principal component axis (Fig. 3). Five Andigena accessions having A1 (PI 473251), C2 (PI 258857 and PI 281088), W2 (PI 498076), or W4 (PI 546017) ctDNA haplotype were separate from the others, which were also plotted in the marginal area of the Andigena variation range in Fig. 2. Although the accessions of *S. stenotomum* overlapped mostly with the northern Andigena accessions, the northern *S. stenotomum* accessions from Peru mainly occupied the right and the southern ones from Bolivia the left along the first principal component axis.

Discussion

Ancestral species of Andigena

Most of Andigena ctDNA haplotypes, except T, were detected in *S. stenotomum* (Table 2). Most of the nDNA RFLP bands, and particularly the high-frequency bands, in Andigena were also shared with *S. stenotomum* (Table 3). Inter- and intraspecific nDNA similarity revealed an extremely close relation between Andigena and *S. stenotomum* (Fig. 1): there was continuous transition in variation from Peruvian wild species with C-type ctDNA to a group of wild species having S-type ctDNA in its variation range (*S. bukasovii*, *S. canasense*, *S. candolleanum*, and *S. multidissectum*), to cultivated diploid species (*S. phureja* and *S. stenotomum*), and to cultivated tetraploid species (Andigena and Chilean

tuberosum) (Fig. 2). These results clearly indicate that Andigena originated monophyletically from *S. stenotomum*. Thus, the present study, using both ctDNA and nDNA information obtained from a large collection of cultivated and closely related wild species accessions, strengthens the monophyletic origin hypothesis proposed by Swaminathan and Magoon (1961), Bukasov (1966), Hosaka and Hanneman (1988*b*) and Hosaka (1995). Involvement of any wild species suses 1986) or *S. vernei* (Brücher 1964) in the mainstream of the origin of Andigena appears to be excluded, because these wild species were clearly separated in nDNA differences from Andigena (Fig. 2) and did not have any of C-, S-, or A-type ctDNA (Hosaka and Hanneman 1988*b*; Sukhotu et al. 2004, this study).

A cultivated diploid species, *S. phureja*, proposed by Matsubayashi (1991) as 1 of the parents involved in the origin of Andigena, is grown in the wetter lower altitude zones of the eastern slopes of the Andes (Hawkes 1956, 1990; Ochoa 1990). The UPGMA dendrogram in this study showed a slight difference in overall nDNA similarity between *S. phureja* and *S. stenotomum* (Fig. 1), whereas the PCA could not differentiate *S. phureja* accessions as a separate group from *S. stenotomum* accessions (Fig. 2). To date, only 2 ctDNA types (S and A) (Hosaka and Hanneman 1988*b*) and no obvious geographic divergence as evaluated by RAPD markers (Ghislain et al. 1999) have been recognized in *S. phureja*, even though *S. phureja* is grown more widely than *S. stenotomum* in a long and narrow strip along the Andes from Venezuela to central Bolivia. These findings indicate that the genetic diversity in *S. phureja* is all shared with that of *S. stenotomum* and support the suggestion that *S. phureja* originated as a non-tuber-dormancy variant from *S. stenotomum* (Hawkes 1988, 1990). Lack of tuber dormancy in *S. phureja* is a dominant character and well marked in the F_1 generation (Hawkes 1956; Freyre et al. 1994), in contrast to Andigena, which has a relatively long tuber dormancy period (Hawkes 1956). Therefore, the involvement of *S. phureja* in the origin of Andigena seems less, if at all, likely.

Successive or multiple domestication versus single domestication

The successive domestication hypothesis (Hosaka 1995) was based entirely on the fact that a larger number of different types of ctDNA restriction fragment patterns were observed in *S. stenotomum* than in any 1 of the putative ancestral wild species, such as *S. bukasovii*, *S. canasense*, *S. candolleanum*, and *S. multidissectum*. However, high-resolution ctDNA markers could differentiate each of W- and C-type ctDNA into many different ctDNA haplotypes (Sukhotu et al. 2004, 2006) and disclosed that *S. stenotomum* possessed only limited ctDNA diversity (Sukhotu et al. 2006). The most prevalent S ctDNA haplotype in *S. stenotomum* was found in putative ancestral wild species distributed mostly in southern Peru (Sukhotu et al. 2006), whereas the second most prevalent A1 ctDNA haplotype was found in 1 accession of *S. bukasovii* from central Peru. Thus, our findings support multiple origins of *S. stenotomum* in Peru (Sukhotu et al. 2006) and suggest its later spread to Bolivia. In addition, nDNA similarity indicated that *S. stenotomum* was apparently differentiated from a group of *S. bukasovii*, *S. canasense*, *S. candolleanum*, and *S. multidissectum* (Fig. 2), all from Peru except *S. candolleanum*. Therefore, we should amend the successive domestication hypothesis to state simply that *S. stenotomum* was successively domesticated from putative ancestral species such as *S. bukasovii*, *S. candolleanum*, *S. canasense*, and *S. multidissectum*

Recently, Spooner et al. (2005) have supported a monophyletic origin of all landrace populations, derived from the northern members of the *S. brevicaule* complex in Peru. Since these *S. brevicaule* northern group member species are poorly defined and ongoing studies may reduce them to a single species, Spooner et al. (2005) proposed a single domestication hypothesis that involves an origin from a single species in the broad area of southern Peru. The successive domestication hypothesis seems to be in accordance with the single domestication hypothesis in the sense that domestication occurred multiple times from nearly the same wild species group in Peru. The difference between the 2 hypotheses is attributed to the controversial taxonomy for a group of ancestral wild species. Indeed, *S. bukasovii*, *S. canasense*, and *S. multidissectum* have already been considered as a single species by Ochoa (1992). Species boundary and substantial grouping of the northern member species of the *S. brevicaule* complex should be argued, but it is outside the scope of this paper.

Tetraploidization process from *S. stenotomum* to Andigena

Andigena shows tetrasomic inheritance, which is due to its auto- or segmental allo-tetraploidy nature (Howard 1970; Matsubayashi 1991). Thus, a higher number of alleles per locus can be postulated in Andigena. This genetic structure was supported by the fact that the number of scored RFLP bands was larger in Andigena (35.8) than in *S. stenotomum* (30.4) (Table 1), which might cause differences between Andigena and *S. stenotomum* on the PCA plot in Fig. 2. This highly heterozygous nature of Andigena supports the idea that Andigena arose through sexual tetraploidization between different genotypes of *S. stenotomum* ($2x \times 2x$) (Hosaka and Hanneman 1988*b*; Hosaka 1995). Since the same series of ctDNA variation was found between Andigena and diploid cultivars (Hosaka and Hanneman 1988*b*), Hosaka (1995) insisted that sexual polyploidization by the union of $2n$ gametes occurred many times in the field of diploid cultivars, resulting in Andigena with wide ctDNA diversity. The occurrence of sexual tetraploidization in potato has been suggested by higher abilities to produce $2n$ pollen and $2n$ eggs in tetraploid potatoes and closely related species (Iwanaga and Peloquin 1982; Watanabe and Peloquin 1989; Werner and Peloquin 1991) and is considered to be the most probable mode of polyploidization in potato (den Nijs and Peloquin 1977; Mendiburu and Peloquin 1977). Therefore, it is most likely that the multiple occurrence of tetraploid forms of *S. stenotomum* through sexual tetraploidization with various combinations of diploid genotypes resulted in tremendous genetic diversity in Andigena.

Parallel differentiation between Andigena and *S. stenotomum*

Did Andigena arise from *S. stenotomum* primarily in Peru with geographic differentiation occurring independently from

that of *S. stenotomum*? We found the same geographic cline from north to south along the Andes in Andigena and *S. stenotomum* (Fig. 3). Therefore, it is suggested that the genetic differentiation of Andigena did not occur independently from that of *S. stenotomum*, that is, as *S. stenotomum* spread and differentiated genetically, sexual tetraploidization likely occurred many times at many places in the fields of *S. stenotomum*, and the mass of collective tetraploid forms of *S. stenotomum* formed an initial Andigena population.

Genetic modification after Andigena arose

ctDNA haplotypes T and W4 and approximately one-quarter of nDNA RFLP bands detected in Andigena were not found in *S. stenotomum*, but were found in various wild species. This is partly because such *S. stenotomum* might have already been extinct. Some Andigena outsiders were more closely plotted to wild species accessions having W-type ctDNA (Fig. 2). The W-type ctDNA in Andigena was found mostly to be from Bolivia or marginal areas of the distribution of Andigena (Sukhotu et al. 2005). These findings support the accepted opinion that genetic modification occurred by hybridization after Andigena arose (Ugent 1970; Bukasov 1978; Oliver and Martínez-Zapater 1984; Hosaka and Hanneman 1988*b*; Sukhotu et al. 2005). Hybridization occurs frequently in cultivated potato fields, and natural hybrids are unintentionally incorporated into the cultivated potato gene pool (Ugent 1970; Jackson et al. 1977, 1980; Huamán et al. 1980; Johns and Keen 1986; Rabinowitz et al. 1990; Zimmerer and Douches 1991; Celis et al. 2004). Furthermore, Andean native farmers occasionally use true botanical seeds to renovate tubers that have degenerated because of virus infection (Quiros et al. 1992). Introgressive hybridization and (or) polyploidization through $2x \times 4x$ or $4x \times 2x$ crosses, including various wild species and *S. stenotomum* as well, could function in Andigena as a genetic sponge (Grun 1990). Andigena with T haplotype could be the result of hybridization between *S. tarijense* females and Andigena males (Hosaka 2004). A widespread Bolivian wild species, *S.* × *sucrense*, is hypothetically a fixed hybrid of Andigena × *S. oplocense* (Astley and Hawkes 1979), and, indeed, one of the *S.* × *sucrense* accessions had an A1 haplotype, a main haplotype in Andigena, but this was very rare in wild species (only this specific accession of *S.* × *sucrense* and one of *S. bukasovii*) and was found among Andigena accessions in the PCA plot (Fig. 2). Hybridization between Andigena and *S. stenotomum* could result in triploid progeny, which are grown with considerable frequency as *S. chaucha* in the native farmers' fields (Hawkes 1958; Jackson et al. 1977). These findings would reinforce the hypothesis that Andigena was modified genetically from the initially occurring tetraploid forms of *S. stenotomum*.

Additional and more frequent genetic modification could occur by intervarietal hybridization ($4x \times 4x$) and by long-distance dispersal of seed-tubers by humans (Sukhotu et al. 2005).

Conclusion

On the basis of the present and previous studies (Sukhotu et al. 2006, 2005), the origin and evolution of Andigena is concluded to be as follows: (*i*) *S. stenotomum* was first domesticated in Peru many times from wild species having S-type ctDNA, such as *S. bukasovii*, *S. canasense*, and *S. multidissectum*, and later spread to Bolivia; (*ii*) Andigena arose from *S. stenotomum* through sexual polyploidization many times at many places in the fields of *S. stenotomum*; and (*iii*) subsequent interspecific and intervarietal hybridization through $4x \times 4x$ crosses and (or) polyploidization through $2x \times 4x$ or $4x \times 2x$ crosses and human activities modified and attenuated the initial tetraploids from *S. stenotomum* alone into the present-day Andigena.

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