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# Successive domestication and evolution of the Andean potatoes as revealed by chloroplast DNA restriction endonuclease analysis

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**Abstract** Five chloroplast DNA (ctDNA) types (W, T, C, S, and A) have previously been identified in the Andean tetraploid cultivated potatoes (*Solanum tuberosum* ssp. *andigena*) and three types (C, S, and A) in diploid cultivated potatoes (*S. stenotomum*). In this study, ctDNA types were determined for an additional 35 accessions of *S. stenotomum* and 97 accessions of putative ancestral wild species (15 of *S. brevicaule*, 26 of *S. bukasovii*, 4 of *S. candolleanum*, 25 of *S. canasense*, 17 of *S. leptophyes*, and 10 of *S. multidissectum*). The first five ctDNA types were also identified in *S. stenotomum*. The wild species were also polymorphic for ctDNA types except for *S. brevicaule*, which had only W-type ctDNA. T-type ctDNA was not found in any of the wild species and could have originated from W-type ctDNA after *S. stenotomum* arose. The other types of ctDNA evolved in wild species. The geographical distribution of each ctDNA type indicated that A-type ctDNA arose in central Peru and T-type ctDNA in the Bolivia-Argentine boundary. It is implied that potatoes were successively domesticated and that, in parallel, several wild species were differentiated from time to time and place to place from the 'ancestral species' complex. Subsequent sexual polyploidization formed a wide ctDNA diversity among the Andean tetraploid potatoes, and selection from them formed the limited ctDNA diversity found in Chilean tetraploid potatoes (ssp. *tuberosum*).

**Key words** Domestication · Potato · Chloroplast DNA · *Solanum stenotomum* · Evolution

Hawkes' (1990) classification system is tentatively adopted throughout this text. Synonyms indicated by Hawkes (1990) for the species names described by various authors are presented in parentheses.

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## Introduction

The potato was raised and domesticated from wild potatoes (tuber-bearing *Solanum* species) in the Andes (Hawkes 1990). The most important Andean cultivated potato is a tetraploid, *S. tuberosum* ssp. *andigena* (referred to as ssp. *andigena* hereinafter), an immediate ancestor of the common potato (*S. tuberosum* ssp. *tuberosum*). *Solanum stenotomum* is a presumed ancestor of ssp. *andigena* and is the most primitive of the cultivated diploid species. *S. phureja* was derived from it as a non-tuber-dormancy variant (Hawkes 1988, 1990). The Andean native farmers grow them together in the fields without being conscious of their ploidy level (Brush et al. 1981; Quiros et al. 1990). In the Peruvian fields, 67% of the native varieties are tetraploids, 14% are triploids, and 13% are diploids (Quiros et al. 1990). Ongoing intogressive hybridization has been reported between *S. stenotomum* and *S. megistacrolobum* (Huamán et al. 1980; Johns and Keen 1986).

Due to the tremendous diversity of the cultivated diploids, various wild species have been suggested to be their ancestors or involved in their origin. These include *S. brevicaule*, *S. bukasovii*, *S. candolleanum*, *S. canasense*, *S. coelestipetalum*, *S. flahaultii*, *S. gourlayi*, *S. leptophyes*, *S. limbaniense*, *S. multidissectum*, *S. multiinterruptum*, *S. neovavilovii*, *S. regularifolium*, *S. sparsipilum*, *S. spegazzinii*, etc. (Hawkes 1958, 1990; Bukasov 1966, 1978; Ugent 1970; Ochoa 1990).

Chloroplast DNA (ctDNA) variation has been investigated by restriction endonuclease analysis in tuber-bearing *Solanum* species (Hosaka et al. 1984; Buckner and Hyde 1985). Five basic ctDNA types (W, T, C, S, and A types) have been identified and their evolutionary relationships elucidated among cultivated potatoes and wild relatives (Fig. 1) (Hosaka 1986). Hosaka and Hanneman (1988a) surveyed 113 accessions of ssp. *andigena* that had been collected throughout its distributional area and found an extensive ctDNA variation among them. A type was typical (62% of the accessions) in ssp.

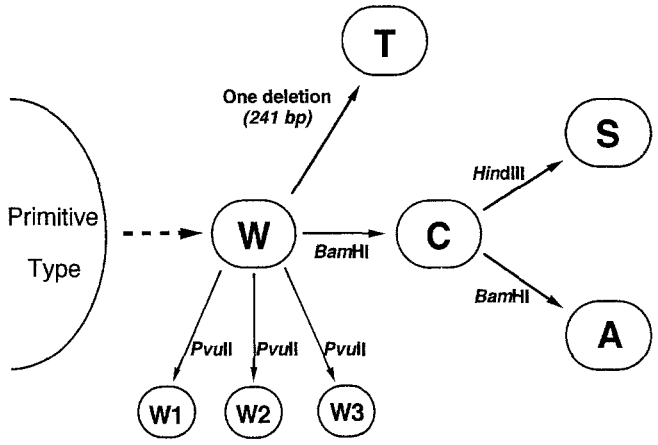


**Fig. 1** Evolutionary pathway of different ctDNA types. W type is the most primitive (Hosaka et al. 1984), from which all other ctDNA types were derived. All ctDNA types, except T type, differ from one another by a single change in the given restriction fragment pattern (Hosaka and Hanneman 1988b). T type was derived from W-type ctDNA through one deletion of 241 bp (Hosaka et al. 1988; Kawagoe and Kikuta 1991)

*andigena*, but the other four ctDNA types were also found among its accessions. A-type ctDNA has also been identified in a few accessions of the Chilean cultivated tetraploid potatoes (*S. tuberosum* ssp. *tuberosum*) and in several accessions of cultivated diploid species (Hosaka and Hanneman 1988b). Further, A-type ctDNA has been discovered in old European and Japanese cultivars, these presumably being descended from the first or very early European potato selections originating from ssp. *andigena* (Hosaka and Hanneman 1988a; Hosaka 1993).

Most of the ctDNA diversity found in ssp. *andigena* seems to be brought from the diploid cultivated species, *S. stenotomum* and *S. phureja*, which have C-, S-, and A-type ctDNAs. However, W- and T-type ctDNAs have not been found among diploid cultivated potatoes (Hosaka and Hanneman 1988b). T-type ctDNA was derived from W-type ctDNA through a single deletion (Hosaka et al. 1988), the size of which was determined to be 241 bp by Kawagoe and Kikuta (1991). T-type ctDNA is the most prevalent type in the common and Chilean potatoes and has been found in a few accessions of ssp. *andigena* but never in diploids. Thus, the deletion has been thought to occur within ssp. *andigena* after it arose (Hosaka and Hanneman 1988b). W-type ctDNA has not been found in diploid cultivated potatoes, but is common in South American wild species (Hosaka 1986). W type, thus, might have been introgressed directly from wild species to ssp. *andigena* (Hosaka and Hanneman 1988b). However, the apparent absence of W-type ctDNA among cultivated diploids might be due to the small number of accessions that have been examined, particularly in *S. stenotomum*, which supposedly has tremendous variation (Hawkes 1990; Ochoa 1990).

In the study presented here, additional accessions of *S. stenotomum* and its presumed ancestral wild species were investigated for ctDNA type in order to answer the following three questions. (1) Did the deletion event from W- to T-type ctDNA occur in ssp. *andigena*? (2) Was the W-type ctDNA in ssp. *andigena* derived from wild species via introgression or from the immediate ancestor, *S. stenotomum*? (3) Did the evolutionary events from C- to either S- or A-type ctDNA occur in *S. stenotomum* under cultivation, or did they already exist in wild species? Domestication and evolution of the potato are discussed with respect to ctDNA analysis.

## Materials and methods

Ninety-seven accessions of wild diploid species (15 of *S. brevicaule*, 26 of *S. bukasovii*, 25 of *S. canasense*, 4 of *S. candolleanum*, 17 of *S. leptophyes*, and 10 of *S. multidissectum*) and 35 accessions of *S. stenotomum* were analyzed (Table 1). The accessions of *S. stenotomum* were obtained as seeds from the International Potato Center, Lima, Peru, and all of the others were from the Potato Introduction Station (NRSP-6), Sturgeon Bay, Wisconsin, USA. Other wild ancestral species proposed by various authors were not available to this study.

For most accessions, fresh leaves were collected from more than ten seedlings and bulked for ctDNA extraction. In a few accessions, ctDNA was extracted from one seedling. CtDNA was extracted from well-expanded leaves, which results in fewer nuclear DNA contaminants, by the method of Hosaka and Hanneman (1987). The ctDNA types were determined as defined by Hosaka and Hanneman (1988b); T and A types were determined by *Bam*HI restriction patterns alone. W or its derived types (W1, W2 or W3) were distinguished by further information from *Pvu*II patterns and C or S types by *Hin*dIII patterns.

## Results

CtDNA types of 94 accessions of the wild diploid species and 35 accessions of *S. stenotomum* are listed in Table 1, together with previously published data (1 accession each for *S. bukasovii*, *S. canasense*, and *S. multidissectum*, cited from Hosaka 1986). One accession (CIP 703803) showed a mixed *Bam*HI restriction fragment pattern of S- and A-type ctDNAs. This is probably because the original seed package contained the two types by error. This accession was treated as an S-type ctDNA holder in the text, since the A-type specific fragment was of less intensity than the S-type fragment.

The number of accessions for each ctDNA type in each species is tabulated in Table 2 together with previously obtained data for *S. tuberosum*, *S. stenotomum*, *S. phureja*, and *S. sparsipilum* (Hosaka and Hanneman 1988a, b). *S. goniocalyx* in the previous paper was classified into *S. stenotomum* in this table in accordance with Hawkes' (1990) classification system. CtDNA polymorphisms existed in all of the wild species used except for *S. brevicaule*, which was monomorphic having W-type ctDNA. Four ctDNA types (W, C, S, and A) were found in *S. bukasovii*, of which the C type was predominant (88%), with W-, S-, and A-type ctDNAs identified in 1 accession each. Both C- and S-type ctDNAs were found in *S. canasense*, *S. multidissectum*, and *S. candolleanum*. Mostly W-type (71%) or C-type (24%) ctDNA was

**Table 1** The accessions of *S. stenotomum* and its presumed ancestral wild species analyzed in this study, and its ctDNA types. The species names are those listed by the NRSP-6 (Bamberg and Martin 1993); J. G. Hawkes has given different names to some accessions

| Accession | Origin[a] | CtDNA type | Accession | Origin[a] | CtDNA type |
|---|---|---|---|---|---|
| *S. brevicaule* | | | *S. candolleanum* | | |
| PI 310929 | B, coc | W | PI 498226 | B, 1p | S |
| PI 310930 | B, coc | W | PI 498227 | B, ? | C |
| PI 473378 | B, coc | W | PI 498313 | B, oru | C |
| PI 498110 | B, coc | W | PI 545972 | B, 1p | C |
| PI 498111 | B, coc | W | *S. leptophyes* | | |
| PI 498112 | B, coc | W | PI 283090 | B, 1p | W |
| PI 498113 | B, coc | W | PI 320340 | B, ? | W |
| PI 498114 | B, coc | W | PI 458378 | P, pun | W |
| PI 498115 | B, coc | W | PI 473445 | P, cuz | C |
| PI 498218 | B, 1p | W | PI 473448 | P, apu | C |
| PI 545967 | B, coc | W | PI 473451 | P, aya | C |
| PI 545968 | B, coc | W | PI 473495 | B, pot | W |
| PI 545969 | B, coc | W | PI 545895 | B, pot | W2 |
| PI 545970 | B, 1p | W | PI 545896 | B, pot | W |
| PI 545971 | B, coc | W | PI 545984 | B, oru | W |
| *S. bukasovii* | | | PI 545987 | B, pot | W |
| PI 210042 | P, jun | C | PI 545990 | B, pot | C |
| PI 210044 | P, jun | C[b] | PI 545991 | B, pot | W |
| PI 210051 | P, jun | C | PI 545992 | B, pot | W |
| PI 230506 | P, jun | C | PI 545994 | B, pot | W |
| PI 265876 | P, aya | C | PI 545995 | B, pot | W |
| PI 266385 | P, jun | C | PI 545997 | B, pot | W |
| PI 275271 | P, huo | C | *S. multidissectum* | | |
| PI 365304 | P. lim | C | PI 210043 | P, jun | C[b] |
| PI 365318 | P, huo | S | PI 210044 | P, jun | C |
| PI 365321 | P, huo | C | PI 210052 | P, aya | C |
| PI 414155 | P, apu | C | PI 210055 | P, cuz | S |
| PI 442698 | P, cuz | C | PI 310955 | P, pun | S |
| PI 458379 | P, apu | C | PI 473349 | P, cuz | C |
| PI 473447 | P, are | C | PI 473352 | P, pun | S |
| PI 473450 | P, aya | C | PI 473353 | P, cuz | S |
| PI 473452 | P, ? | C | PI 473354 | P, apu | C |
| PI 473453 | P, aya | C | PI 498304 | P, cuz | S |
| PI 473469 | P, cuz | C | *S. stenotomum* | | |
| PI 473491 | P, cuz | W | CIP 700348 | P, jun | S |
| PI 473492 | P, huv | A | CIP 700670 | P, jun | S |
| PI 473493 | P, huv | C | CIP 701165 | P, jun | S |
| PI 473494 | P, ? | C | CIP 701243 | P, jun | A |
| PI 498219 | P, jun | C | CIP 701960 | P, jun | A |
| PI 498220 | P, jun | C | CIP 701985 | P, jun | S |
| PI 498221 | P, jun | C | CIP 702033 | P, jun | A |
| PI 498222 | P, jun | C | CIP 702172 | P, jun | A |
| *S. canasense* | | | CIP 702243 | P, aya | A |
| PI 210035 | P, cuz | C | CIP 702249 | P, aya | S |
| PI 230511 | P, pun | S | CIP 702286 | B, coc | S |
| PI 246533 | P, cuz | C | CIP 702353 | P, cuz | S |
| PI 265863 | P, pun | S | CIP 702464 | P, huo | S |
| PI 265864 | P, cuz | C | CIP 702583 | B, pot | W |
| PI 265875 | P, cuz | C | CIP 702834 | P, pun | S |
| PI 283074 | P, cuz | C | CIP 703034 | P, jun | S |
| PI 283080 | P, cuz | C | CIP 703088 | P, jun | C |
| PI 283084 | P, pun | S | CIP 703151 | P, jun | S |
| PI 310937 | P, cuz | C | CIP 703197 | P, huv | S |
| PI 310938 | P, cuz | C | CIP 703225 | P, pun | S |
| PI 310939 | P, cuz | C | CIP 703286 | B, 1p | S |
| PI 310940 | P, cuz | C | CIP 703287 | P, cuz | S |
| PI 310941 | P, cuz | C | CIP 703311 | P, huv | S |
| PI 310956 | P, pun | S | CIP 703313 | P, jun | A |
| PI 442695 | P, pun | S | CIP 703319 | P, pun | S |
| PI 442696 | P, pun | C | CIP 703470 | P, aya | S |
| PI 458375 | P, pun | C[b] | CIP 703624 | P, pun | S |
| PI 458376 | P, pun | S | CIP 703637 | P, pun | S |
| PI 458377 | P, pun | C | CIP 703698 | P, jun | S |
| PI 473345 | P, pun | C | CIP 703707 | P, jun | S |
| PI 473346 | P, pun | C | CIP 703708 | P, jun | W |
| PI 473347 | P, cuz | S | CIP 703803 | P, pun | S[c] |
| PI 473348 | P, cuz | C | CIP 703843 | P, huo | A |
| PI 473355 | P, aya | C | CIP 703933 | P, cuz | A |
| | | | CIP 704089 | B, pot | T |

[a] Country codes: B, Bolivia; P, Peru
State or Department codes: Apu, Apurímac; are, Arequipa; aya, Ayacucho; coc, Cochabamba, cuz, Cusco; huv, Huancavelica; huo, Huánuco; jun, Junín; lp, La Paz; lim, Lima; oru, Oruro; pot, Potosí; pun, Puno

[b] Data cited from Hosaka (1986)

[c] Slightly mixed with A-type ctDNA. See text

**Table 2** The number of accessions with different ctDNA types in wild and cultivated species of the South American potato. Previous data (Hosaka and Hanneman 1988a, b) are included

| Species | Chloroplast DNA type | | | | | | |
|---|---|---|---|---|---|---|---|
| | T | A | S | C | W | W2 | W?[a] |
| 1) Cultivated tetraploid species | | | | | | | |
| *S. tuberosum* | | | | | | | |
| ssp. *andigena* | 5 | 70 | 14 | 16 | 5 | 0 | 3 |
| ssp. *tuberosum* | 30 | 2 | 0 | 0 | 1 | 0 | 0 |
| 2) Cultivated diploid species | | | | | | | |
| *S. stenotonum* | 1 | 10 | 39 | 2 | 2 | 0 | 0 |
| *S. phureja* | 0 | 6 | 33 | 0 | 0 | 0 | 0 |
| 3) Wild presumed ancestral species | | | | | | | |
| *S. bukasovii* | 0 | 1 | 1 | 23 | 1 | 0 | 0 |
| *S. canasense* | 0 | 0 | 7 | 18 | 0 | 0 | 0 |
| *S. multidissectum* | 0 | 0 | 5 | 5 | 0 | 0 | 0 |
| *S. leptophyes* | 0 | 0 | 0 | 4 | 12 | 1 | 0 |
| *S. candolleanum* | 0 | 0 | 1 | 3 | 0 | 0 | 0 |
| *S. brevicaule* | 0 | 0 | 0 | 0 | 15 | 0 | 0 |
| *S. sparsipilum* | 0 | 0 | 0 | 0 | 33 | 2 | 2 |

[a] Either W or W derivative type (*Pvu*II pattern not available)

found in *S. leptophyes*, but a rare W2-type ctDNA was also found in 1 accession of this species. In *S. stenotomum*, for which C-, S-, and A-type ctDNAs have previously been recognized (Hosaka and Hanneman 1988b), one T and two W types were also found. All of the five ctDNA types present in ssp. *andigena* were identified in *S. stenotomum*, and these were also identified in wild diploid species except for the T-type ctDNA.

The geographical distribution of different ctDNA types in cultivated diploid and presumed wild ancestors is shown in Table 3. Of the 10 accessions of *S. stenotomum* having A-type ctDNA, 9 were from Peru, from central Peru in particular. Six accessions of *S. phureja* having A-type ctDNA were all from Colombia, although their detailed passport data were not available. The only accession having A-type ctDNA in the wild species was *S. bukasovii* (PI 473492) from the Department of Huancavelica, Peru (Table 1). The S- and C-type ctDNAs were obtained throughout their distributional area of the cultivated and wild species. T-type ctDNA

**Table 3** Geographical distribution of different ctDNA types in the Andean diploid potatoes. The number of each ctDNA type is given by states or departments, which are arranged approximately from north to south

| Location | *S. stenotomum* | *S. phureja* | *S. bukasovii* | *S. canasense* | *S. multidissectum* | *S. leptophyes* | *S. candolleanum* | *S. brevicaule* | *S. sparsipilum* |
|---|---|---|---|---|---|---|---|---|---|
| Colombia | | | | | | | | | |
| ? | 1S | 3S, 6A | | | | | | | |
| Boyacá | | 1S | | | | | | | |
| Valle | | 1S | | | | | | | |
| Cauca | | 4S | | | | | | | |
| Nariño | | 20S | | | | | | | |
| Ecuador | | | | | | | | | |
| ? | | 1S | | | | | | | |
| Pichincha | | 1S | | | | | | | |
| Peru | | | | | | | | | |
| ? | | | 2C | | | | | | 1W |
| Amazonas | 1A | | | | | | | | |
| Huánuco | 3S, 1A | | 2C, 1S | | | | | | |
| Lima | 1S | | 1C | | | | | | |
| Junín | 9S, 5A, 1W, 1C | | 9C | | 2C | | | | |
| Huancavelica | 2S | | 1C, 1A | | | | | | |
| Ayacucho | 4S, 1A | | 3C | 1C | 1C | 1C | | | |
| Cusco | 4S, 1A | | 2C, 1W | 12C, 1S | 3S, 1C | 1C | | | 8W, 1W? |
| Apurímac | | 1S | 2C | | 1C | 1C | | | |
| Arequipa | | | 1C | | | | | | |
| Puno | 6S | | | 6S, 5C | 2S | 1W | | | |
| Bolivia | | | | | | | | | |
| ? | 6S, 1C, 1A | 1S | | | | 1W | 1C | | 3W, 1W2 |
| La Paz | 2S | | | | | 1W | 1S, 1C | 2W | 1W? |
| Cochabamba | 1S | | | | | | | 13W | 21W, 1W2 |
| Oruro | | | | | | 1W | 1C | | |
| Potosí | 1W, 1T | | | | | 8W, 1W2, 1C | | | |

was found in 1 accession of *S. stenotomum* (CIP 704089), which was collected from the Department of Potosí, Bolivia, the most southern area of the distribution of *S. stenotomum*.

## Discussion

### Origin of the five basic ctDNA types

This study showed that the five different ctDNA types found in ssp. *andigena* also exist in *S. stenotomum* (Table 2). It is therefore most likely that the wide ctDNA diversity of the cultivated tetraploid potato was introduced from cultivated diploid potatoes via sexual polyploidization (Hosaka and Hanneman 1988b). In a previous study in which we were not able to find W-type ctDNA in cultivated diploid species, we suggested the possible introgression of W-type ctDNA from wild species. However, the findings of this study expand the number of possible explanations.

All ctDNA types possessed by *S. stenotomum* were found in the wild species except for the T-type ctDNA (Table 2). Thus, ctDNA differentiation from C to either A or S occurred not under cultivation as previously thought (Hosaka and Hanneman 1988b), but instead these three types already existed among the wild species. The T-type ctDNA, or a deletion event, occurred apparently at a diploid level prior to the development of tetraploid forms (Table 2). Before we can draw the conclusion that T-type ctDNA originated after cultivated forms arose, it may be necessary to investigate more extensively intraspecific ctDNA variation in some wild species from southern Bolivia and northern Argentina, e.g., *S. kurtzianum*, *S. gourlayi*, *S. spegazzinii*, and *S. vernei*, since only 1 or 2 accessions of each have been analyzed and all had W-type ctDNA (Hosaka 1986). Brücher (1964) mentioned that on the basis of morphological similarity *S. vernei* played a very important role in the phylogeny of the cultivated potato.

### Geographical differentiation of ctDNA

*S. stenotomum* is distributed mostly from central Peru to central Bolivia at high altitudes (Hawkes 1990; Ochoa 1990). The A-type ctDNA found in this species was mostly found in accessions from central Peru, the northern edge of its distributional area. The A-type ctDNA found in *S. phureja* was all from Colombia. Thus, A-type ctDNA at a diploid level is distributed in the northern Andean region, in accordance with a geographical cline of ctDNA types disclosed among the cultivated tetraploid potatoes (Hosaka and Hanneman 1988a). The place where A-type ctDNA originated from C-type ctDNA can now be pinpointed with some accuracy to be somewhere in the central Peru because A-type ctDNA was discovered in 1 accession of *S. bukasovii* from the Department of Huancavelica, Peru (Table 3).

W-type ctDNA is common throughout the Andean region in diverse wild species, i.e., *S. chacoense*, *S. gourlayi*, *S. kurtzianum*, *S. microdontum*, *S. oplocense*, *S. spegazzinii*, and *S. vernei* (Hosaka 1986). The distribution of C- and S-type ctDNA did not indicate any obvious geographical tendency. The ctDNA differentiation from W to S via C type, therefore, might have occurred in a very early stage of species differentiation among these wild species. In contrast, the only accession having T-type ctDNA in *S. stenotomum* was from the most southern distributional area of this species in the Department of Potosí, Bolivia (southern boundary with Argentina) and was co-distributed with W-type ctDNA from which the T type originated. This coincides with the distributional pattern of T-type ctDNA at a tetraploid level: the T type was found in several accessions of ssp. *andigena* all from Argentina and prevails in Chile as Chilean ssp. *tuberosum*. Therefore, it is likely that T-type ctDNA originated in relatively recent times somewhere along the Bolivia-Argentine boundary area.

### Ancestral wild species of cultivated diploids

Hawkes (1988, 1990) stated that the most primitive-looking cultivated diploid potato is *S. stenotomum*, from which *S. phureja* was selected as non-tuber-dormancy variants adapted to lower altitudes. The evolutionary pathway from *S. stenotomum* to *S. phureja* can be supported by the fact that among the five ctDNA types of *S. stenotomum* only the most advanced S- and A-type ctDNAs were found in *S. phureja* (Hosaka and Hanneman 1988b).

The ancestral wild species which gave rise to *S. stenotomum* have long been argued over. Hawkes (1958) suggested *S. canasense*, *S. leptophyes*, and *S. soukupii* (= *S. canasense*) as the ancestral species of *S. stenotomum*. Later, he favored *S. leptophyes*, because the wildest-looking varieties of *S. stenotomum* are found at the same altitude in the same phytogeographical region with *S. leptophyes* from northern Bolivia (Hawkes 1988, 1990; Hawkes and Hjerting 1989). Since *S. stenotomum* is highly polymorphic (Hawkes 1956, 1990; Bukasov 1978; Ochoa 1990), Ugent (1970) proposed its ancestor to be a single superspecies called the '*S. brevicaule* complex', which included, as microspecies, *S. abbottianum* (= *S. bukasovii*), *S. brevicaule*, *S. bukasovii*, *S. canasense*, *S. leptophyes*, *S. liriunianum* (= *S. brevicaule*), *S. multidissectum*, *S. multiinterruptum*, *S. ochoae* (= *S. coelestipetalum*), *S. soukupii* (= *S. canasense*), *S. spegazzinii*, and *S. vidaurrei* (= *S. gourlayi*). The independent origins of *S. stenotomum* and *S. phureja* have also been proposed by Bukasov (1966, 1978); *S. canasense* and *S. leptophyes* (Bukasov 1966), or *S. brevicaule*, *S. bukasovii*, *S. candolleanum*, *S. leptophyes*, *S. soukupii*, (= *S. canasense*), *S. sparsipilum* (Bukasov 1978) giving rise to *S. stenotomum*; *S. multiinterruptum* to *S. goniocalyx* (= *S. stenotomum*); *S. candolleanum* and *S. leptophyes* (Bukasov 1966), or *S. candolleanum* alone (Bukasov 1978) to *S. phureja*; *S.*

*flahaultii* and *S. regularifolium* (Bukasov 1966), or in addition to the 2 species, *S. pascoense*, *S. paucijugum*, and *S. solisii* (Bukasov 1978) to *S. rybinii* (= *S. phureja*). Ochoa (1990) also claimed independent origins: *S. brevicaule*, *S. bukasovii*, and *S. soukupii* (= *S. canasense*) for *S. stenotomum*, and *S. candolleanum*, *S. limbaniense*, and *S. neovavilovii* for *S. phureja*. Most of the above-listed wild species are closely related to each other and many controversies exist with respect to their taxonomy (Correll 1962; Bukasov 1978; Hawkes 1990; Ochoa 1990). Regular meiosis and good pollen fertility have been reported in the diploid hybrids among these species (Swaminathan and Howard 1953; Matsubayashi 1991). Molecular approaches have not yet contributed significantly to disclosing the genetic relationships among them, although restriction fragment length polymorphism (RFLP) analysis of nuclear DNA has shown a close relationship among ssp. *andigena*, *S. stenotomum*, and *S. canasense* (Debener et al. 1990).

This study, although it does not cover all of the wild species listed above, provides genetic insights into the wild ancestral species of *S. stenotomum*. At first glance, *S. bukasovii* resembles *S. stenotomum* the most in terms of ctDNA diversity because four of the five ctDNA types are shared between them (Table 2). *S. canasense*, *S. multidissectum*, and *S. candolleanum* share C- and S-type ctDNA with *S. stenotomum*. *S. leptophyes* shares W- and C-type ctDNA with S. *stenotomum* and has W2-type ctDNA. The W2-type ctDNA has previously been identified in *S. tarijense* (Hosaka 1986) and in 2 accessions of *S. sparsipilum* (Table 2) (Hosaka and Hanneman 1988b). Thus, *S. leptophyes* seems to link the group of *S. canasense*, *S. multidissectum*, and *S. candolleanum* with *S. sparsipilum*.

The majority of the *S. brevicaule* and *S. sparsipilum* accessions have the most primitive W-type ctDNA, which is common in wild species in South America. Thus, the present study indicates that there is no positive possibility of these species being involved as a female parent in the origin of cultivated potatoes. Ugent (1970) suggested that *S. sparsipilum* could not have been involved in the initial origins of the cultivated diploid potatoes, but instead acted as a genetic bridge between the wild species and the cultivated potatoes.

On the basis of the results from this study, *S. bukasovii* seems to be the most likely ancestral speices of *S. stenotomum* (Table 2). It should be possible to confirm this in future analyses comparing the frequency of A-type ctDNA in *S. bukasovii* and other species. It should be mentioned that a series of diploid wild species, i.e., *S. bukasovii*, *S. canasense*, *S. candolleanum*, *S. leptophyes*, and *S. multidissectum*, remain as possible candidates contributing to the origin of cultivated potatoes.

## Successive domestication of potatoes

As is shown schematically in Fig. 2, the spectra of ctDNA diversity are parallel in wild species, cultivated diploids and cultivated tetraploids. The most probable domestication process of the Andean potatoes could be inferred to be as follows: potato tubers were first taken from the 'ancestral species' complex and raised as cultivated forms by human beings; these should have had the W-type ctDNA that is common to other South American wild species. As the 'ancestral species' evolved, C-type ctDNA was derived from the W type, which was then domesticated and incorporated into the stock of cultivated forms. If the old stocks were not too clearly inferior to the newly domesticated ctDNA genotypes, both could be vegetatively maintained by farmers. At this stage, some genotypes of the 'ancestral species' that adapted well to a particular environment became *S. leptophyes*. After S-type ctDNA occurred in the 'ancestral species', these genotypes were introduced into the cultivated gene pool. On the other hand, some genotypes were differentiated into *S. canasense* and *S. multidissectum* in Peru and *S. candolleanum* in Bolivia. The most recent event that occurred in the 'ancestral species' in terms of ctDNA evolution was the derivation of A-type ctDNA in central Peru, thereby giving rise to cultivated forms with A-type ctDNA. *S. bukasovii* might have differentiated at this stage from the 'ancestral species'.

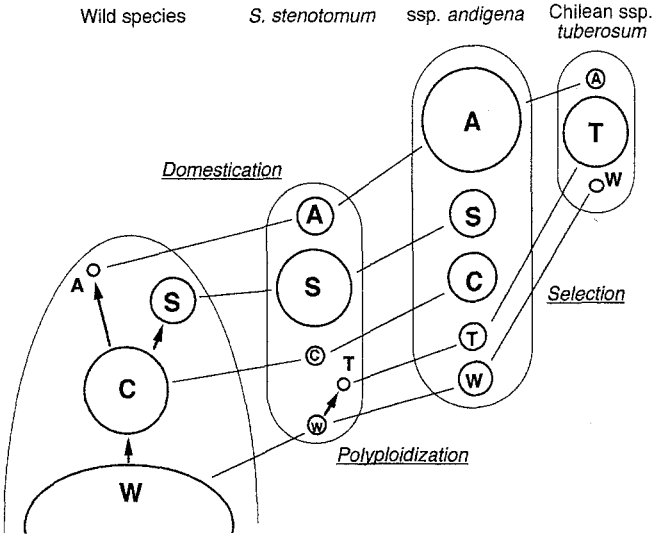


**Fig. 2** A schematic representation of the evolution of Andean and Chilean potatoes. The size of *circles* occupied by the respective ctDNA types corresponds to the number of accessions except for that of W type in the wild species

It seems more appropriate to assume the existence of a primitive 'ancestral species' as the common ancestor to both wild and cultivated species than to assume that existing wild species were the ancestors of cultivated species. Thus, the Andean potatoes might have been successively domesticated and, in parallel, several wild species might have differentiated from time to time and place to place from the 'ancestral species' complex. Differentiation of ctDNA could have occurred in the 'ancestral species' complex. This successive domestication over a long period of time might have accumulated different genotypes that have been maintained vegeta-

tively as cultivated stocks, resulting in a highly polymorphic cultigen. Ochoa (1990) has stated "...the origin of this species (*S. stenotomum*) may have been polyphyletic. Possibly, the plants comprising this cultigen may have arisen in more than one place and at more than one time from such commonly distributed wild species as *S. bukasovii*, *S. soukupii* (= *S. canasense*), and *S. brevicaule*."

## Evolutionary process and genetic diversity

Subsequent polyploidization occurred many times in the field of diploid cultivars (Swaminathan and Magoon 1961) and formed a series of ctDNA variation in ssp. *andigena* similar to that in diploid cultivars (Hosaka and Hanneman 1988b). It is highly probable that sexual polyploidization by the union of 2n gametes contributed to the origin and evolution of the cultivated tetraploid potatoes. Higher abilities to produce 2n pollens and eggs in tetraploid potatoes have been reported (Iwanaga and Peloquin 1982; Watanabe and Peloquin 1989; Werner and Peloquin 1991). Various combinations of diploid genotypes through sexual polyploidization could result in tremendous genetic diversity in ssp. *andigena* (den Nijs and Peloquin 1977).

Chilean ssp. *tuberosum* probably originated from ssp. *andigena* by selection (Hawkes 1956, 1990; Brücher 1963; Hosaka and Hanneman 1988a). An independent origin from wild species (Bukasov 1966, 1978; Sykin 1971; Ugent et al. 1987) or a hybrid origin between ssp. *andigena* and an unknown species as a female (Grun 1990) has also been proposed as the origin of Chilean ssp. *tuberosum*. As summarized in Fig. 2, the spectrum of ctDNA diversity in Chilean ssp. *tuberosum* appears to be parallel with that in ssp. *andigena* but is significantly limited. This supports the hypothesis that the Chilean ssp. *tuberosum* was essentially a collection of selected forms of the southern ssp. *andigena*.

Therefore, it can be suggested that the majority of genetic diversity seen in present-day Andean and Chilean native cultivars might be attributed to the evolutionary process giving rise to their origin; that is, successive domestication for diploid cultigens, sexual polyploidization for ssp. *andigena*, and selection for Chilean ssp. *tuberosum*. Further genetic diversity could have been introduced by introgressive hybridization and/or polyploidization (Ugent 1970; Bukasov 1978; Hosaka and Hanneman 1988b; Grun 1990). It has been demonstrated that gene flow occurred from both *S. sparsipilum* (Rabinowitz et al. 1990) and *S. megistacrolobum* (Huamán et al. 1980; Johns et al. 1987) to cultivated diploid potatoes. Grun (1990) described ssp. *andigena* as genetic sponge that absorbs genes via introgression from any wild and cultivated species with which it hybridized via 2x × 4x or 4x × 2x crosses.

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