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Origin of chloroplast DNA diversity in the Andean potatoes

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Summary. Wide chloroplast DNA (ctDNA) diversity has been reported in the Andean cultivated tetraploid potato, *Solanum tuberosum* ssp. *andigena.* Andean diploid potatoes were analyzed in this study to elucidate the origin of the diverse ctDNA variation of the cultivated tetraploids. The ctDNA types of 58 cultivated diploid potatoes (*S. stenotomum, S. goniocalyx* and *S. phureja*), 35 accessions of *S. sparsipilum,* a diploid weed species, and 40 accessions of the wild or weed species, *S. chacoense,* were determined based on ctDNA restriction fragment patterns of *Bam*HI, *Hin*dIII and *Pvu*II. Several different ctDNA types were found in the cultivated potatoes as well as in weed and wild potato species; thus, intraspecific ctDNA variation may be common in both wild and cultivated potato species and perhaps in the higher plant kingdom as a whole. The ctDNA variation range of cultivated diploid potatoes was similar to that of the tetraploid potatoes, suggesting that the ctDNA diversity of the tetraploid potato could have been introduced from cultivated diploid potatoes. This provided further evidence that the Andean cultivated tetraploid potato, ssp. *andigena,* could have arisen many times from the cultivated diploid populations. The diverse but conserved ctDNA variation noted in the Andean potatoes may have occurred in the early stage of species differentiation of South American tuber-bearing Solanums.

Key words: Chloroplast DNA – Intraspecific variation – *Solanum tuberosum* ssp. *andigena* – Tuber-bearing solanums – Potato

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Introduction

Interspecific chloroplast DNA (ctDNA) variation is common, although the evolutionary change of ctDNA is much slower than that of nuclear and mitochondrial DNA (Banks and Birky 1985; Palmer et al. 1985; Zurawski et al. 1984). By using such interspecific variation, various crop species have been investigated for their maternal phylogenetic relationships, i.e., *Brassica* (Erickson et al. 1983; Palmer et al. 1983), *Coffea* (Berthou et al. 1983), *Cucumis* (Perl-Treves and Galun 1985), *Lycopersicon* (Palmer and Zamir 1982), *Nicotiana* (Kung et al. 1982), *Pisum* (Palmer et al. 1985), tuber-bearing *Solanum* (Hosaka et al. 1984), *Triticum* and *Aegilops* (Ogihara and Tsunewaki 1982; Bowman et al. 1983), etc. In these studies, however, relatively small sample size, frequently only one accession, was used to represent each species. Clegg et al. (1984b) detected no ctDNA variation in fairly large samples of pearl millet in which 12 geographically diverse collections were analyzed. Scowcroft (1979) reported the first instance of intraspecific ctDNA variation in *Nicotiana debneyi.* Since then, intraspecific variation has been reported by others (Timothy et al. 1979; Clegg et al. 1984a).

In the potato, great ctDNA diversity was found in the Andean cultivated tetraploid potato, *Solanum tuberosum* ssp. *andigena,* in contrast to a uniform ctDNA type of *S. tuberosum* ssp. *tuberosum,* the common and Chilean potato (Hosaka and Hanneman 1988). The ctDNA variation of the Andean tetraploid potato demonstrated a geographical cline, i.e. the frequency of the most typical ssp. *andigena* type ctDNA (A type) decreased from north to south in the Andes, and other ctDNA types (S, C, W and T types) increased gradually. This finding raised the question as to whether the observed ctDNA variation was introduced from dif-

Table 1. CtDNA restriction fragment pattern types and their proposed ctDNA types among diploid potatoes. WRF 1567, WRF 1152, WRF 1846 and WRF 2406 consist of mixed seeds of reciprocal crosses between those of given PI numbers. The samples from each WRF number yielded only a single ctDNA pattern in spite of the mixed sample used; thus, both parental PIs must have the same ctDNA type. Other WRF lines used have the female parent PI as indicated

Accession	Origin	Bam	Hin	Pvu	CtDNA type
S. stenotomum					
WRF 380 (PI 195204×205526)	Peru	4	3	1	S
PI 205526	Peru	4	3	1	S
PI 205527	Peru	4	3	1	S
PI 234007	Bolivia	4	3	1	S
PI 234008	Bolivia	4	3	1	S
PI 234009	Bolivia	4	1	1	C
PI 234010[a]	Bolivia	4	3	1	S
PI 234011	Bolivia	3	1	1	A
PI 234012	Bolivia	4	3	1	S
PI 234013	Bolivia	4	3	1	S
PI 234015[a]	Bolivia	4	3	1	S
PI 283141	Colombia	4	3	1	S
WRF 2406 (PI 292099×292110)	Peru	4	3	1	S
PI 365344	Peru	4	3	1	S
PI 458393	Bolivia	4	3	1	S
S. goniocalyx					
WRF 146 (PI 195186×195188)	Peru	4	3	1	S
PI 195188	Peru	4	3	1	S
PI 195214	Peru	4	3	1	S
PI 230512	Peru	3	1	1	A
S. phureja					
PI 195191	Ecuador	4	3	1	S
PI 225665	Colombia	4	3	1	S
PI 225667	Colombia	4	3	1	S
PI 225668	Colombia	4	3	1	S
PI 225669	Colombia	4	3	1	S
PI 225670	Colombia	4	3	1	S
PI 225671	Colombia	4	3	1	S
PI 225674	Colombia	4	3	1	S
PI 225676	Colombia	4	3	1	S
PI 225677	Colombia	4	3	1	S
PI 225678	Colombia	4	3	1	S
PI 225683	Colombia	4	3	1	S
PI 225695	Colombia	4	3	1	S
WRF 936 (PI 230867×230586)	?	4	3	1	S
PI 243461	Colombia	4	3	1	S
PI 243463	Colombia	4	3	1	S
PI 243464	Colombia	4	3	1	S
PI 243465	Colombia	4	3	1	S
PI 243466	Colombia	4	3	1	S
PI 243468	Colombia	4	3	1	S
PI 243469	Peru	4	3	1	S
PI 258855	Bolivia	4	3	1	S
PI 275110	Colombia	4	3	1	S
PI 283116	Colombia	4	3	1	S
PI 283118	Colombia	4	3	1	S
PI 283119	Colombia	3	1	1	A
PI 283120	Colombia	3	1	1	A
PI 283121	Colombia	3	1	1	A
PI 283123	Ecuador	4	3	1	S
PI 283125	Colombia	3	1	1	A
PI 283126	Colombia	3	1	1	A
PI 283135	Colombia	3	1	1	A
PI 320348	Colombia	4	3	1	S
PI 320349	Colombia	4	3	1	S
PI 320351	Colombia	4	3	1	S
PI 320354	Colombia	4	3	1	S
PI 320358	Colombia	4	3	1	S
PI 320359	Colombia	4	3	1	S
PI 320360[a]	Colombia	4	3	1	S
S. sparsipilum					
PI 210039[b]	Bolivia	1	1	–	W?
PI 230502	Peru	1	1	1	W
WRF 1152 (PI 233693×233692)	?	1	1	–	W?
PI 234014	Bolivia	1	1	1	W
PI 246536	Peru	1	1	1	W
PI 275276	Bolivia	1	1	1	W
WRF 1846 (PI 290955×230502)	Peru	1	1	1	W
PI 310933	Bolivia	1	1	1	W
PI 310957	Peru	1	1	1	W
PI 310958	Peru	1	1	1	W
PI 310959	Peru	1	1	1	W
PI 310972	Bolivia	1	1	1	W
PI 310984	Bolivia	1	1	3	W2
PI 311001	Peru	1	1	1	W
PI 365343	Peru	1	1	1	W
PI 414151	Bolivia	1	1	1	W
PI 458385	Bolivia	1	1	1	W
PI 458386	Bolivia	1	1	1	W
PI 458387	Bolivia	1	1	1	W
PI 458388	Bolivia	1	1	1	W
PI 473373	Bolivia	1	1	1	W
PI 473375	Bolivia	1	1	1	W
PI 473376	Bolivia	1	1	1	W
PI 473377	Bolivia	1	1	1	W
PI 473385	Peru	1	1	1	W
PI 473503	Bolivia	1	1	1	W
PI 473504	Bolivia	1	1	1	W
PI 473505	Bolivia	1	1	1	W
PI 473530	Bolivia	1	1	1	W
PI 498072	Bolivia	1	1	1	W
PI 498073	Bolivia	1	1	1	W
PI 498074	Bolivia	1	1	1	W
PI 498131	Bolivia	1	1	1	W
PI 498132	Bolivia	1	1	3	W2
PI 498133	Bolivia	1	1	1	W
PI 498134	Bolivia	1	1	1	W
PI 498135	Bolivia	1	1	1	W
S. chacoense f. *gibberulosum*					
WRF 317[a] (PI 133073×133664)	Argentina	1	1	4	W1
WRF 320 (PI 133619×133664)	Argentina	1	1	4	W1
S. chacoense					
WRF 324 (PI 189215×133664)	?	1	1	4	W1
PI 230580[b]	?	1	–	–	W?

Table 1 (continued)

Accession	Origin	Bam	Hin	Pvu	CtDNA type
PI 230582	?	1	–	–	W?
PI 265576	Argentina	1	1	4	W1
PI 275136	Argentina	1	1	1	W
WRF 1567 (PI 275137×275140)	Argentina	1	1	4	W1
PI 275138	Argentina	1	1	1	W
PI 275141	Argentina	1	1	4	W1
PI 320281	Argentina	1	1	4	W1
PI 320282	Argentina	1	1	4	W1
PI 320283	Argentina	1	1	4	W1
PI 320286	Argentina	1	1	4	W1
PI 320288	Argentina	1	1	4	W1
PI 320291	Argentina	1	1	4	W1
PI 320292	Argentina	1	1	1	W
PI 320293	Argentina	1	1	1	W
PI 414143	Argentina	1	1	1	W
PI 414144	Argentina	1	1	4	W1
PI 414153	Paraguay	1	1	4	W1
PI 458308	Argentina	1	1	1	W
PI 458310	Argentina	1	1	4	W1
PI 458311	Argentina	1	1	1	W
PI 458312	Argentina	1	1	4	W1
PI 458313	Argentina	1	1	4	W1
PI 458314	Argentina	1	1	5	W3
PI 458315	Argentina	1	1	4	W1
PI 458316	Argentina	1	1	4	W1
PI 472810	Argentina	1	1	4	W1
PI 472813	Argentina	1	1	4	W1
PI 472816	Argentina	1	1	1	W
PI 472817	Argentina	1	1	4	W1
PI 472819	Argentina	1	1	4	W1
PI 472820[a]	Argentina	1	1	1	W
PI 472821	Argentina	1	1	1	W
PI 472831	Argentina	1	1	4	W1
PI 473402	Argentina	1	1	4	W1
PI 473404	Argentina	1	1	4	W1
PI 473405	Argentina	1	1	4	W1
PI 473406	Argentina	1	1	4	W1
PI 498317	Argentina	1	1	1	W

[a] data cited from Hosaka (1986)
[b] data cited from Hosaka et al. (1984)
– = no data
Bam = *Bam*HI; Hin = *Hin*dIII; Pvu = *Pvu*II

ferent species with different ctDNA types or whether a presumed ancestor, perhaps the cultivated diploid population, already possessed such variation.

In this paper, the ctDNA type of diverse collections of the diploid cultivated potatoes and two wild relatives was determined, and the origin of ctDNA diversity of Andean potatoes is discussed.

Materials and methods

Three closely related cultivated diploid species, *S. stenotomum*, *S. goniocalyx* and *S. phureja*, and two wild or weed diploid species, *S. sparsipilum* and *S. chacoense*, were used (Table 1). Seeds were supplied by the Inter-Regional Potato Introduction Project (IR-1), Sturgeon Bay, Wisconsin, USA. Fresh leaves were collected from 24 seedlings per accession for *S. sparsipilum* and from 16 seedlings per accession of other species, and bulked for ctDNA extraction. For some accessions, fewer plants were used. Each plant was about 50 days old at sampling time. CtDNA extraction and restriction enzyme analysis methods have been described elsewhere (Hosaka and Hanneman 1987). CtDNA type was determined based on the restriction fragment patterns of *Bam*HI, *Hin*dIII and *Pvu*II restriction endonucleases (Hosaka 1986).

Results

The ctDNA extracted from bulked seedlings yielded specific restriction fragment patterns that did not overlap with other pattern types, indicating no ctDNA variation within an accession.

The ctDNA types of 58 cultivated diploid, 35 *S. sparsipilum* and 40 *S. chacoense* accessions were determined from a total of 137 ctDNA sources (Table 1). Two accessions each of *S. chacoense* and *S. sparsipilum* were not completed with three restriction enzyme digestions; thus, their ctDNA types were not determined. Three types (1, 3 and 4) were distinguished by *Bam*HI digestion and two types (1 and 3) by *Hin*dIII. Restriction fragment patterns of their respective types are shown in Hosaka (1986) and Hosaka and Hanneman (1988). The *Pvu*II restriction fragment pattern revealed four types 1 and 3–5 (Fig. 1). Type 5 of *Pvu*II is a new restriction fragment pattern type not detected previously, and is described for the first time in this paper having been found in a *S. chacoense* accession (PI 458314). This type was probably derived from type 1 by one point mutation, and the new *Pvu*II recognition site appeared in the 14.8 kilobase pair (kbp) fragment and produced two smaller fragments, 8.7 kbp and 5.3 kbp (Fig. 1). Another mechanism, such as a deletion or an additional point mutation, could also explain this observation, since the sum of the size of two smaller fragments is considerably less than that of the large fragment.

The ctDNA type of each accession was determined based on Hosaka's description (Hosaka 1986). The combination of restriction fragment pattern types, 1-1-1 for *Bam*HI-*Hin*dIII-*Pvu*II is W; 3-1-1 is A; 4-1-1 is C, and 4-3-1 is S type ctDNA. Three types derived from the W type were detected: 1-1-3, 1-1-4 and 1-1-5 for *Bam*HI-*Hin*dIII-*Pvu*II combination. Previously, two derivative types were identified as W″ and W′, respectively. Now, these latter derivative types have been renamed W2 and W1, respectively, since many other W derivative types may be discovered in the future. In this context, the above mentioned new ctDNA type of *S. chacoense* (1-1-5 combination) has been named W3. The data summarized are shown in Table 2, along with

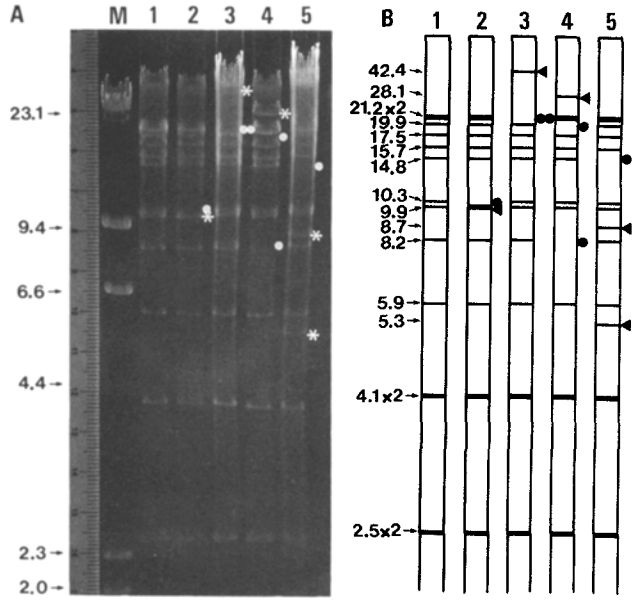

Fig. 1 A and B. A photograph of an agarose gel (**A**) and its diagramatic representation (**B**) of *Pvu*II restriction fragment patterns. Type 1, *S. chacoense* (PI 320293); type 2, *S. tuberosum* ssp. *tuberosum* (WRF 1748 (PI 245795×245314)); type 3, *S. sparsipilum* (PI 498132); type 4, *S. chacoense* (PI 414144) and type 5, *S. chacoense* (PI 458314). The *Hin*dIII digested λDNA was used as a marker DNA (M). Fragment changes were compared with type 1 pattern. A loss or a gain of a fragment is shown by a *circle* or an *asterisk* in (**A**) and by a *circle* or a *triangle* in (**B**), respectively. The fragment size is indicated in kilobase pairs. Type 2 pattern was not seen in any ctDNAs of the present study

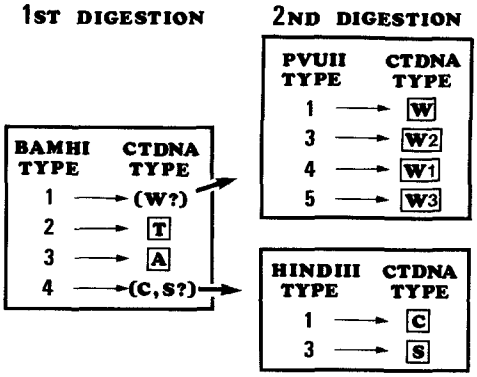

Fig. 2. A simple determination system of ctDNA types for cultivated potatoes and their relatives. T type ctDNA was not found in this report

Table 2. The number of accessions with different ctDNA types

Species	T	A	S	C	W	W1	W2	W3	W?
S. tuberosum[a]									
ssp. *andigena*	5	70	14	16	5	0	0	0	3
ssp. *tuberosum*	30	2	0	0	1	0	0	0	0
S. stenotomum	0	1	13	1	0	0	0	0	0
S. goniocalyx	0	1	3	0	0	0	0	0	0
S. phureja	0	6	33	0	0	0	0	0	0
S. sparsipilum	0	0	0	0	33	0	2	0	2
S. chacoense	0	0	0	0	11	28	0	1	2

[a] The *S. tuberosum* data is cited from Hosaka and Hanneman (1988). In that paper, nine accessions were determined as W type, based on *Bam*HI restriction fragment pattern. In the later experiment, six of the accessions were confirmed as W type by *Pvu*II digestion, but the remaining three accessions were not available for analyses

those of *S. tuberosum* ssp. *andigena* and ssp. *tuberosum* reported elsewhere (Hosaka and Hanneman 1988).

In a previous paper (Hosaka 1986), only the S type was assigned to the cultivated diploid species *S. stenotomum, S. goniocalyx* and *S. phureja.* But several other types, A (14%) and C (2%) have now been found among them (Table 1). Out of 40 *S. chacoense* accessions, 11 (28%) have W type, 28 (70%) have W1 type, and one (3%) has W3 type ctDNA, a new type. Previously, the W type was assigned to *S. chacoense* and W1 to *S. chacoense* f. *gibberulosum* (Hosaka 1986). But, the results of this study indicate that W1 type ctDNA is a major type for *S. chacoense* rather than W type, and that its distribution is not confined to f. *gibberulosum.* Among *S. sparsipilum* accessions, 33 (94%) had W type ctDNA and two (6%) had W2 type ctDNA, which had been found in *S. tarijense* (Hosaka 1986).

Discussion

CtDNA determination

A total of 133 ctDNAs were completed with all three restriction enzyme digestions. The *Hin*dIII digest provided the only information for distinguishing S or C type ctDNA, while *Pvu*II was useful to distinguish among W derivative types (Table 1). A previously proposed simple determination method for potato ctDNA types (Hosaka and Hanneman 1987) was also effective in this study. As shown in Fig. 2, first the ctDNA sample was subjected to *Bam*HI digestion, and only if it showed a type 1 or a type 4 pattern was it subjected to *Pvu*II or *Hin*dIII digestion. *Pvu*II was used to distinguish among type 1 patterns and *Hin*dIII among type 4. This determination system is simple and may be useful for large scale experiments.

Intraspecific variation of ctDNA type

In general, ctDNA is thought to evolve very slowly and is much less polymorphic than mitochondrial or nuclear

Table 3. Intraspecific variation revealed by restriction enzyme analysis of ctDNA for other genera. ctDNA variation detected, based on the comparison of cytoplasmic male sterile lines with normal lines, are not listed in this table

Species	Ploidy	Accessions[a]	Reference
Aegilops aucheri	2x	3 (2–1)	Nakamichi and Tsunewaki (1986)
Aegilops bicornis	2x	4 (2–2)	Nakamichi and Tsunewaki (1986)
Aegilops speltoides	2x	6 (5–1)	Bowman et al. (1983)
Aegilops speltoides	2x	11 (7–2–1–1)	Nakamichi and Tsunewaki (1986)
Aegilops squarrosa	2x	16 (11–4–1)	Terachi et al. (1985)
Aegilops triuncialis	4x	3 (2–1)	Ogihara and Tsunewaki (1982)
Aegilops triuncialis	4x	20 (13–6–1)	Murai and Tsunewaki (1984)
Beta macrocarpa	2x, 4x	2 (1–1)	Kishima et al. (1987)
Beta maritima	2x	6 (4–2)	Kishima et al. (1987)
Brassica campestris	2x	4 (3–1)	Palmer et al. (1983)
Brassica campestris	2x	8 (6–1–1)	Kemble (1987)
Brassica juncea	4x	7 (5–1–1)	Kemble (1987)
Brassica napus	4x	3 (2–1)	Palmer et al. (1983)
Brassica napus	4x	97 (45–30–9–7–1–1–4)	Kemble (1987)
Brassica nigra	2x	3 (1–1–1)	Palmer et al. (1983)
Clarkia biloba	2x	2 (1–1)	Sytsma and Gottlieb (1986)
Cucumis melo	2x	6 (5–1)	Perl-Treves and Galun (1985)
Glycine gracilis	2x	5 (3–1–1)	Shoemaker et al. (1986)
Glycine max	2x	26 (16–7–3)	Shoemaker et al. (1986)
Hordeum spontaneum	2x	11 (4–3–3–1)	Clegg et al. (1984a)
Hordeum vulgare	2x	9 (7–2)	Clegg et al. (1984a)
Lisianthius skinneri	4x	3 (1–1–1)	Sytsma and Schaal (1985)
Lupinus texensis	2x	100 (88–11–1–1)?	Banks and Birky (1985)
Lycopersicon peruvianum	2x	6 (3–2–1)	Palmer and Zamir (1982)
Nicotiana debneyi	4x	9 (7–2)	Scowcroft (1979)
Oryza latifolia	4x	2 (1–1)	Ichikawa et al. (1986)
Oryza sativa	2x	22 (15–7)	Ishii et al. (1986)
Pelargonium zonale	2x	16 (13–2–1)	Metzlaff et al. (1981)
Pisum elatius	2x	2 (1–1)	Palmer et al. (1985)
Pisum humile	2x	12 (5–4–3)	Palmer et al. (1985)
Pisum sativum	2x	13 (4–3–3–2–1)	Palmer et al. (1985)
Pisum sativum	2x	48 (24–14–6–3–1)	Teeri et al. (1985)
Zea mays (annual teosinte)	2x	7 (4–2–1)	Timothy et al. (1979)

[a] First figure indicates the number of accessions used. In parentheses, the number of accessions in each different ctDNA type are shown

DNA (Banks and Birky 1985; Palmer et al. 1985). Nevertheless, it has become increasingly apparent that ctDNA variation within a species is common (Scowcroft 1979; Timothy et al. 1979). In *Nicotiana,* the ctDNA restriction fragment pattern was altered rapidly when the nuclear genome of wild species was replaced by that of cultivated tobacco in order to produce the male sterile lines (Frankel et al. 1979; Kung et al. 1981). In pea, the loss of an inverted repeat generated a high rate of ctDNA change (Palmer and Thompson 1982; Teeri et al. 1985). The intraspecific variation reported so far is summarized in Table 3.

In the potato, both the previous (Hosaka and Hanneman 1988) and the present study show large variation for ctDNA types within *S. tuberosum* as well as in the cultivated diploid and wild relatives (Table 2). Five ctDNA types were detected in the Andean tetraploid cultivated potatoes, *S. tuberosum* ssp. *andigena* (hereinafter designated only as ssp. *andigena*). At the diploid level, both cultivated and wild potatoes revealed polymorphic ctDNA, but a diploid weed species, *S. sparsipilum,* exhibited relatively low polymorphism, i.e., 94% of *S. sparsipilum* accessions had a common W type ctDNA. It is concluded that ctDNA variation within a

species may be a general rule in the plant kingdom, as tentatively described by Timothy et al. (1979).

Origin of the intraspecific variation of ssp. andigena ctDNA

Clegg et al. (1984a) reported that cultivated barley, *Hordeum vulgare* (2*x*), exhibited a single ctDNA type, whereas its land races and wild progenitor, *H. spontaneum* (2*x*), exhibited five, so they suggested that the level of cytoplasmic diversity was markedly restricted during domestication. In contrast, a large number of different ctDNA types has been reported in the garden pea (*Pisum sativum*) and in the soybean (*Glycine max*) compared with their wild progenitors (Palmer et al. 1985; Shoemaker et al. 1986). The large variation observed seems to have occurred during domestication. Thus, the mode of ctDNA variation during evolution and domestication might be different for each species. However, there is the possibility of broadening the ctDNA variation of a species through polyploidization or cross-breeding over centuries. Various ctDNA types exist in the different *Brassica* species (Erickson et al. 1983; Palmer et al. 1983) and seem to have been incorporated into *B. napus* (4*x*) via amphidiploidization. CtDNA variation of *Aegilops triuncialis* (4*x*) has been explained by the incorporation of different ctDNAs from the parental species *Ae. caudata* and *Ae. umbellulata* by reciprocal crosses followed by polyploidization (Murai and Tsunewaki 1984).

In the potato, it is suggested that the large ctDNA variation found in ssp. *andigena* was apparently introduced from the diploid species that already had considerable variation. Out of A, S, C, W and T type ctDNAs maintained by the ssp. *andigena* population, A, S and C type ctDNAs could have been derived from diploid cultivated species (Table 2). W type ctDNA was not found in the cultivated diploid population, probably because the present sample size of *S. stenotomum* was too small to cover the whole range of ctDNA diversity of *S. stenotomum,* which is tremendously polymorphic (Hawkes 1958). But another possibility can not be excluded: ssp. *andigena* with W type ctDNA might be a result of introgression from wild species after ssp. *andigena* arose, since W type ctDNA is distributed predominantly in the wild species of South America (Hosaka 1986). T type ctDNA derived from W type through a 400 base pairs deletion change (Hosaka et al. 1988) could be an exceptional case, which occurred within the ssp. *andigena* population after it arose. This is because T type ctDNA has not been found in any diploid or tetraploid wild species, but only in five accessions of ssp. *andigena* and in ssp. *tuberosum* (Table 2), which is a derived form of ssp. *andigena* (Hosaka and Hanneman 1988).

The A type ctDNA, a typical ctDNA type of ssp. *andigena,* has also been found in ssp. *tuberosum* and in some diploid cultivated species, and also in *S. maglia,* a Chilean wild triploid species (also in the diploid cytotype) (Hosaka 1986). This indicates that some maternal link may underlie Andean cultivated potatoes and this Chilean wild species. The same situation is true for W2 type ctDNA, which has been found in two accessions of *S. sparsipilum* (Table 2) and in *S. tarijense,* a constituent of a different taxonomic series (Hawkes 1978). The W2 type ctDNA is distinguished from W type by a single point mutation in one of the *Pvu*II recognition sites. Thus, the occurrence of the same *Pvu*II restriction fragment pattern in these two wild species might be the result of a parallel mutation that occurred in the same restriction site (convergence), since convergence of the restriction sites is known to occur with some frequency in ctDNA (Palmer et al. 1983; Sytsma and Gottlieb 1986). *Solanum chacoense* accessions have one of three ctDNA types: W, W1 and W3, irrespective of the morphological diversity within the species. W type ctDNA has been broadly maintained in many South American species, *S. chacoense, S. gourlayi, S. kurtzianum, S. leptophyes, S. microdontum, S. oplocense, S. sparsipilum, S. spegazzinii, S. sucrense* and *S. vernei,* and even in the Mexican hexaploid species, *S. demissum* (Hosaka 1986). This evidence suggests that ctDNA variation may have occurred during the very early stages of species differentiation of South American tuber-bearing *Solanum* species. In contrast to such ctDNA conservatism in wild species, a fairly rapid ctDNA change is inferred in the cultivated diploid potatoes, i.e. during the evolution of *S. stenotomum,* S and A type ctDNAs seem to have been derived from the more primitive C type. The question is whether those advanced type ctDNAs of *S. stenotomum* already existed in the wild population or evolved and were selected for under cultivation.

Origin of S. tuberosum ssp. andigena

The Andean tetraploid potato, ssp. *andigena,* has been widely grown in the Andean highlands by native farmers. It displays large variation in morphological and physiological traits (Salaman 1946; Hawkes 1956) as well as for ctDNA types (Hosaka and Hanneman 1988). Many hypotheses have been proposed for its origin (Hosaka 1986), however, they tentatively can be combined into two essentially different ideas: ssp. *andigena* originated via polyploidization (1) from an inter-varietal or inter-species cross within cultivated diploid potatoes (Swaminathan and Magoon 1961; Matsubayashi 1981; Hosaka 1986); or (2) from an inter-species cross between cultivated diploid species and a particular wild diploid species (Hawkes 1958, 1978; Brücher 1964; Cribb and Hawkes 1986).

The present results revealed that three (A, S and C) of the five ctDNA types (A, S, C, W and T) found in ssp. *andigena* are present in the cultivated diploid potatoes, whereas only one type (W) is found in the wild diploid species. This finding strongly supports our previous proposal that cultivated diploid potatoes functioned many times as the ctDNA donor parent, irrespective of what the male parent was, yielding the ssp. *andigena* complex with various ctDNA types (Hosaka et al. 1984; Hosaka 1986).

It is probable that tetraploids arose (and probably are still arising) continuously in the fields, where the cultivated diploid potatoes are grown, by bilateral sexual polyploidization via the union of 2*n* gametes of different genotypes of cultivated diploid potatoes. From these tetraploid materials, some might be selected and maintained by clonal propagation under cultivation, finally being established as a member of ssp. *andigena* complex. Many years of human selection for preferable tetraploids could generate the ssp. *andigena* complex; its origin reflected in the large ctDNA variation. Additional ctDNA diversity could have been provided by introgression from the cultivated diploid potatoes as well as wild species via the union of *n* and 2*n* gametes.

The ctDNA data, however, does indicate various possibilities for the origin of ssp. *andigena*: it might have originated from cultivated diploid species (♀) × wild diploid species (♂), and moreover, some ssp. *andigena* might originate from the reciprocal crosses for the origin of W type ssp. *andigena*. Those that arose from hybridization between cultivated potatoes and wild diploid species most likely would express the unfavorable agronomic characteristics of latter species, i.e., long stolons, bitter taste, etc. (Matsubayashi 1981), but this need not be the case as reported by Hermundstad and Peloquin (1985). The direct nuclear DNA comparison, such as those of rDNA restriction fragment patterns (Doyle and Beachy 1985) or RFLP markers (Beckmann and Soller 1986), or the comparison of artificial polyploids obtained through the schemes proposed by the respective hypotheses, could provide further information to elucidate the origin of ssp. *andigena*.

Species differentiation of the cultivated diploid potatoes

A very close relationship has been confirmed among cultivated diploid potatoes, *S. stenotomum*, *S. goniocalyx* and *S. phureja*, based upon morphological, genetic and biochemical traits (Hawkes 1958; Dodds and Paxman 1962; Hosaka and Matsubayashi 1983; Hosaka 1986). The present data provides further evidence that *S. stenotomum* is the most probable ancestral type from which *S. goniocalyx* and *S. phureja* were derived by mutation and selection (Hawkes 1978), since C type ctDNA, which is an ancestral type of A and S type ctDNAs, was found only in *S. stenotomum*. This strongly supports the idea of Hawkes (1978) rather than the idea of independent origin of cultivated diploid species from different wild species (Bukasov 1966).

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References

Banks JA, Birky CW Jr (1985) Chloroplast DNA diversity is low in a wild plant, *Lupinus texensis*. Proc Natl Acad Sci USA 82:6950–6954

Beckmann JS, Soller M (1986) Restriction fragment length polymorphisms and genetic improvement of agricultural species. Euphytica 35:111–124

Berthou F, Mathieu C, Vedel F (1983) Chloroplast and mitochondrial DNA variation as indicator of phylogenetic relationships in the genus *Coffea* L. Theor Appl Genet 65:77–84

Bowman CM, Bonnard G, Dyer TA (1983) Chloroplast DNA variation between species of *Triticum* and *Aegilops*. Location of the variation on the chloroplast genome and its relevance to the inheritance and classification of the cytoplasm. Theor Appl Genet 65:247–262

Brücher H (1964) El origen de la papa (*Solanum tuberosum*). Physis 24:439–452

Bukasov SM (1966) Die Kulturarten der Kartoffel und ihre wildwachsenden Vorfahren. Z Pflanzenzücht 55:139–164

Clegg MT, Brown AHD, Whitfeld PR (1984a) Chloroplast DNA diversity in wild and cultivated barley: Implications for genetic conservation. Genet Res Camb 43:339–343

Clegg MT, Rawson JR, Thomas K (1984b) Chloroplast DNA variation in pearl millet and related species. Genetics 106:449–461

Cribb PJ, Hawkes JG (1986) Experimental evidence for the origin of *Solanum tuberosum* subspecies *andigena*. In: D'Arcy WG (ed) *Solanaceae:* biology and systematics. Columbia University Press, New York, pp 383–404

Dodds KS, Paxman GJ (1962) The genetic system of cultivated diploid potatoes. Evolution 16:154–167

Doyle JJ, Beachy RN (1985) Ribosomal gene variation in soybean (*Glycine*) and its relatives. Theor Appl Genet 70:369–376

Erickson LR, Straus NA, Beversdorf WD (1983) Restriction patterns reveal origins of chloroplast genomes in *Brassica* amphiploids. Theor Appl Genet 65:201–206

Frankel R, Scowcroft WR, Whitfeld PR (1979) Chloroplast DNA variation in isonuclear male-sterile lines of *Nicotiana*. Mol Gen Genet 169:129–135

Hawkes JG (1956) Taxonomic studies on the tuber-bearing Solanums. 1. *Solanum tuberosum* and the tetraploid species complex. Proc Linn Soc London 166:97–144

Hawkes JG (1958) Kartoffel. 1. Taxonomy, cytology and crossability. In: Kappert H, Rudorf W (eds) Handb Pflanzenzücht vol 3. Parey, Berlin, pp 1–43

Hawkes JG (1978) Biosystematics of the potato. In: Harris PM (ed) The potato crop. Chapman and Hall, London, pp 15–69

Hermundstad SA, Peloquin SJ (1985) Germplasm enhancement with potato haploids. J Hered 76:463–467

Hosaka K (1986) Who is the mother of the potato? – Restriction endonuclease analysis of chloroplast DNA of cultivated potatoes. Theor Appl Genet 72:606–618

Hosaka K, Hanneman RE Jr (1987) A rapid and simple method for determination of potato chloroplast DNA type. Am Potato J 64:345–353

Hosaka K, Hanneman RE Jr (1988) The origin of the cultivated tetraploid potato based on chloroplast DNA. Theor Appl Genet 76:172–176

Hosaka K, Matsubayashi M (1983) Studies on the phylogenetic relationships in tuberous Solanums by isozyme analysis. 3. Inter-specific differences between each of four cultivated diploid species. Rep Soc Crop Sci Breed Kinki 28:28–32

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

Hosaka K, de Zoeten GA, Hanneman RE Jr (1988) Cultivated potato chloroplast DNA differs from the wild type by one deletion – Evidence and implications. Theor Appl Genet 75:741–745

Ichikawa H, Hirai A, Katayama T (1986) Genetic analyses of *Oryza* species by molecular markers for chloroplast genomes. Theor Appl Genet 72:353–358

Ishii T, Terachi T, Tsunewaki K (1986) Restriction endonuclease analysis of chloroplast DNA from cultivated rice species, *Oryza sativa* and *O. glaberrima.* Jpn J Genet 61:537–541

Kemble RJ (1987) A rapid, single leaf, nucleic acid assay for determining the cytoplasmic organelle complement of rapeseed and related *Brassica* species. Theor Appl Genet 73:364–370

Kishima Y, Mikami T, Hirai A, Sugiura M, Kinoshita T (1987) *Beta* chloroplast genomes: analysis of Fraction I protein and chloroplast DNA variation. Theor Appl Genet 73:330–336

Kung SD, Zhu YS, Chen K, Shen GF, Sisson VA (1981) *Nicotiana* chloroplast genome. 2. Chloroplast DNA alteration. Mol Gen Genet 183:20–24

Kung SD, Zhu YS, Shen GF (1982) *Nicotiana* chloroplast genome. 3. Chloroplast DNA evolution. Theor Appl Genet 61:73–79

Matsubayashi M (1981) Species differentiation in tuberous *Solanum* and the origin of cultivated potatoes. Rec Adv Breed 22:86–106

Metzlaff M, Börner T, Hagemann R (1981) Variations of chloroplast DNAs in the genus *Pelargonium* and their biparental inheritance. Theor Appl Genet 60:37–41

Murai K, Tsunewaki K (1984) Intraspecific variation of chloroplast DNAs (ctDNAs) in *Aegilops triuncialis* and geographical distribution of cytoplasmic types. Jpn J Breed (Suppl 1) 34:280–281

Nakamichi O, Tsunewaki K (1986) Restriction enzyme analysis of ctDNA in *Aegilops* species of section *Sitopsis.* Jpn J Breed (Suppl 1) 36:238–239

Ogihara Y, Tsunewaki K (1982) Molecular basis of the genetic diversity of the cytoplasm in *Triticum* and *Aegilops.* 1. Diversity of the chloroplast genome and its lineage revealed by the restriction pattern of ct-DNAs. Jpn J Genet 57:371–396

Palmer JD, Thompson WF (1982) Chloroplast DNA rearrangements are more frequent when a large inverted repeat sequence is lost. Cell 29:537–550

Palmer JD, Zamir D (1982) Chloroplast DNA evolution and phylogenetic relationships in *Lycopersicon.* Proc Natl Acad Sci USA 79:5006–5010

Palmer JD, Shields CR, Cohen DB, Orton TJ (1983) Chloroplast DNA evolution and the origin of amphidiploid *Brassica* species. Theor Appl Genet 65:181–189

Palmer JD, Jorgensen RA, Thompson WF (1985) Chloroplast DNA variation and evolution in *Pisum:* patterns of change and phylogenetic analysis. Genetics 109:195–213

Perl-Treves R, Galun E (1985) The *Cucumis* plastome: physical map, intrageneric variation and phylogenetic relationships. Theor Appl Genet 71:417–429

Salaman RN (1946) The early European potato: its character and place of origin. J Linn Soc Lond Bot 53:1–27

Scowcroft WR (1979) Nucleotide polymorphism in chloroplast DNA of *Nicotiana debneyi.* Theor Appl Genet 55:133–137

Shoemaker RC, Hatfield PM, Palmer RG, Atherly AG (1986) Chloroplast DNA variation in the genus *Glycine* subgenus *Soja.* J Hered 77:26–30

Swaminathan MS, Magoon ML (1961) Origin and cytogenetics of the commercial potato. In: Advances in genetics, vol 10. Academic Press, London, pp 217–256

Sytsma KJ, Gottlieb LD (1986) Chloroplast DNA evolution and phylogenetic relationships in *Clarkia* sect. *Peripetasma* (*Onagraceae*). Evolution 40:1248–1261

Sytsma KJ, Schaal BA (1985) Phylogenetics of the *Lisianthius skinneri* (*Gentianaceae*) species complex in Panama utilizing DNA restriction fragment analysis. Evolution 39:594–608

Teeri TH, Saura A, Lokki J (1985) Insertion polymorphism in pea chloroplast DNA. Theor Appl Genet 69:567–570

Terachi T, Kataoka J, Tsunewaki K (1985) Intraspecific variation of organelle DNAs in *Aegilops squarrosa.* Jpn J Breed (Suppl 2) 35:194–195

Timothy DH, Levings CS III, Pring DR, Conde MF, Kermicle JL (1979) Organelle DNA variation and systematic relationships in the genus *Zea:* Teosinte. Proc Natl Acad Sci USA 76:4220–4224

Zurawski G, Clegg MT, Brown AHD (1984) The nature of nucleotide sequence divergence between barley and maize chloroplast DNA. Genetics 106:735–749