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Phylogenetic relationship between the tuberous *Solanum* species as revealed by restriction endonuclease analysis of chloroplast DNA[1)]

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ABSTRACT

Cytoplasmic relationship between 37 tuberous *Solanum* accessions and hybrids and two *Lycopersicon* species was investigated by restriction fragment pattern analysis of chloroplast DNA using eight endonucleases. Variations found in their chloroplast DNAs were subjected to mutation analysis, from which a phylogenetic tree was constructed as shown in Fig. 4. The 30 species studied are classified into four groups from their chloroplast DNA similarities: (1) South American species and Mexican polyploid species, (2) Mexican diploid species, (3) *S. etuberosum*, and (4) *S. lycopersicoides* and *Lycopersicon* species. These groups correspond well to the groups established from crossability by Hawkes (1978). Cytoplasmic differences between 22 species in the first group are so small that these species seem to be of recent origin. Mexican diploid species in the second group greatly differ from those in the first group, and also from each other. *S. etuberosum* differs from all others by, at least, 21 mutational changes. *S. lycopersicoides* is rather closely related to the *Lycopersicon* species, and is different from other *Solanum* species by, at least, 21 mutational changes. Cytoplasms of all cultivated species except common potato, *S. tuberosum* ssp. *tuberosum* have originated monophyletically from *S. stenotomum*. The cytoplasm of common potato is revealed to have derived from Chilean *tuberosum* as pointed out by some workers, but the cytoplasm donor to Chilean *tuberosum* remains unidentified.

1. INTRODUCTION

Tuberous *Solanum* species have been classified into 18 taxonomic series comprising about 160 wild and eight cultivated species, mainly distributed in South America and Mexico through Central America (Hawkes 1978). Nuclear genome constitutions of cultivated potato and close relatives have

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been identified by cytogenetic analyses (Matsubayashi 1981), but, to what extent these species are related genetically to Mexican diploid species and to non-tuberous species involving tomato, and how wild relatives have evolved remain unknown.

Recently, restriction fragment pattern analysis of chloroplast DNA has become a powerful tool for studying the phylogenetic relationship between related species. Due to the specific nature of chloroplast DNA, *i.e.*, its extreme conservatism, compared to nuclear DNA, and maternal inheritance in most cases (Atchison *et al.* 1976; Palmer and Zamir 1982), this approach provides phylogenetic information on both the macro-evolution, *i.e.*, interspecific as well as intergeneric relationship (Vedel *et al.* 1980; Kung *et al.* 1982; Ogihara and Tsunewaki 1982; Palmer and Zamir 1982; Berthou *et al.* 1983; Bowman *et al.* 1983), and on the cytoplasm donor to polyploid species (Tsunewaki and Ogihara 1983; Erickson *et al.* 1983; Palmer *et al.* 1983).

In this paper, we present our results on the cytoplasmic relationship between tuberous *Solanum* and *Lycopersicon* species which were obtained by restriction fragment pattern analysis of chloroplast DNA (hereinafter referred to ctDNA), and will discuss the origin of cultivated potato.

2. MATERIALS AND METHODS

The species and varieties used as the source of ctDNA and their introduction numbers are shown in Table 1. In this paper, all species and varieties are referred to by the abbreviations indicated in Table 1. *Ver*, which is distributed in Mexico (Hawkes 1956a), is conveniently dealt as a South American species because it has the same genome as the latter species (Matsubayashi and Misoo 1979).

In most cases, the leaf material used for ctDNA isolation was obtained from a single clone having the same genotype, but in some species they were prepared from a few different clones having the same introduction number.

All the species and varieties were grown in a glasshouse when they were young, then moved outdoors in spring or summer.

Intact chloroplasts were isolated from the homogenate of mature leaves or small whole plants, without destarching, and were purified using a discontinuous gradient made with 15, 30 and 60% sucrose solutions which was centrifuged at 25000 rpm for 30 min at 4°C. Other procedures of ctDNA isolation were the same as those of Tsunewaki and Ogihara (1983).

The following eight restriction enzymes were used; *Bam*HI, *Bgl*II, *Eco*RI, *Hin*dIII, *Kpn*I, *Pst*I, *Xba*I and *Xho*I. CtDNA was digested with these enzymes according to the directions given by the supplier, Takara Shuzo Co. Ltd., Kyoto, Japan. The DNA fragments were separated by electrophoresis at 1V/cm for 40–48 hr or 2V/cm for 20 hr, using 0.5 to 1.2% agarose slab gels containing 40 mM Tris (pH7.8), 20 mM sodium acetate, 2 mM EDTA and 0.5

Table 1. *Materials used in the present study*

Genus and series	Species	2n	Abbrev.*	Remarks**	
Genus *Lycopersicon*					
	L. esculentum	24	*le*	cv. 'Sekaiichi'	
	L. peruvianum	24	*lp*	LA 1283	6)
Genus *Solanum*					
Non-tuberous species					
Juglandifolia	*S. lycopersicoides*	24	*lyc*	Och 2712	1)
Etuberosa	*S. etuberosum*	24	*etb*	GLKS 13/2	1)
Mexican diploid species					
Bulbocastana	*S. bulbocastanum*	24	*blb*	WAC 3027	4)
Pinnatisecta	*S. cardiophyllum*	24	*cph*	P. I. 184762	1)
	S. pinnatisectum	24	*pnt*	P. I. 186554	1)
Polyadenia	*S. polyadenium*	24	*pld*	P. I. 275238	1)
Mexican polyploid species					
Longipedicellata	*S. stoloniferum*	48	*sto*	P. I. 161178	1)
Demissa	*S. demissum*	72	*dms*	P. I. 160230	1)
South American species					
Commersoniana	*S. chacoense*	24	*chc*	P. I. 230580	2)
	S. commersonii	24	*cmm*	P. I. 243503	1)
Circaeifolia	*S. capsicibaccatum*	24	*cap*	P. I. 205560	1)
Conicibaccata	*S. santolallae*	24	*san*	HHC 5103	1)
	S. moscopanum	72	*msp*	P. I. 230517	1)
Piurana	*S. piurae*	24	*pur*	P. I. 365365	1)
Acaulia	*S. acaule*	48	*acl*		3)
Cuneoalata	*S. infundibuliforme*	24	*ifd*	Hoff. 1626	1)
Megistacroloba	*S. boliviense*	24	*blv*	P. I. 265860	1)
Tuberosa (Wild)	*S. leptophyes*	24	*lph*	HHC 5057	1)
	S. multidissectum	24	*mlt*	P. I. 210043	1)
	S. sparsipilum	24	*spl*	P. I. 210039	1)
	S. spegazzinii	24	*spg*	Hoff. 1754	1)
	S. vernei	24	*vrn*	D/1421	2)
	S. verrucosum	24	*ver*	WAC 3337 (P.I. 275258)	4)
(Cultivated)	*S. goniocalyx*	24	*gon*	P. I. 195188	2)
	S. phureja	24	*phu*	Ivp 35	2)
	S. stenotomum	24	*stn* 1	P. I. 205527	2)
	S. stenotomum	24	*stn* 2	P. I. 205526	1)
	S.×chaucha	36	*cha* 1	from C. Ochoa	
	S.×chaucha	36	*cha* 2	T-AY-43	3)

Table 1. (*Continued*)

Genus and series	Species	2n	Abbrev.*	Remarks**
	S. tuberosum			
	ssp. *tuberosum*	48	*tbr* 1	cv. 'May Queen'
	ssp. *tuberosum*	48	*tbr* 2	cv. 'Early Rose' 2)
	ssp. *tuberosum*	48	*tbr* 3	cv. 'Greta'
	ssp. *tuberosum*	48	C*tbr*	Chilean *tuberosum*, UA-1234 (cv. 'Huilcaña') 5)
	ssp. *andigena*	48	*adg* 1	T-AY-22 3)
	ssp. *andigena*	48	*adg* 2	T-AY-28 3)
F_1 hybrids				
S. infundibuliforme×*S. spegazzinii*		24	*ifd*/*spg*	
S. spegazzinii×*S. infundibuliforme*		24	*spg*/*ifd*	

* Abbreviation proposed by Simmonds (1963) and by the present authors.

** Seeds or tubers were supplied from the following;

1) Potato Introduction Station, Wisconsin, U. S. A.
2) Y. Irikura, Shimamatsu Potato Branch, Hokkaido National Agricultural Experiment Station, Japan.
3) Collection of the Expedition of Cultivated Plants in the Andean Areas, Kyoto University (1971).
4) J. G. Th. Hermsen, Institute of Plant Breeding, Wageningen, The Netherlands.
5) A. Contreras M., Universidad Austral de Chile, Chile.
6) C. M. Rick, University of California, Davis, U. S. A.

μg/ml ethidium bromide for DNA staining. The DNA fragments were made visible and were photographed under long wave UV light. From the photographs, the restriction fragment pattern of ctDNA was drawn by measuring the distance of each fragment from the origin. The molecular size of each restriction fragment was estimated from its mobility, as compared with those of the non-digested λDNA and its *Hin*dIII-digested fragments having known molecular weights.

3. RESULTS

1) *CtDNAs of tuberous* Solanum *and* Lycopersicon *species*

CtDNAs were extracted from a total of 39 sources (Table 1). Several samples of ctDNAs particularly from *sto* and *stn*2 seemed to have been contaminated with nuclear or mitochondrial DNA, or to contain some inhibitory substance to restriction enzymes, because partial digestion or smeared background was observed. Estimated chloroplast genome size varied from 110.7 kbp to 154.9 kbp depending on the enzyme used. As very small fragments could not be observed in all digests except with *Pst*I and *Xho*I, chloroplast genome size of tuberous *Solanum*s is presumed to be about 155 kbp, which is quite close to the 156 kbp reported by Schiller *et al.* (1982). The reciprocal hybrids between *ifd* and *spg* gave the same patterns as their respective female parent

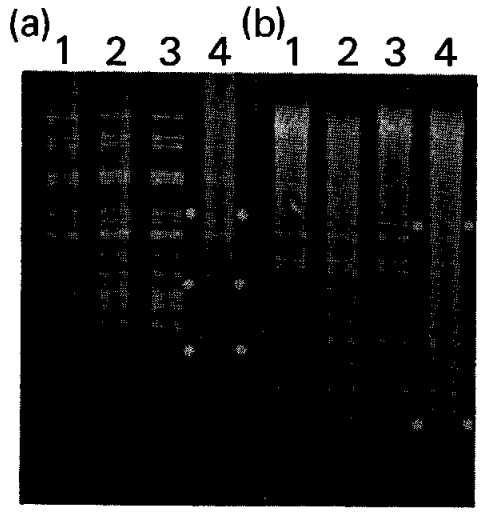

Fig. 1. Restriction fragment patterns of ctDNAs from *spg* (1), *spg*/*ifd* (2), *ifd*/*spg* (3) and *ifd* (4), showing the maternal inheritance of the unique fragments (marked with asterisks). (a) and (b): *Eco*RI and *Kpn*I pattern, respectively.

(Fig. 1). This confirms that ctDNA is maternally inherited. Autotetraploids of *ifd* and *spg* which were produced by colchicine treatment also gave the same pattern as their respective diploid parent (the patterns are not shown here).

2) *Interspecific variation in restriction fragment pattern*

In the foregoing sections, the restriction fragment patterns of ctDNAs from 37 sources will be reported in two parts. All the fragment patterns are classified into different types. The most commonly observed type was named Type 1, and was used as the standard type. The mutational change seen in the restriction fragment of all restriction pattern types, as compared with Type 1, is analyzed, and classified into two types, *i.e.*, informative and independent mutation, according to Palmer and Zamir (1982), informative mutation referring to a mutation occurring in more than one species, thus useful for grouping ctDNA sources, and independent mutation being specific to a given species. This type of analysis will hereafter be referred to as ctDNA mutation analysis.

Bam*HI restriction fragment pattern*: The *Bam*HI pattern of the ctDNA from 37 sources could be classified into 12 types, as shown in Fig. 2. In the Type 1 pattern, 27 fragments, including multiple copies, were observed, the total molecular size of which amounted to 128.3 kbp. Besides these fragments, at least six fragments, which were smaller than 2 kbp were detected. The

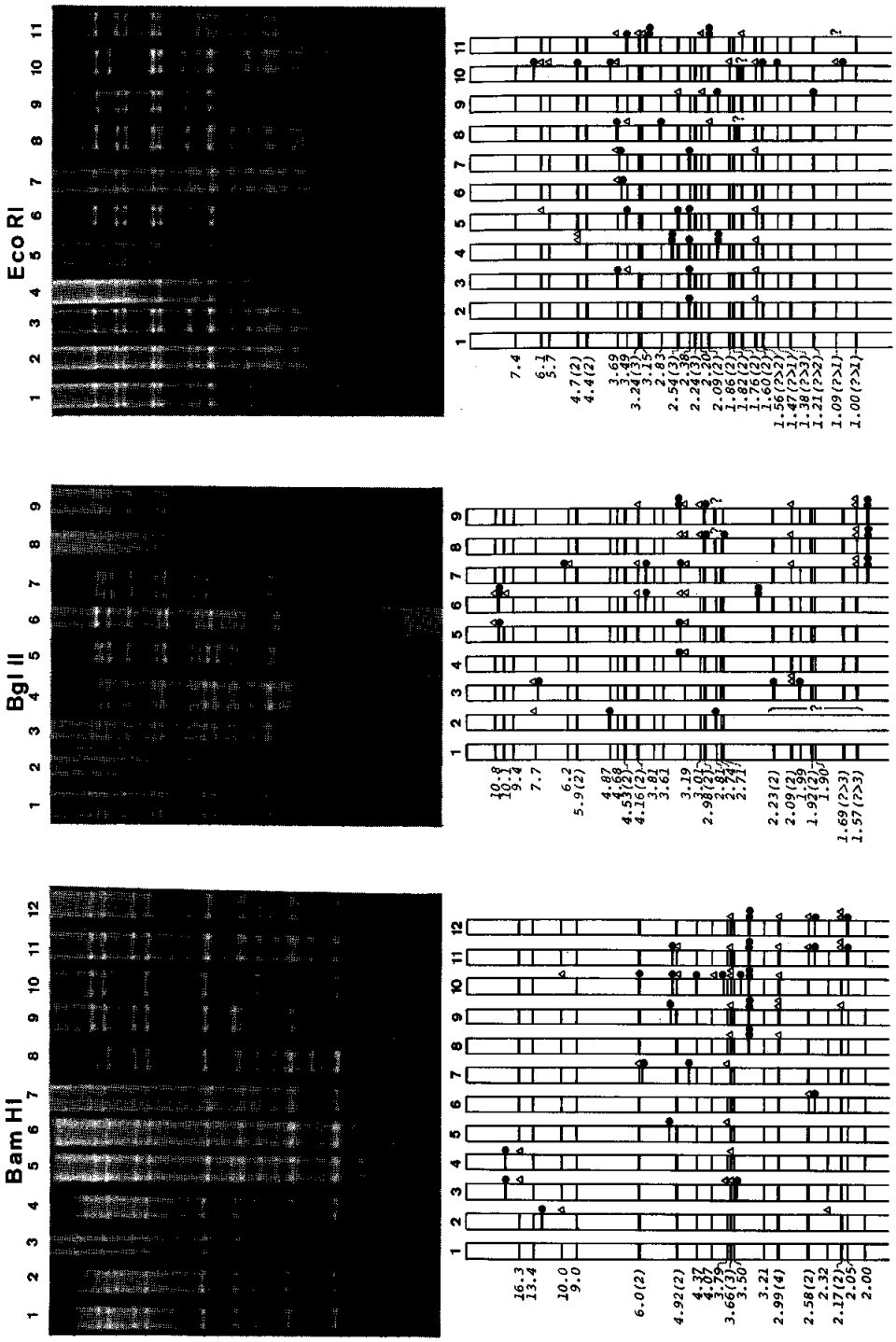

Fig. 2. Types of *Bam*HI, *Bgl*II and *Eco*RI restriction fragment patterns observed among ctDNAs from 37 sources and their schematic representation under each picture. Molecular sizes (kbp) of all fragments observed in Type 1 pattern are indicated at the left side of each schematic pattern. The figure given in parentheses indicates copy number of the fragment. Lost or gained fragment, as compared with Type 1 pattern, is indicated by △ or ●, respectively, on the right-hand side of the respective pattern.

Table 2. *CtDNA mutation analysis on the BamHI restriction fragment patterns*

Type	Informative mutation*		Independent mutation*		CtDNA source
	Loss	Gain	Loss	Gain	
1	—	—	—	—	**
2	—	—	10.0+2.32	12.2	*tbr* 1, *tbr* 2, C*tbr*
3	16.3+3.66	19.5	3.79	3.44	*adg* 1, *adg* 2
4	16.3+3.66	19.5	—	—	*acl, mlt, gon, phu, stn* 1, *stn* 2, *cha* 1, *cha* 2
5	—	—	3.79+1.40	5.19	*cap*
6	2.58	2.45	—	—	*pur*
7	—	—	6.0 3.79	5.89 4.60	*msp*
8	3.66+2.99	3.36×2	—	—	*cph, pnt, pld*
9	3.66+2.99	3.36×2	2.99+2.17	5.14	*blb*
10	4.92 3.66+2.99	5.02 3.36×2	10.0 4.07 3.66	6.0+3.85 4.37 3.45	*etb*
11	4.92 3.66+2.99 2.58 2.17×2	5.02 3.36×2 2.45 2.05+?	—	—	*lyc*
12	3.66+2.99 2.58 2.17×2	3.36×2 2.45 2.05+?	—	—	*le, lp*

*: Loss or gain of a fragment is determined in comparison with Type 1. Each fragment is indicated by its molecular size in kbp. Number of multiple copies is given with the× sign. ? shows unidentified fragment(s).

**: CtDNA sources of Type 1 are *sto, dms, chc, cmm, san, ifd, blv, lph, spl, spg, vrn, ver* and *tbr* 3.

restriction fragment changes are summarized in Table 2. All Mexican diploids (Types 8 and 9), non-tuberous species (Types 10 and 11) and *Lycopersicon* species (Type 12) differed from the South American species (Types 1–7) in their ctDNA by possessing two copies of 3.36 kbp fragments which had originated from 3.66 and 2.99 kbp fragments of Type 1, probably through a small inversion as described by Palmer *et al.* (1983); however, we deal with these fragment changes as two independent mutations.

Bgl*II restriction fragment pattern*: The *Bgl*II pattern was classified into nine types, as shown in Fig. 2. In Type 1, 36 restriction fragments, the total molecular size of which amounted to 136.9 kbp were observed. In addition, at least eight bands, all of which were smaller than 1.5 kbp in molecular size, were noticed but could not be analyzed with certainty, and were thus excluded from the present analysis. The fragment changes observed in Type 2 to 9, as

Table 3. *CtDNA mutation analysis on the BglII restriction fragment patterns*

Type	Informative mutation*		Independent mutation*		CtDNA source
	Loss	Gain	Loss	Gain	
1	—	—	—	—	**
2	—	—	7.7	4.87+2.81	*cmm*
3	—	—	7.7 2.09×2	7.6 2.23+1.99	*pur*
4	3.19	3.23	—	—	*blb, cph, pnt*
5	10.8 3.19	10.5 3.23	—	—	*pld*
6	10. 8 4.16 3.19	10.5 3.98 3.23	10.1 3.23	10.5 2.36×2	*etb*
7	4.16 3.19 2.09 1.57×2	3.98 3.23 ? 1.50×2	6.2	6.3	*lyc*
8	3.19 3.01 2.09 1.57×2	3.23 2.98 ? 1.50×2	3.23	2.71	*le*
9	3.19 3.01 2.09 1.57×2	3.23 2.98 ? 1.50×2	4.16	3.23	*lp*

*: Refer to a footnote of Table 2.
**: CtDNA sources of Type 1 are *dms, cap, san, msp, acl, ifd, blv, lph, mlt, spl, spg, ver, stn* 2, *cha* 1, *cha* 2, *tbr* 1, *tbr* 3, *adg* 1 and *adg* 2.

compared with the standard Type 1, are collectively shown in Table 3.

Eco*RI restriction fragment pattern*: Eleven different types were found, as shown in Fig. 2. In Type 1, at least, 42 fragments, including multiple copies, which amounted to 110.7 kbp were observed: Fragments smaller than 1 kbp were not accounted for it. The fragment changes observed in Type 2 to 11, as compared with Type 1, are collectively shown in Table 4.

Hind*III restriction fragment pattern*: Eight different types were found among the *Hind*III pattern of 27 ctDNA sources (Fig. 3). In Type 1, 21 restriction fragments, the total molecular size of which amounted to 143.5 kbp were analyzable. In addition, at least, 12 fragments, the molecular size of which was smaller than 1.2 kbp, were noticed but could not be analyzed with certainty, thus being excluded from the present analysis. The fragment changes observed in Type 2 to 8, as compared with Type 1, are collectively shown in Table 5.

Table 4. *CtDNA mutation analysis on the EcoRI restriction fragment patterns*

Type	Informative mutation*		Independent mutation*		CtDNA source
	Loss	Gain	Loss	Gain	
1	—	—	—	—	**
2	1.60	2.38	—	—	*dms, chc, san, blv, spg, tbr* 3, *adg* 1, *adg* 2
3	1.60	2.38	3.49	3.69	*tbr* 1, *tbr* 2, *Ctbr*
4	1.60	2.38	4.7×2	2.65×2+ 1.98×2	*ver*
5	1.60	2.38	6.1	3.49+2.54	*cmm*
6	—	—	3.69	3.65	*ifd*
7	1.60	2.38	3.69	3.67	*pur*
8	—	—	3.49 2.09	3.69 2.83	*blb*
9	—	—	2.54 2.20	1.98 1.21	*cph*
10	—	—	6.1 5.7 3.69 1.86 1.60 1.09	6.3 4.7 3.76 1.56 1.47 1.06	*etb*
11	3.69 3.24 3.15 2.20 1.76	3.49 3.11 3.11 2.09 2.09	—	—	*lyc, lp*

*: Refer to a footnote of Table 2.

**: CtDNA sources of Type 1 are *pnt, pld, cap, acl, lph, mlt, spl, vrn, gon, phu, stn* 1, *cha* 1 and *cha* 2.

Kpn*I restriction fragment pattern*: The patterns obtained from the *Kpn*I digests were the simplest of all the digests with the eight endonucleases we used (Fig. 3). In Type 1, 11 fragments, the total molecular size of which was 150.9 kbp were analyzed. In addition, at least, one fragment which was smaller than 2 kbp, was noticed but excluded from the present analysis. The fragment changes observed in Type 2 to 5, as compared with Type 1, are compiled in Table 6.

Pst*I restriction fragment pattern*: The *Pst*I pattern was classified into seven types (Fig. 3). In Type 1, 14 fragments, the total molecular size of which amounted to 154.9 kbp were analyzable. The fragment changes observed in Type 2 to 7, as compared with Type 1, are collectively shown in Table 7.

Xba*I restriction fragment pattern*: The *Xba*I fragment pattern was classi-

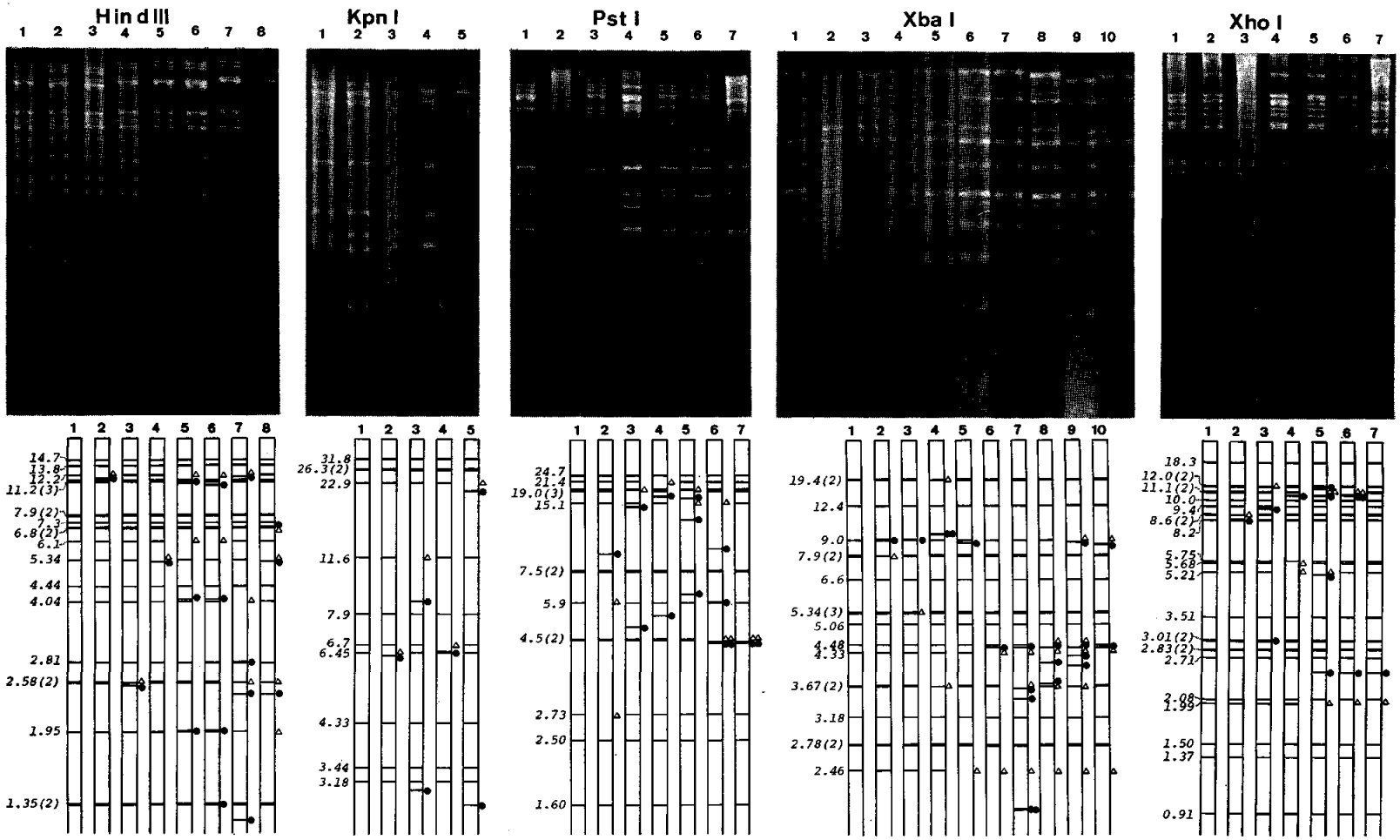

Fig. 3. Types of *Hin*dIII, *Kpn* I, *Pst*I, *Xba*I and *Xho*I restriction fragment patterns observed among ctDNAs from 37 sources and their schematic representation under each picture. For the numbers and marks given in the schemes, see the explanations in Fig. 2.

Table 5. *CtDNA mutation analysis on the HindIII restriction fragment patterns*

Type	Informative mutation*		Independent mutation*		CtDNA source
	Loss	Gain	Loss	Gain	
1	—	—	—	—	**
2	—	—	12.2	11.7	*tbr* 1
3	—	—	2.58	2.54	*cha* 1, *cha* 2
4	—	—	5.34	5.23	*pur*
5	(12.2, 6.1)	(11.2, 4.14+1.95)	—	—	*cph*, *pnt*
6	6.1	4.14+1.95	12.2	10.9+1.35	*pld*
7	2.58	2.37	(12.2, 4.04)	(11.9, 2.81+1.22)	*etb*
8	2.58	2.37	(6.8, 5.34, 1.95)	(7.0, 5.22, ?)	*lyc*

*: Refer to a footnote of Table 2.
**: CtDNA sources of Type 1 are *blb*, *dms*, *cmm*, *cap*, *san*, *msp*, *acl*, *ifd*, *blv*, *lph*, *mlt*, *spl*, *spg*, *ver*, *stn* 2, *tbr*3, *adg* 1 and *adg* 2.

Table 6. *CtDNA mutation analysis on the KpnI restriction fragment patterns*

Type	Informative mutation*		Independent mutation*		CtDNA source
	Loss	Gain	Loss	Gain	
1	—	—	—	—	**
2	—	—	6.45	6.15	*tbr* 1
3	—	—	11.6	8.8+2.87	*ifd*
4	6.7	6.45	—	—	*etb*, *pld*
5	—	—	22. 9	20.7+2.25	*lyc*

*: Refer to a footnote of Table 2.
**: CtDNA sources of Type 1 are *blb*, *pnt*, *sto*, *dms*, *cmm*, *cap*, *san*, *pur*, *acl*, *blv*, *lph*, *mlt*, *spl*, *spg*, *ver*, *stn* 2, *cha* 1, *cha* 2, *tbr* 3, *adg* 1 and *adg* 2.

fied into ten types, as shown in Fig. 3. Type 1 pattern consisted of 19 fragments, including multiple copies, total size of which amounted to 131.0 kbp: Fragments smaller than 2 kbp were not analyzed. All the fragment changes observed in Type 2 to 10, as compared with Type 1, are collectively shown in Table 8.

Xho*I restriction fragment pattern*: The *Xho*I pattern was classified into seven types (Fig. 3). In Type 1, 24 fragments, the total molecular size of which amounted to 151.7 kbp were analyzed. The fragment changes in Type 2

Table 7. *CtDNA mutation analysis on the PstI restriction fragment patterns*

Type	Informative mutation*		Independent mutation*		CtDNA source
	Loss	Gain	Loss	Gain	
1	—	—	—	—	**
2	—	—	5.9+2.73	9.2	*cmm*
3	—	—	19.0	14.3+5.0	*san*
4	—	—	21.4	16.2+5.3	*pld*
5	—	—	19.0 15.1	12.8+6.2 15.6	*etb*
6	4.5×2	4.4×2	15.1	9.4+5.9	*lyc*
7	4.5×2	4.4×2	—	—	*le, lp*

*: Refer to a footnote of Table 2.
**: CtDNA sources of Type 1 are *blb, cph, pnt, sto, dms, cap, msp, pur, acl, ifd, blv, lph, mlt, spl, spg, ver, stn* 2, *cha* 1 *cha* 2, *tbr* 1, *adg* 1 and *adg* 2.

Table 8. *CtDNA mutation analysis on the XbaI restriction fragment patterns*

Type	Informative mutation*		Independent mutation*		CtDNA source
	Loss	Gain	Loss	Gain	
1	—	—	—	—	**
2	—	—	7.9	9.0	*stn* 2
3	5.43	9.0	—	—	*sto, ver*
4	—	—	19.4 3.67	9.5×2 ?	*msp*
5	2.46	?	?	8.7	*pur*
6	4.33 2.46	4.42 ?	—	—	*cph, pnt*
7	4.33 2.46	4.42 ?	3.67×2 ?	3.63+3.43 2.01×2	*blb*
8	4.33 2.46	4.42 ?	4.48 3.67	4.11 3.75	*pld*
9	4.33 2.46	4.42 ?	9.0 4.48 3.67	8.8 4.23 4.04	*etb*
10	4.33 2.46	4.42 ?	9.0	8.7	*lyc*

*: Refer to a footnote of Table 2.
**: CtDNA sources of Type 1 are *dms, cap, san, acl, ifd, blv, lph, mlt, spl, spg, cha* 1, *cha* 2, *tbr* 1, *tbr* 3, *adg* 1 and *adg* 2.

Table 9. *CtDNA mutation analysis on the XhoI restriction fragment patterns*

Type	Informative mutation*		Independent mutation*		CtDNA source
	Loss	Gain	Loss	Gain	
1	—	—	—	—	**
2	—	—	8.6	8.2	*tbr* 1, *tbr* 2
3	—	—	12.0	9.1+3.01	*stn* 2
4	—	—	5.68+5.21	10.9	*blb*
5	1.99	2.45	(11.1×2 / 5.21)	(11.9+10.9 / 5.16)	*etb*
6	1.99	2.45	11.1×2	10.9×2	*lyc*
7	1.99	2.45	—	—	*le*, *lp*

*: Refer to a footnote of Table 2.

**: CtDNA sources of Type 1 are *cph*, *pnt*, *pld*, *sto*, *dms*, *chc*, *cmm*, *cap*, *san*, *msp*, *pur*, *acl*, *ifd*, *blv*, *lph*, *mlt*, *spl*, *spg*, *ver*, *cha* 1, *cha* 2, *tbr* 3, *adg* 1 and *adg* 2.

to 7 are summarized in Table 9, as compared with Type 1.

3) *Differences between ctDNAs of 28* Solanum *and 2* Lycopersicon *species*

The restriction fragment pattern types of ctDNA digested with eight restriction endonucleases are collectively shown in Table 10. Interspecific relationship between these chloroplast genomes is shown in Fig. 4, in the form of a phylogenetic tree that was obtained by the following clustering method. Firstly, the order of clustering the species, the root of the tree and the similarity distances among clusters were determined by the group-average method, using the matrix of similarity distances calculated from the number of identical informative mutations and total number of mutations between the two species. Secondly, each branch was extended by the number of independent mutations in each species.

4. DISCUSSION

1) *Phylogenetic relationships revealed from the ctDNA resemblance*

As shown in Fig. 4, 28 tuberous *Solanum* species and two *Lycopersicon* species were classified into four groups based on their ctDNA resemblance; (1) South American species and Mexican polyploid species, (2) Mexican diploid species, (3) *etb*, and (4) *lyc* and *Lycopersicon* species. These groups correspond well to the groups established from crossability by Hawkes (1978). Interspecific relationships within each group are discussed below.

South American species and Mexican polyploid species: Although *chc* and

Table 10. *Restriction fragment pattern types of ctDNAs of tuberous Solanum and Lycopersicon species*

CtDNA source	Restriction fragment pattern type*							
	*Bam*HI	*Bgl*II	*Eco*RI	*Hin*dIII	*Kpn*I	*Pst*I	*Xba*I	*Xho*I
le	12	8	—	—	—	7	—	7
lp	12	9	11	—	—	7	—	7
lyc	11	7	11	8	5	6	10	6
etb	10	6	10	7	4	5	9	5
blb	9	4	8	1	1	1	7	4
cph	8	4	9	5	—	1	6	1
pnt	8	4	1	5	1	1	6	1
pld	8	5	1	6	4	4	8	1
sto	1	—	—	—	1	1	3	1
dms	1	1	2	1	1	1	1	1
chc	1	—	2	—	—	—	—	1
cmm	1	2	5	1	1	2	—	1
cap	5	1	1	1	1	1	1	1
san	1	1	2	1	1	3	1	1
msp	7	1	—	1	—	1	4	1
pur	6	3	7	4	1	1	5	1
acl	4	1	1	1	1	1	1	1
ifd	1	1	6	1	3	1	1	1
blv	1	1	2	1	1	1	1	1
lph	1	1	1	1	1	1	1	1
mlt	4	1	1	1	1	1	1	1
spl	1	1	1	1	1	1	1	1
spg	1	1	2	1	1	1	1	1
vrn	1	—	1	—	—	—	—	—
ver	1	1	4	1	1	1	3	1
gon	4	—	1	—	—	—	—	—
phu	4	—	1	—	—	—	—	—
stn 1	4	—	1	—	—	—	—	—
stn 2	4	1	—	1	1	1	2	3
cha 1	4	1	1	3	1	1	1	1
cha 2	4	1	1	3	1	1	1	1
tbr 1	2	1	3	2	2	1	1	2
tbr 2	2	—	3	—	—	—	—	2
tbr 3	1	1	2	1	1	—	1	1
C*tbr*	2	—	3	—	—	—	—	—
adg 1	3	1	2	1	1	1	1	1
adg 2	3	1	2	1	1	1	1	1

* : Refer to Fig. 2 and 3 for the individual restriction fragment pattern types.
—: Not analyzed.

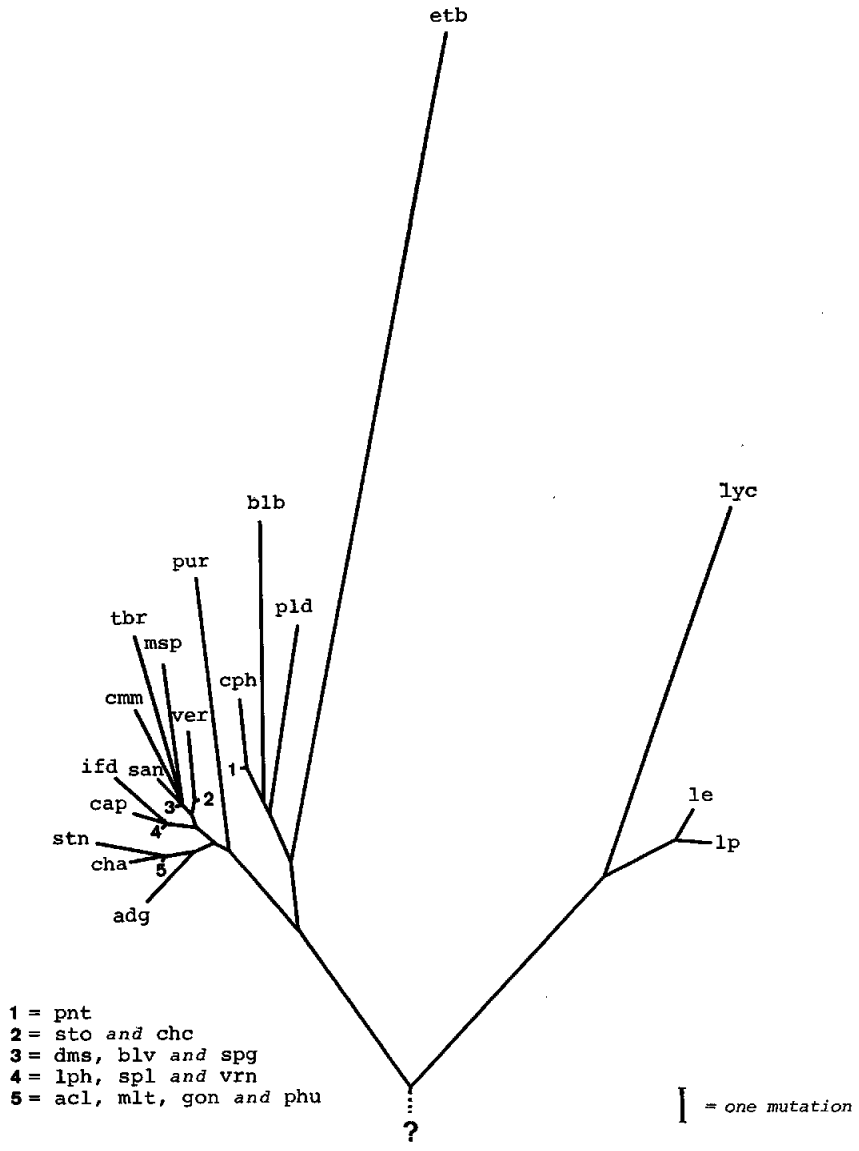

Fig. 4. A dendrogram showing the phylogenetic relationship between chloroplast genomes of tuberous *Solanum* and *Lycopersicon* species; obtained by the analysis of ctDNA restriction fragment patterns. The interspecific ctDNA difference between two given species is estimated by the sum of the length of all branches lying between the two species, divided by the unit length corresponding to one mutation that is indicated at the right-hand side.

cmm are included in the series *Commersoniana*, the ctDNA of *cmm* shows three specific mutations, and is different from that of *chc* by, at least, one mutation. The nuclear genome of *cmm* differs from those of *chc* and *Tuberosa* species by small structural changes of chromosomes (Matsubayashi 1983). Therefore, *cmm* is slightly differentiated from other South American species in both the cytoplasmic and nuclear genome.

A *Circaeifolia* species, *cap*, differs from other South American species only by one ctDNA mutation from three *Tuberosa* species. Thus, the cytoplasm of *cap* is very closely related to those of the *Tuberosa* species, but, it has low crossability and clear morphological differences to other South American species (Hawkes, personal communication). No cytological data are available on their relationship.

Of two *Conicibaccata* species, *san* has almost the same ctDNA restriction fragment pattern as *dms*, *chc*, *blv* and *spg*. On the other hand, *msp* differs from *san* by five ctDNA mutations. Since one of the *msp* genomes is identical to *san* genome (López 1979), the cytoplasm donor to *msp* seems to be the donor of its second or third genome, providing *san* is the first genome donor to *msp*. The other possibility that ctDNA mutations have occurred in *msp* and *san* after their speciation can not be excluded because according to Hawkes (1979) this series differentiated a long time ago.

CtDNA of *pur* of the series *Piurana* greatly differs from those of all other South American species, at least, by eight mutations. Accordingly, *pur* shows low crossability to *ifd*, and their F_1 hybrid exhibits more irregular meiotic behavior than those observed in F_1 hybrids between *ifd* and the other South American diploid species (Nagasawa and Matsubayashi, personal communication).

CtDNA of *acl* in the series *Acaulia* shows the same restriction fragment pattern as those of *mlt*, *gon* and *phu* in the series *Tuberosa*. This species is a wild or weed tetraploid species possessing a genome constitution AAA^aA^a (Matsubayashi 1982). Thus, the *acl* cytoplasm seems to have derived from *mlt*, *gon*, *phu*, or some unidentified species closely related to them.

As to two species of the series *Cuneoalata* and *Megistacroloba*, *ifd* has ctDNA different from those of *lph*, *spl* and *vrn* by two mutations, and *blv* has the same ctDNA as that of *spg*. Therefore, *ifd* and *blv* are cytoplasmically closely related to *Tuberosa* species, though their chromosomes have cryptic structural differences from those of *Tuberosa* (Matsubayashi 1981).

Close ctDNA resemblance is found among 10 *Tuberosa* species except *ver* and *tbr*. CtDNA of *ver* that distributes in Mexico (Hawkes 1956a) differs from those of other *Tuberosa* species, at least, by three mutations. These mutations might have occurred after this species was geographically isolated from the South American species. CtDNA of *tbr* will be discussed later in relation to those of other cultivated species in this series.

As stated above, all South American species show obvious ctDNA resemblance with each other, irrespective of the taxonomic series they belong to (Fig. 4). This fact supports the previous view that all South American species possess the A genome or its slightly modified genomes by multiple gene substitution or cryptic structural differentiation of the chromosomes (Howard and Swaminathan 1952; Hawkes 1958, 1978; Irikura 1976; Matsubayashi 1981). The present results are also in accordance with the data of peroxidase isozyme analysis (Hosaka and Matsubayashi 1983).

The two Mexican polyploid species, *sto* and *dms*, are very closely related to the South American species in their ctDNA. This fact suggests that these Mexican polyploid species originated recently in Mexico by amphidiploidization, having a South American species as the female parent. In fact, *sto* and *dms* are allotetra- and allohexaploid, respectively (Hawkes 1958, 1978; Matsubayashi 1981), and one of their genomes seems to have derived from *ver* (Irikura 1976). As *ver* is known to distribute in Mexico as previously described and has ctDNA closely resembling that of *sto*, it is highly probable that *ver* provided both the A genome and cytoplasm to *sto*.

Mexican diploid species: The ctDNAs of all Mexican diploid species, *i.e.*, *blb*, *cph*, *pnt* and *pld*, differ from those of the South American species and Mexican polyploid species by about 10 mutations. The difference in ctDNA between Mexican diploid species is rather large, *i.e.*, seven to 14 mutations, except between two *Pinnatisecta* species, which differ by only two mutations. The greater cytoplasmic differentiation between the series of Mexican diploid species than that between the series of South American species is well correlated with the greater genomic differentiation in the former than in the latter that have been revealed by cytogenetical investigations (Matsubayashi and Misoo 1977; Ramanna and Hermsen 1979; Matsubayashi 1981), as well as by peroxidase isozyme analysis (Hosaka and Matsubayashi 1983).

S. etuberosum: CtDNA of a non-tuberous species *etb* shows 21 specific mutations and is most closely related to that of *pnt*, revealing 29 mutational changes from the latter. Evidently *etb* is cytoplasmically very far from all tuberous *Solanums*. This is in good accordance with the results of genome analysis that a unique genome, E, present in *etb* does not exist in *pnt* (Ramanna and Hermsen 1981). CtDNA of *etb* possesses five to seven informative mutations in common with Mexican diploid species. Hence, *etb* seems to have, in part, a common evolutionary history with the Mexican diploid species, though at present they distribute in different subcontinents.

S. lycopersicoides *and* Lycopersicon *species*: The present ctDNA data indicate that *lyc* is cytoplasmically more closely related to *Lycopersicon* species

than to tuberous *Solanums*. Palmer and Zamir (1982) already investigated the ctDNA restriction fragment patterns of seven *Lycopersicon* species and their three close relatives, including *lyc*, with 25 endonucleases reporting much less ctDNA difference between them than in the present results.

Resemblance of *lyc* to *Lycopersicon* species was already pointed out by Rick (1951) from the crossability test and by Hawkes (1956a) from morphological comparison. Thus, *lyc* seems to have evolved independently from both the tuberous *Solanums* and non-tuberous *etb*.

2) *Origin of the cytoplasm of cultivated potato*

Diploid cultivated species: CtDNAs of *gon*, *phu* and *stn* are almost identical, though two other mutations are *stn*-specific. This fact supports Hawkes' view that *gon*, *phu* and *stn* derived from a common ancestral type (Hawkes 1956a).

Triploid cultivated species: The ctDNAs of two *cha* clones resembled those of *gon*, *phu* and *stn* more closely than they did that of *adg*, implying that *cha* may have occurred from one of the first three species as the female parent. Hawkes (1956a) and Jackson *et al.* (1977) considered its origin to be natural crosses between *adg* as female and *stn* as male parent. Recently, Hawkes (1978) suggested the possibility that it originated by the union of reduced and unreduced gametes in *stn*. The present results support the latter possibility.

Tetraploid cultivated species:

(i) Ssp. *andigena*—Various hypotheses have been proposed on the origin of *adg*. Based on the morphological data, Hawkes (1956b) suggested its origin as an amphidiploid between *stn* and *spl*, while Brücher (1964) suggested its amphidiploid origin between *stn* and *vrn*. Both of them did not state which species was the female parent. On the contrary, Matsubayashi (1981) assumed that *adg* originated as an amphidiploid between *phu* as female and *stn* as male parent, based on the morphologies and cytological behaviors of the F_1 hybrid and colchicine-induced amphidiploid between *phu* and *stn*. In addition, the autotetraploid origin of *adg* from *stn* was also suggested by some workers (Swaminathan and Magoon 1961; Gatenby and Cocking 1978).

CtDNA of *adg* differs from those of *gon* and *phu* by two fragment mutations, and by three fragment mutations from those of *spl* and *vrn*. In addition, one common mutation is found among ctDNAs of *gon*, *phu*, *stn* and *adg* (Table 2). Thus, it is concluded that the female parent of *adg* is neither *spl* nor *vrn*, but one of cultivated diploid species.

(ii) Ssp. *tuberosum*—The ctDNAs of four *tbr* strains have been analyzed with two to eight restriction endonucleases. Of these, two cultivars and

Chilean *tbr* have identical patterns with all enzymes, whereas one cultivar 'Greta' (*tbr*3) has a pattern identical to that of *dms*. Their differences are simply explained by the fact that 'Greta' was bred from the cross between *dms* as female and *tbr* as male in order to introduce late blight resistant genes of the former into common potato (Toxopeus 1964).

Salaman (1937) and Hawkes (1956b) suggested that European and Chilean *tbr* derived independently from *adg* by selection for long-day adaptation in Europe and Chile, respectively. Nevertheless, the present data reveal that the cytoplasm of *tbr* is remarkably different from those of *adg* and other cultivated species. The fact that European *tbr* and *adg* have a different large subunit of Fraction I protein (Gatenby and Cocking 1978) also supports the present data. Their cytoplasmic difference was already pointed out by Grun (1979) who compared the reciprocal hybrids between *tbr* and *adg*, and suggested that the cytoplasm of European *tbr* is derived from *S. chacoense* f. *gibberulosum* through Chilean *tbr*. The present data, however, do not support his view, because the *chc* ctDNA (but not of f. *gibberulosum*) differs from that of *tbr* by three ctDNA mutations.

All these facts seem to suggest that Chilean *tbr* originated as a hybrid between an unidentified species as female and *adg* as male in the process migrating southward to Chile, though its nuclear genome has already been replaced mostly with the *adg* genome by backcrossing.

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