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Who is the mother of the potato? – restriction endonuclease analysis of chloroplast DNA of cultivated potatoes*

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Summary. Chloroplast DNA from 44 lines of 16 wild and 7 cultivated *Solanum* species were compared by restriction endonuclease analysis. Seven chloroplast genome types were identified among them by 5 restriction enzymes: Type A (*S. tuberosum* ssp. *andigena* and *S. maglia*); Type S (*S. goniocalyx, S. phureja, S. stenotomum, S.* × *chaucha* and a line of ssp. *andigena*); Type C (*S. acaule, S. bukasovii, S. canasense, S. multidissectum* and *S.* × *juzepczukii*); Type T (*S. tuberosum* ssp. *tuberosum*); Type W (other wild species); Type W′ (*S. chacoense* f. *gibberulosum*) and Type W″ (*S. tarijense*). From this cytoplasmic identification, it was concluded that *S. goniocalyx, S. phureja, S.* × *chaucha* and ssp. *andigena* were derived from *S. stenotomum* or its primitive type, which may have originally evolved itself from *S. canasense.* The chloroplast genome of the European potato, however, was introduced from the Chilean potato, which might have been primarily constructed with the nuclear genome from ssp. *andigena* and with cytoplasm from other species. The cytoplasmic donor of the Chilean potato could not be determined.

Key words: *Solanum tuberosum* ssp. *tuberosum* – Chloroplast DNA – Restriction endonuclease analysis – Cytoplasmic origin – Potato

Introduction

The European[1] and some Andean and Chilean potatoes are known as cultivated potatoes throughout the world. According to Hawkes (1956a, 1978), the Andean potato includes diploid (*Solanum stenotomum, S. phureja, S. goniocalyx* and *S. ajanhuiri*), triploid (*S.* × *chaucha* and *S.* × *juzepczukii*), tetraploid (*S. tuberosum* ssp. *andigena,* designated only as Andigena in this paper) and pentaploid (*S.* × *curtilobum*) species. Some Chilean and all European potatoes are taxonomically identified as *S. tuberosum* ssp. *tuberosum* and thought to have been independently selected as long-day adapted types from Andigena in Chile and Europe, respectively (Hawkes 1956b).

In a previous report (Hosaka et al. 1984), we indicated that chloroplast DNA (ctDNA) restriction endonuclease analysis was a useful method for the phylogenetic study of tuber-bearing *Solanum* species, and we obtained several interesting results, namely, that the cytoplasmic genome of all Andean cultivated potatoes were differentiated monophyletically from that of *S. stenotomum,* whereas both the Chilean and European potatoes have a distinctly different cytoplasmic genome from that of the above group. Buckner and Hyde (1985), however, also reporting the results of ctDNA restriction endonuclease analysis of the common potato and some relatives described their Andigena ctDNA to be the same as that of the common potato.

In this paper an attempt is made to verify the proposal of Hosaka et al. (1984) by including a study of *S.* × *juzepczukii* and *S.* × *curtilobum,* both of which were not analyzed previously. The cytoplasmic donor species of these Andean potatoes as well as those of the European and Chilean potatoes will also be discussed.

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[1] 'European' potato means the so-called common potato that became widespread throughout the world after its introduction into Europe in the sixteenth century

Table 1. Restriction fragment pattern types of ctDNAs from cultivated potatoes and their relatives and ct-genome types proposed for each line

Species	Identity	Source[a]	Code	2n	Bam	Hin	Kpn	Pvu	Xho	Ct-genome type
I Cultivated species										
S. goniocalyx	PI 195188	4)	–	24	4*	–	–	–	–	S
S. phureja	PI 320360	1)	01	24	4	3	1	1	1	S
S. phureja	Ivp 35	4)	–	24	4*	–	–	–	–	S
S. stenotomum	PI 205527	4)	–	24	4*	–	–	–	–	S
S. stenotomum	PI 234010	1)	02	24	4	3	1	1	1	S
S. stenotomum	PI 234015	1)	03	24	4	3	1	1	1	S
S. × *chaucha*	T-110-a	2)	04	36	4	3	1	1	1	S
S. × *chaucha*	T-AY-43	3)	–	36	4*	3*	1*	–	1*	S
S. × *juzepczukii*	T-109	2)	05	36	4	1	1	1	1	C
S. tuberosum										
ssp. *andigena*	T-AY-5	3)	06	48	3	1	1	1	1	A
	T-AY-6	3)	07	48	4	3	1	1	1	S
	T-AY-19	3)	08	48	3	1	1	1	1	A
	T-AY-22	3)	–	48	3*	1*	1*	–	1*	A
	T-AY-28	3)	09	48	3	1	1	1	1	A
ssp. *tuberosum*										
(European)	cv. 'Early Rose'	4)	–	48	2*	–	–	–	–	T
	cv. 'Greta'		–	48	1*	1*	1*	–	1*	W
	cv. 'Irish Cobbler'		10	48	2	2	2	2	2	T
	cv. 'May Queen'		11	48	2	2	2	2	2	T
(Chilean)	cv. 'Chona'	5)	12	48	2	2	2	2	2	T
	cv. 'Huilcana'	5)	–	48	2*	–	–	–	–	T
(Wild Chilean)	PI 208563	4)	13	48	2	2	2	2	2	T
	PI 133667	4)	14	48	2	2	2	2	2	T
S. × *curtilobum*	T-86-f	2)	15	60	4	3	1	1	1	S
II Wild species										
S. acaule	1-t	3)	–	48	4*	1*	1*	–	1*	C
S. bukasovii	PI 210044	1)	16	24	4	1	1	1	1	C
S. canasense	PI 458375	1)	17	24	4	1	1	1	1	C
S. chacoense	PI 472820	1)	18	24	1	1	1	1	1	W
S. chacoense	PI 230580	4)	–	24	1*	–	–	–	1*	W
S. chacoense f. *gibberulosum*	PI 133073 × 133664	1)	19	24	1	1	–	4	1	W′
S. demissum	PI 160230	1)	–	60	1*	1*	1*	–	1*	W
S. gourlayi	PI 473059	1)	20	24	1	1	–	1	1	W
S. kurtzianum	PI 442678	1)	21	24	1	1	1	1	1	W
S. leptophyes	PI 458378	1)	22	24	1	1	1*	–	1	W
S. maglia	PI 245087	1)	23	36	3	–	1	1	1	A
S. maglia	PI 407408	1)	24	24	3	1	1	1	1	A
S. microdontum	PI 473176	1)	25	24	1	1	1	1	1	W
S. multidissectum	PI 210043	1)	–	24	4*	1*	1*	–	1*	C
S. oplocense	PI 473499	1)	26	24	1	1	1	1	1	W
S. sparsipilum	PI 210039	1)	–	24	1*	1*	1*	–	1*	W
S. sparsipilum	PI 473305	1)	27	24	1	1	1	1	1	W
S. spegazzinii	PI 472966	1)	–	24	1*	1*	1*	–	1*	W
S. tarijense	PI 265577	1)	28	24	1	1	1	3	1	W″
S. vernei	PI 473308	1)	29	24	1	1	1	1	1	W
S. vernei	D/1421	4)	–	24	1*	–	–	–	–	W

[a] Seeds or tubers were supplied from the following: 1) Inter-Regional Potato Introduction Project (IR-1), Potato Introduction Station, Wisconsin, USA; 2) Ochoa CM, International Potato Center, Lima, Peru; 3) Collection of the Expedition of Cultivated Plants in the Andean areas, Kyoto University (1971); 4) Irikura Y, Shimamatsu Potato Branch, Hokkaido National Agricultural Experiment Station, Japan; 5) Contreras A, Universidad Austral de Chile, Chile

For each restriction fragment pattern type, see Fig. 6

Bam = *Bam*HI; Hin = *Hin*dIII; Kpn = *Kpn*I; Pvu = *Pvu*II; Xho = *Xho*I; * = data cited from Hosaka et al. (1984); – = not analyzed

Note: Species names used for each accession were those which the IR-1 listed them under, although Prof. J. G. Hawkes indicated that *S. multidissectum* (PI 210043) and *S. tarijense* (PI 265577) were *S. bukasovii* and *S. tarijense* × *S. berthaultii,* respectively

Materials and methods

The *Solanum* species used are listed in Table 1 and follow the Hawkes' (1978) classification system. Of the eight accessions of *S. tuberosum* ssp. *tuberosum,* one is var. *guaytecarum* Hawkes (PI 208563), and another had been named *S. leptostigma* Juzepczuk (PI 133667), both of which are tetraploid wild types occurring in the coastal region of southern Chile.

Some ctDNAs were extracted by the same method described by Hosaka et al. (1984), whereas many of them were obtained by the following simple method referred to as the Palmer (1982) method: 50 to 100 g fresh leaves were homogenized with three 3 s bursts in a Waring blender with 3 volumes of cold A buffer (0.44 M mannitol, 3 mM EDTA, 1 mM 2-mercaptoethanol, and 0.1% bovine serum albumin in 50 mM Tris-HCl buffer, pH 8.0). The homogenate was filtered through four layers of gauze and two layers of Miracloth, all kept in ice, and then centrifuged at 3,500 rpm for 10 min at 4 °C. The pellet obtained was suspended gently by a soft brush in 12 ml B buffer (the same as A buffer except without 2-mercaptoethanol and bovine serum albumin) and loaded on six tubes of a step gradient consisting of 15 ml of a 60% sucrose solution and 15 ml of a 30% sucrose solution, each made in B buffer minus the mannitol. The gradient was centrifuged at 25,000 rpm for 50 min at 4 °C. The chloroplast band seen at the 30–60% interface was removed by pipetting and was gently diluted in 3 volumes of B buffer before centrifuging at 4,000 rpm for 10 min at 4 °C. The chloroplast pellet was resuspended in 2 ml of TE buffer (50 mM Tris-HCl buffer, pH 8.0, containing 20 mM EDTA) and after adding 0.5 ml of 10% sodium lauryl sarcosinate solution, it was rotated for 30 min with 2.5 ml of phenol saturated with TE buffer. After centrifuging the emulsion at 3,500 rpm for 10 min, the aqueous portion was collected. The solution was rotated again for 30 min with 2.5 ml of phenol and chloroform (1 : 1). The aqueous solution was collected after centrifugation at 3,500 rpm for 10 min and 1/10 volume of 3 M sodium acetate and 3 volumes of cold ethanol (–20 °C) were added. The ethanol solution was kept overnight at –20 °C for DNA precipitation and was centrifuged at 10,000 rpm for 15 min at –10 °C. The DNA pellet was dissolved in 100 to 300 µl DNA buffer (1 mM KCl and 0.1 mM EDTA in 1 mM Tris-HCl buffer, pH 7.9) and stored at 4 °C until use.

The restriction enzymes used were *Bam*HI, *Hin*dIII, *Kpn*I, *Pvu*II and *Xho*I. Digestion was done following the directions given by the supplier (Takara Shuzo Co. Ltd., Kyoto, Japan). After digestion, the ctDNA fragments mixture was further incubated with RNase at a concentration of 1 mg/ml at 37 °C for 30 min.

CtDNA fragments were separated by semi-submarine type electrophoresis at 50 V for 20–24 h in the agarose slab gel containing 20 mM sodium acetate and 2 mM EDTA in 40 mM Tris-HCl buffer (pH 7.8), the concentration of which was 0.5–1.0% depending on the enzymes used for digestions. DNA bands were observed and photographed under long wave UV light.

If the above DNA isolation method was not pure enough for digestion, one more cycle of phenol extraction and ethanol precipitation was usually sufficient. This simple purification procedure was adapted for digestion using the above five enzymes.

Results

Restriction fragment patterns among the ctDNAs used

Restriction fragment patterns of 29 ctDNAs digested by five enzymes are shown in Figs. 1 to 5. As indicated schematically in Fig. 6, four types of restriction fragment patterns were distinguished by each of *Bam*HI and *Pvu*II, three types by *Hin*dIII, and two types by each of *Kpn*I and *Xho*I. Note that the respective types except that of *Pvu*II correspond with the types found in the previous paper (Hosaka et al. 1984). In Table 1, all the restriction pattern types of the present materials are listed, to which some previous data shown with asterisks are also included. Considering the present data, the restriction pattern types of *S. stenotomum* given in the previous report (stn 2, PI 205526) must have been in error, probably caused by the impurity of the DNA, so that it did not digest well.

Relationships between the chloroplast genomes distinguished

Based on the similarity of the restriction fragment patterns, seven types of ctDNAs, which are dealt with as chloroplast genomes hereafter (ct-genomes), were identified (Table 1): ct-genome types T (named after ssp. *tuberosum*), A (after Andigena), S (after *S. stenotomum*), C (after *S. canasense*), and W (after wild species), W′ (*S. chacoense* f. *gibberulosum*) and W″ (*S. tarijense*). Among these ct-genome types, W is the most primitive, as its restriction fragment patterns show the closest pattern to the Mexican diploid species as well as to non-tuber-bearing species, both of which are phylogenically far from the species used here (Hosaka et al. 1984). Thus, the direction and the number of mutational changes between each ct-genome types were determined and counted compared with the W type ct-genome, based on the mutation analysis (Hosaka et al. 1984) (Table 2). Mutational changes between the respective ct-genomes are schematically indicated in Fig. 7; A, S and C changed from W by a common point mutation (16.3 kbp + 3.66 kbp → 19.5 kbp) (Table 2) occurred in one of *Bam*HI recognition sites. A and S also possessed fragment size reduction in each of *Bam*HI and *Hin*dIII fragments, respectively. W′ and W″, found in *S. chacoense* f. *gibberulosum* and *S. tarijense,* respectively, were derived from W by a different point mutation in the *Pvu*II recognition site. T, found in ssp. *tuberosum* except in cv. 'Greta', is thought to be changed from W by one point mutation occurring in one of *Bam*HI recognition sites and additionally by a fragment size reduction in each of *Hin*dIII, *Kpn*I, *Pvu*II and *Xho*I digests (Table 2). Theoretically, if one physical deletion really occurred, one or more fragment size reductions should be detected in every enzyme digest. T type ct-genome, therefore, seems to have one physical deletion because nearly the same size reductions (about 400 bp) were detected in the above four enzyme digests. This point will be tested by a further study to construct the physical map of potato ctDNA.

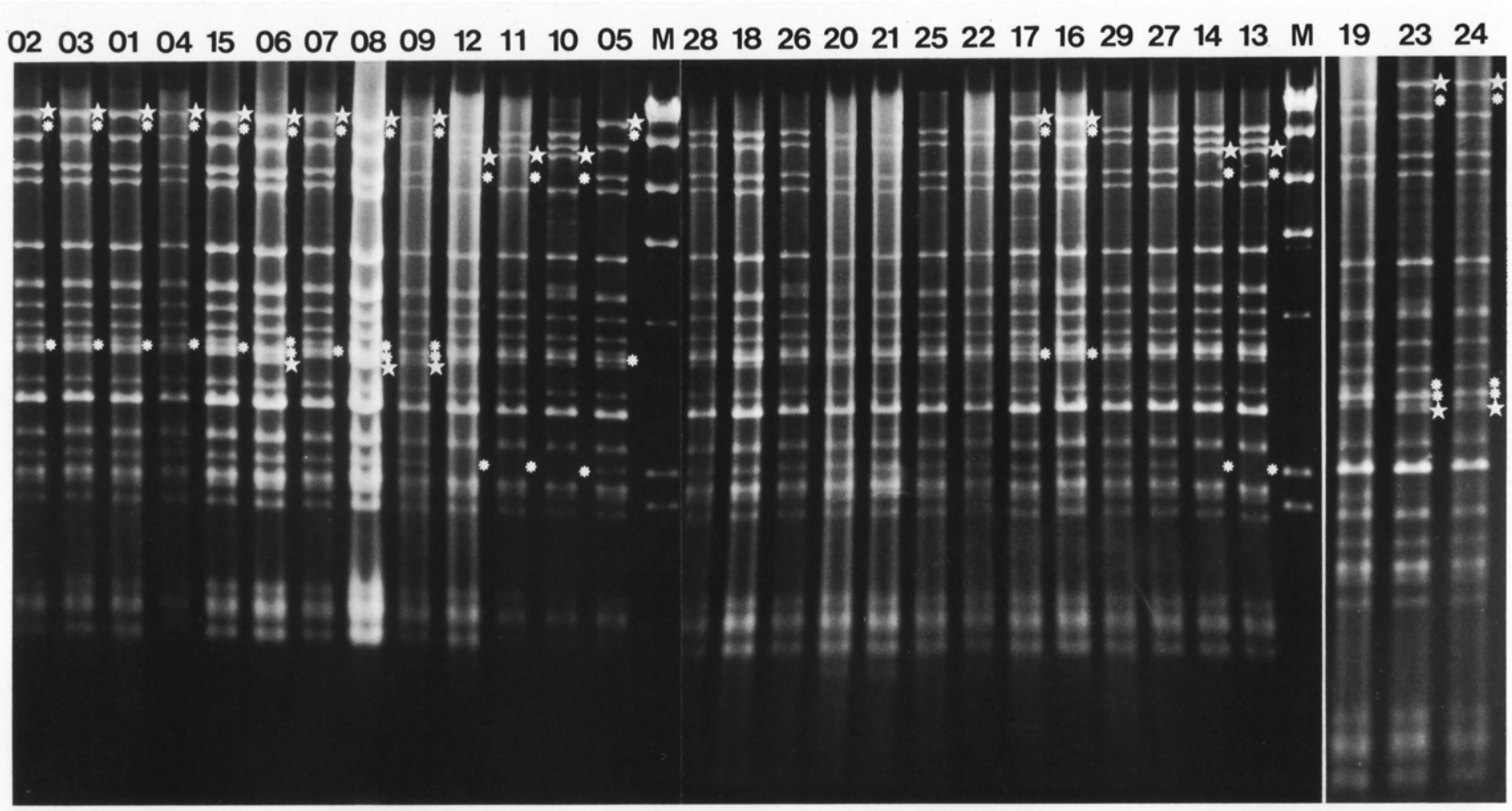

Fig. 1. *Bam*HI restriction fragment patterns of ctDNAs from cultivated species and their wild relatives. See Table 1 for accession code of each lane. M is a *Hin*dIII digestion pattern of λDNA. Electrophoresis was done on 0.8% agarose gels. Loss or gain of a fragment is indicated by * or ★, respectively, compared with that of most wild species

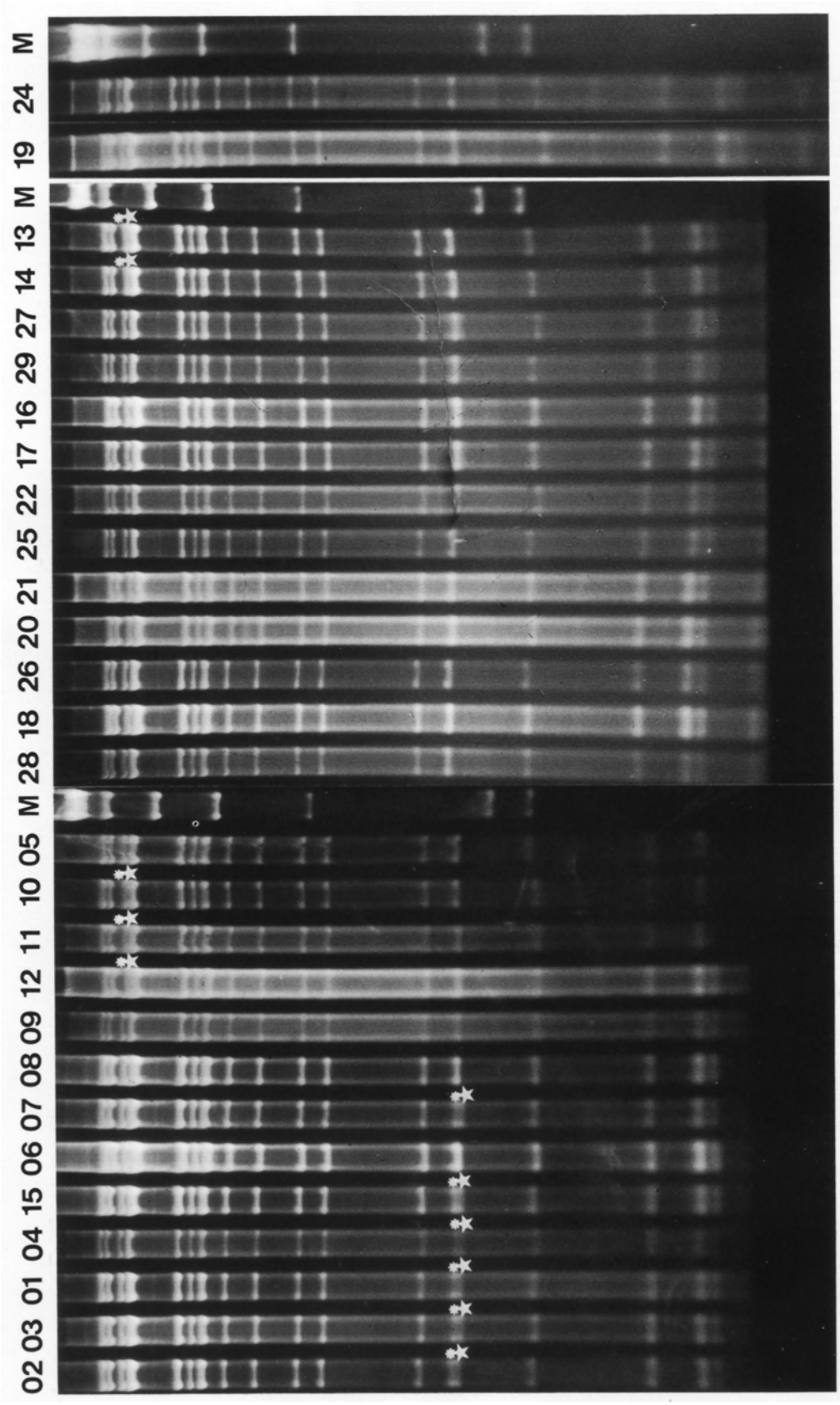

Fig. 2. *Hin*dIII restriction fragment patterns obtained on 1% agarose gels. See Fig. 1 for explanation of symbols

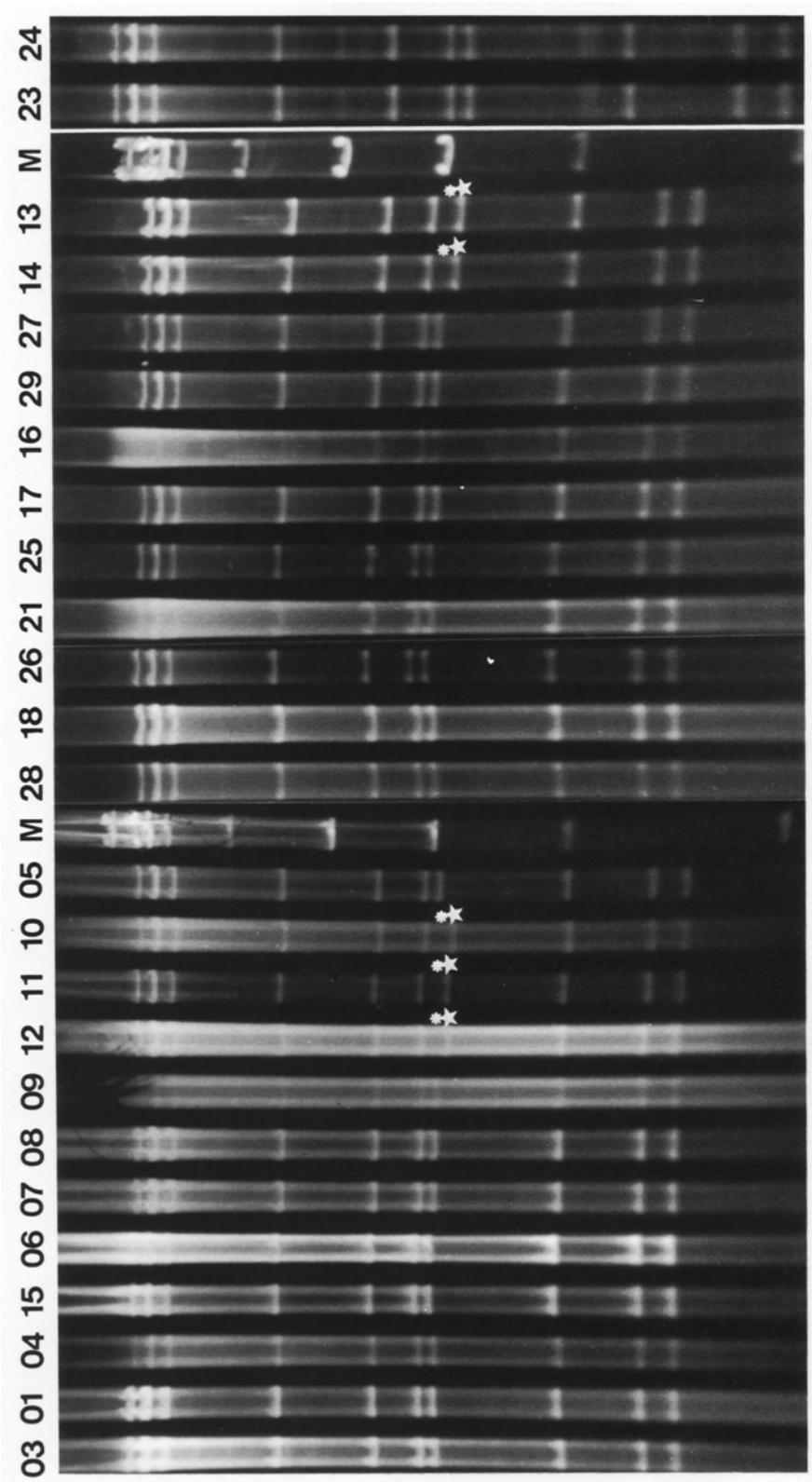

Fig. 3. *Kpn*I restriction fragment patterns obtained on 0.5% agarose gels. See Fig. 1 for explanation of symbols

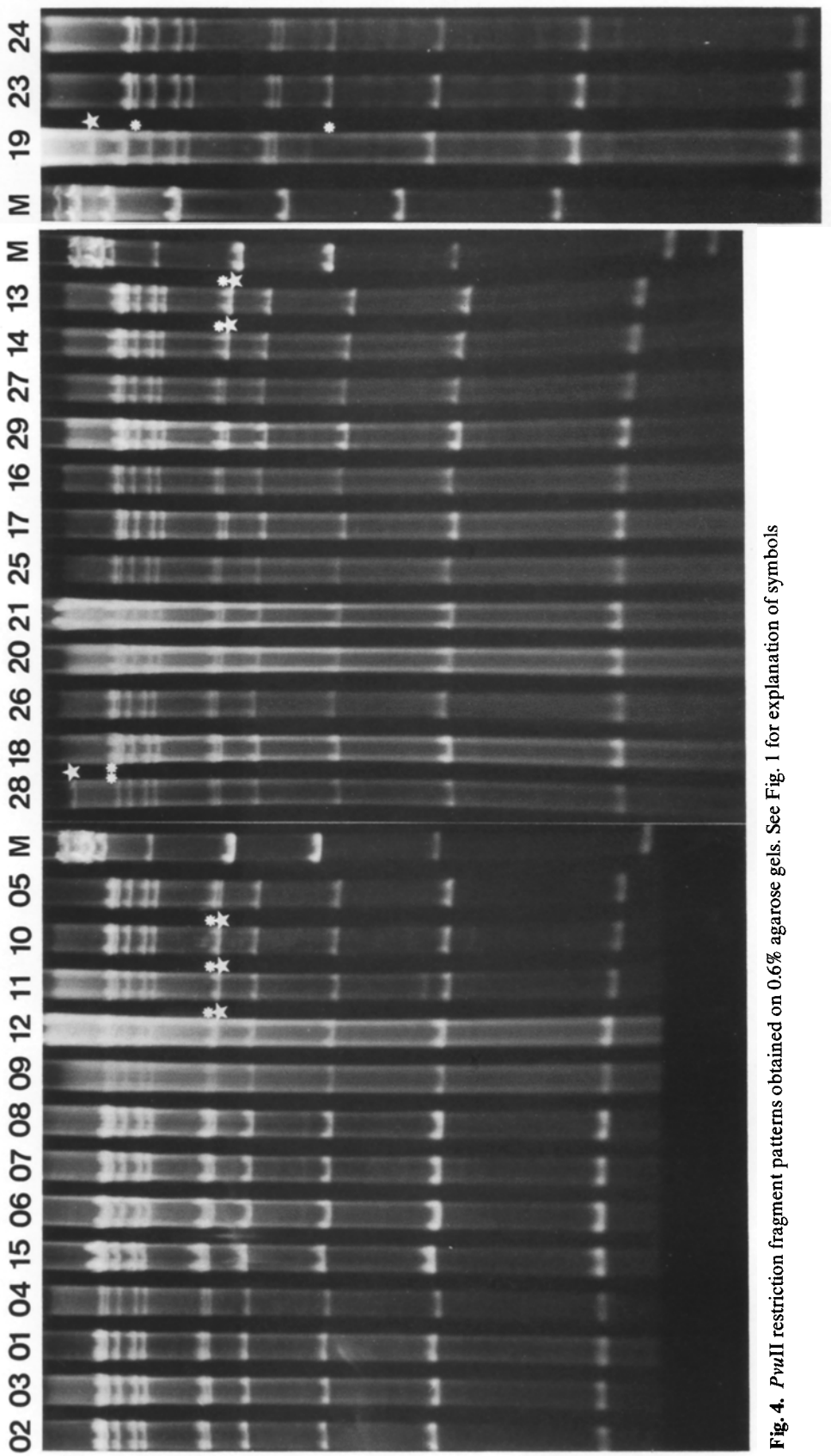

Fig. 4. *Pvu*II restriction fragment patterns obtained on 0.6% agarose gels. See Fig. 1 for explanation of symbols

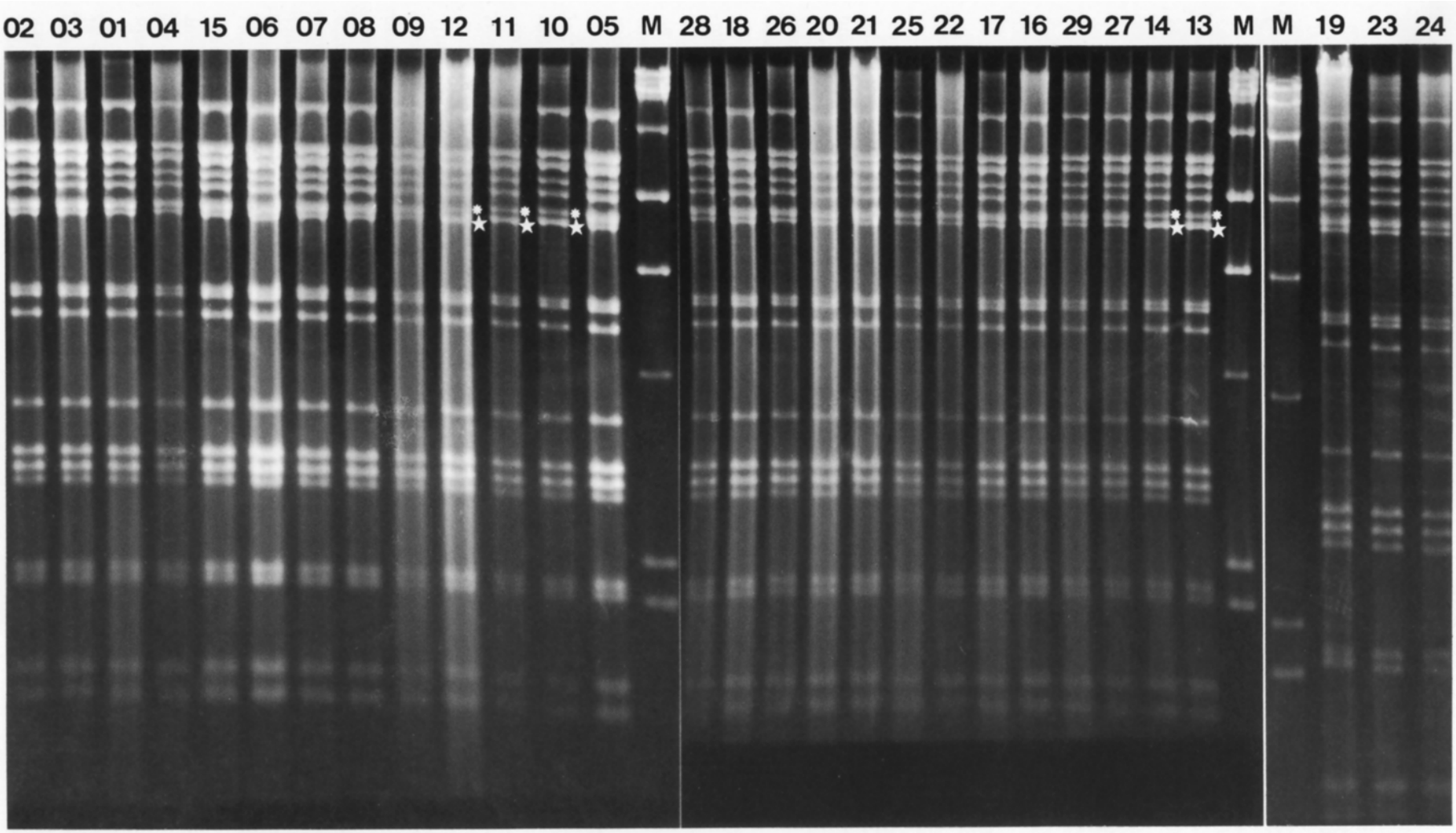

Fig. 5. *Xho*I restriction fragment patterns obtained on 0.8% agarose gels. See Fig. 1 for explanation of symbols

Table 2. CtDNA mutation analysis among six different ct-genome types

Ct-genome type	*Bam*HI	*Hin*dIII	*Kpn*I	*Pvu*II	*Xho*I
Type T	10.0+2.32 → 12.2	12.2 → 11.7	6.45 → 6.15	10.3 → 9.88	8.6 → 8.2
Type A	(16.3+3.66 → 19.5 3.79 → 3.44)	–	–	–	–
Type S	16.3+3.66 → 19.5	2.58 → 2.54	–	–	–
Type C	16.3+3.66 → 19.5	–	–	–	–
Type W″	–	–	–	21.2×2 → 42.4	–
Type W′	–	–	–	19.9+8.3 → 28.4	–
Type W	–	–	–	–	–

Loss or gain of a fragment is determined in comparison with ctDNA of Type W genome. Each fragment is indicated by its molecular size in kilo base pairs (kbp)

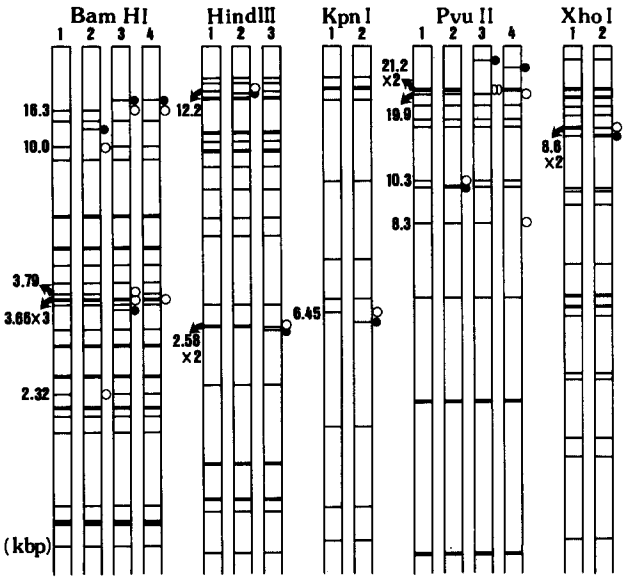

Fig. 6. Restriction fragment pattern types by *Bam*HI, *Hin*dIII, *Kpn*I, *Pvu*II and *Xho*I. The loss or gain of a fragment, as compared with the Type 1 pattern, is indicated by ○ or ●, respectively. The molecular size of a fragment change is indicated in kilo base pairs (kbp); the number of multiple copies is indicated by ×

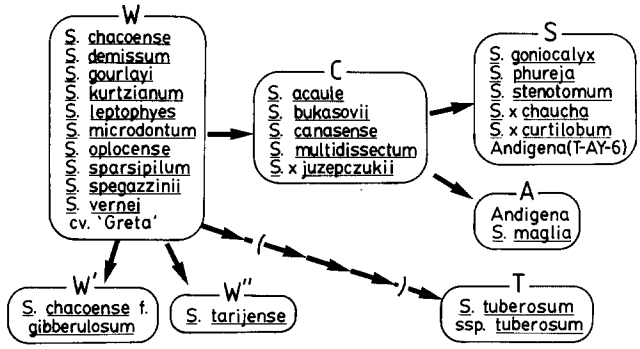

Fig. 7. The relationships of seven ct-genomes. *Arrows* indicate the number and direction of mutational changes

Discussion

Ct-genome of cultivated diploid species and its origin

According to Hawkes (1978), *S. stenotomum* is the most primitive cultivated diploid species, from which *S. goniocalyx* and *S. phureja* were derived by mutation and selection. Thus, the high degree of relationships among them has been morphologically, genetically and biochemically confirmed (Hawkes 1958; Dodds and Paxman 1962; Hosaka and Matsubayashi 1983). As shown in Table 1 and Fig. 7, *S. stenotomum, S. goniocalyx* and *S. phureja* possess a common ct-genome, type S. Therefore, as described previously (Hosaka et al. 1984), it is confirmed further that the cytoplasmic genomes in these species are differentiated only to a limited extent.

Some authors have hypothesized the ancestor of these cultivated diploid species on the basis of morphological similarity. Hawkes (1958) suggested that *S. stenotomum,* the most primitive cultivated diploid, was derived from such wild diploid species as *S. canasense, S. leptophyes* and/or *S. soukupii*, but he particularly favored *S. canasense* (Hawkes 1978). Independent origins were proposed for these cultivated diploid species by Bukasov (1966) who suggested that *S. goniocalyx* originated from *S. multiinterruptum, S. phureja* from *S. candolleanum, S. leptophyes* and other allied species, and *S. stenotomum* from *S. canasense* and other allied species. Bukasov (1968) (in Ugent 1970) also suggested the participation of *S. soukupii* and *S. sparsipilum* in their origin as well. Because of the tremendous diversity in the cultivated diploid species, Ugent (1970) suggested that the '*S. brevicaule* complex', consisting of all the wild species stated above except *S. candolleanum* and *S. sparsipilum* and, moreover, several other species, were involved in the origin of cultivated diploid species. Out of the presumed ancestral species proposed by the above three authors, *S. canasense* is certainly one of the most probable

ancestors, as *S. canasense* has a C type ct-genome which is ancestral to the S type, common in cultivated diploid species (Fig. 7).

Ct-genome of the cultivated triploid, S. × chaucha, and its origin

S. × chaucha has the S type ct-genome similar to that of the cultivated diploid species. Thus, this species is presumed to be of an autotriploid origin from *S. stenotomum* (Hosaka et al. 1984). However, one of the Andigena accessions (T-AY-6) has the S type ct-genome. This leads to another possibility of *S. × chaucha*'s origin, which has already been proposed by Hawkes (1956a, 1963, 1978), that is, that *S. × chaucha* originated as a triploid hybrid from Andigena as female and *S. stenotomum* as male parent.

Ct-genome of the cultivated triploid, S. × juzepczukii, and its origin

S. × juzepczukii, which originated as a triploid hybrid from the wild frost resistant tetraploid species, *S. acaule* as female and *S. stenotomum* as a male parent, is adapted to high altitudes because of its frost resistance (Hawkes 1956a, 1958; Bukasov 1966). This idea has been confirmed by the production of artificial triploids from *S. acaule* and *S. stenotomum* which were morphologically similar to *S. × juzepczukii* (Hawkes 1962; Schmiediche et al. 1982). The present data strongly support the above idea, as the ct-genome type of *S. × juzepczukii* is C, which was seen in *S. acaule* but not in other cultivated species.

Ct-genome of the cultivated tetraploid Andigena and its origin

As to the origin of Andigena, many authors have proposed various ideas: an autotetraploid origin from *S. stenotomum* (Swaminathan and Magoon 1961; Gatenby and Cocking 1978), or from *S. vernei* (Brücher 1954); an amphidiploid origin between *S. stenotomum* and *S. sparsipilum* (Hawkes 1956b, 1958, 1963, 1978, 1979; Cribb 1972), between *S. stenotomum* and *S. vernei* (Brücher 1964), or between *S. phureja* as female and *S. stenotomum* as male parent (Matsubayashi 1981); and also an amphidiploid origin among various genotypes of cultivated diploid species by the union of the respective unreduced gametes (Hosaka and Matsubayashi, unpublished). In any case, the ct-genome donor, namely a female parent of Andigena, has been thought to be one of the cultivated diploid species (Hosaka et al. 1984). It became obvious, however, that out of five Andigena accessions, one (T-AY-6) has the same S type ct-genome as that of the cultivated diploid species while the remaining four have a unique A type ct-genome (Fig. 7, Table 1). The same ct-genome as that of the European potato was not detected in Andigena in this nor in a more recent study (Hosaka 1985), although Buckner and Hyde (1985) reported that the ctDNA of an accession of Andigena that they used was the same as that of the American potato variety 'Kennebec'.

A tremendously large variation, including cytoplasmic differentiation in Andigena, could be explained as follows: if the polyploid hybrid occurred in an indian's field, the chance to be selected and preserved had to be higher in the even-numbered polyploids than in the odd-numbered one because the former could propagate itself by self-pollination. Thus, some Andigenas might be derived from a primitive cultivated diploid species as the female parent, which probably had the C type ct-genome as described above, and then differentiated cytoplasmically to have a unique A type ct-genome, while others were derived recently from the present type of cultivated diploid species with S type ct-genome. Consequently, many kinds of Andigena varieties exist at present, compared with the small number of odd-numbered polyploid varieties of *S. × chaucha, S. × juzepczukii* and *S. × curtilobum* (Jackson et al. 1977; Schmiediche et al. 1980). Therefore, it could be concluded that Andigena is a collective species assigned to all cultivated tetraploid clones in the Andes, and its origin parallels the evolution of cultivated diploid species.

Ct-genome of cultivated tetraploid, subsp. tuberosum, and its origin

It is postulated that the ct-genome of European and Chilean potatoes are both T type (Table 1) except for that of cv. 'Greta', the cytoplasm of which was derived from *S. demissum* (Hosaka et al. 1984). The T type ct-genome was not identified among the Andigena used in this study. Nevertheless, the possibility remains that some Andigena has T type ct-genome, since Andigena is a collective species showing ct-genomic variation. Actually, Buckner and Hyde (1985) identified such an Andigena. The following discussion is based on the present data.

Grun et al. (1977) and the present writer (Hosaka and Kamijima 1985) have stated, based on the analyses of cytoplasmic factors and of ctDNA restriction fragment patterns, that the present common potato took in the cytoplasmic genome of Chilean cultivated potato later than the mid-nineteenth century.

On the origin of the Chilean potato itself, three hypotheses have been proposed. It originated from: 1) *S. leptostigma* or *S. molinae* (Bukasov 1933, 1966) (*S. molinae* has been named as *S. tuberosum* ssp. *tuberosum* var. *guaytecarum* by Hawkes (1956b) and Correll (1962)); 2) Andigena as a long-day

adapted type (Hawkes 1956b); and 3) a hybrid between some wild species as female and Andigena as male (Irikura 1976; Grun 1979; Hosaka et al. 1984). The third idea seems the most probable for the following two reasons: 1) artificially selected Tuberosum-like Andigena, so-called Neo-tuberosum (Simmonds 1966; Glendinning 1975) strongly indicates the possibility that the Chilean potato could be originated by the selection from Andigena populations. 2) As already pointed out by some workers (Gatenby and Cocking 1978; Hosaka and Kamijima 1985), a unique ct-genome in the Chilean potato can not be explained simply by mutation from Andigena ct-genome. As indicated in Fig. 7, T type ct-genome of the Chilean potato is differentiated by at least two mutations from W, which was seen in many wild species, whereas the ct-genome of other cultivated species, including Andigena, all could have evolved from W to another type via the C type. Considering these aspects, most of the genetic information in the Chilean potato might have been introduced from Andigena, but so far as that encoded in the cytoplasmic genome, it did not come from Andigena but from some other species.

The problem is what was the female parent, the ct-genome donor of the Chilean potato? Most of the wild species used in the present study were species distributed in or near the Chilean region. Among these wild species, Grun (1979) considered *S. chacoense* f. *gibberulosum* to be a cytoplasmic donor parent as it revealed common cytoplasmic factors with the Chilean potato. From the present results his idea can not be accepted, for the reasons already described by Buckner and Hyde (1985), because although *S. chacoense* f. *gibberulosum*'s cytoplasm was originally derived from the same accession as that of Grun's (PI 133073), it possessed W′ type ct-genome distinctly different from that of the Chilean potato. No wild diploid species having the T type ct-genome were found among the materials used, including *S. maglia,* which is the only wild tuber-bearing species in Chile (Table 1).

It was shown that *S. leptostigma* (PI 133667) and var. *guaytecarum* (PI 208563) both possessed the same ct-genome, T type, as the Chilean potato (Table 1). These two species, both of which are tetraploid, were said to be truly wild under the present conditions in Chile (Correll 1962), so that Bukasov (1933, 1966) and Sykin (1971) considered these so-called wild indigenous species to have been the immediate ancestors of the Chilean potato. On the other hand, Irikura (1976) observed that a haploid induced from var. *guaytecarum* produced many, small tubers, and based on the cytogenetic data, he implied that the Chilean potato was derived from the cross between Andigena as male parent and var. *guaytecarum* as female parent. Hawkes (1956b), however, has proposed that both *S. leptostigma* and var. *guaytecarum* are not wild species but escaped types from the Chilean potato. Based on Brücher's (1963) thorough explorations in Chiloé, he also emphasized that they were not true wild species. Therefore, it seems possible to suppose that such a tetraploid as *S. leptostigma* or var. *guaytecarum* was the derivative, rather than the wild ancestor of the Chilean potato. Thus, the Chilean potato was probably derived from a cross between Andigena and an unidentified wild species, expressing strong wild characters – Andigena having functioned as a male parent with an unidentified wild species as female in the process of expanding its distribution southward into the Chilean region. Two kinds of tetraploids developed: one took on much adaptability for wild conditions (*S. leptostigma* or var. *guaytecarum*), while the other took on more favorable cultivated characters such as long-day adaptability and became the present Chilean potato, ssp. *tuberosum.* Consequently, it still remains a question as to which diploid ancestor donated the cytoplasm to the Chilean potato.

Ct-genome of the cultivated pentaploid, S. × curtilobum, and its origin

As generally known, *S. × curtilobum* is a much less variable species. Schmiediche et al. (1980) have recognized morphologically and electrophoretically only two morphotypes in *S. × curtilobum:* one a natural hybrid, from which another originated by somatic mutation for pigmentation. This species has been thought to be of hybrid origin from the fertilization between an unreduced gamete of *S. × juzepczukii* and a normal gamete of Andigena (Bukasov 1939, 1966; Hawkes 1956a, 1958). This idea has been strengthened by the resemblance between natural *S. × curtilobum* and an artificially synthesized pentaploid made by the above scheme (Hawkes 1962). An alternative hypothesis has been proposed by Schmiediche et al. (1980) that *S. × curtilobum* resulted from the union of unreduced gametes from both *S. × juzepczukii* and a cultivated diploid species, based on the fact that the flowering period of *S. × juzepczukii* corresponds with that of other cultivated diploid species, but not with that of Andigena. Both hypotheses imply that *S. × juzepczukii* played a role as a female parent because of its presumed male-sterility caused by triploidy. Thus, *S. × curtilobum* can be expected to have C type ct-genome the same as that of *S. × juzepczukii.* The fact, however, is that *S. × curtilobum* has the S type ct-genome. To solve this contradiction, further study is needed, but the alternative possibility that *S. × juzepczukii* functioned as a male parent can be presumed because *S. × juzepczukii* has been used successfully as a male parent (Estrada and Landeo pers. comm. in Schmiediche et al. (1982)) and because *S. × chaucha,* a cultivated triploid, produced some hybrid seeds when used as either male or female parent (Jackson et al. 1978).

Ct-genome of S. acaule, and its origin

It became evident that the Andean weed tetraploid, *S. acaule* had the C type ct-genome. In the previous report (Hosaka et al. 1984), possible candidates for its ancestral species were given as *S. goniocalyx, S. multidissectum* and *S. phureja.* The present results, however, show more precisely that the cytoplasm of *S. acaule* was derived from a wild diploid species with C type ct-genome, for example, *S. bukasovii, S. canasense* or *S. multidissectum.*

Ct-genome of S. maglia, and its origin

As stated already, *S. maglia* is the only wild species distributed in Chile, and all clones collected so far are triploid except for two diploids (Hawkes and Hjerting 1969). This fact led to the idea on its origin that *S. maglia* occurred as an autotriploid from the union of reduced and unreduced gametes from a diploid clone, and then the vigorous triploid eliminated the original

parental diploid colonies (Hawkes and Hjerting 1969; Hawkes 1979). It is evident that both ploidy types of *S. maglia* have curiously enough the same ct-genome, type A, as that of Andigena (Table 1). This ct-genomic resemblance between *S. maglia* and Andigena suggests the following two possibilities concerning its origin; (1) *S. maglia* was derived from a triploid hybrid in the cross between Andigena as female and an unidentified diploid species as male parent, or (2) *S. maglia* was an immediate cytoplasmic ancestor of Andigena. The second idea has no prior basis except from the present data, as summarized on the origin of Andigena earlier. The first possibility seems to be more probable, but can not explain the existence of diploid clones of *S. maglia*. Though a diploid could be obtained from the cross of Andigena with an unidentified diploid species, as in the case of the cross of Andigena by a certain line of *S. phureja* (Hanneman and Ruhde 1978), it would not become diploid *S. maglia* but a haploid of Andigena.

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