

Molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops* species. IV. CtDNA variation in *Ae. triuncialis**

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Restriction endonuclease analysis of chloroplast DNA of *Ae. triuncialis* was carried out. Thirteen accessions had the type 2a chloroplast genome derived from *Ae. caudata*, eight possessed type 3 chloroplast genome of *Ae. umbellulata*, and the remaining five contained a new chloroplast genome (named type 2b) differing from the former two, by a 0.3 kbp insertion and four base substitutions, respectively. The accessions with type 2a and type 3 chloroplast genomes distribute in wide areas, and in both of its subspecies, *eu-triuncialis* and *orientalis*, whereas those having the type 2b chloroplast genome occur only locally in Azerbaijan, Transcaucasus. From these results, the following two conclusions are drawn; (a) *Ae. triuncialis* originated from the reciprocal crosses between *Ae. caudata* and *Ae. umbellulata*, and (b) the type 2b chloroplast genome was arisen from type 2a chloroplast genome by a 0.3 kbp insertion.

INTRODUCTION

Aegilops triuncialis, a tetraploid species ($2n = 28$, genome constitution $C^u C^u CC$) is known to have originated as an amphidiploid between two diploids, *Ae. caudata* ($2n = 14$, CC) and *Ae. umbellulata* ($2n = 14$, $C^u C^u$) (Kihara, 1940; Kihara and Kondo, 1943). This species is adapted to a wide range of environments, and is more widely distributed than the two parental species, i.e., from the western Mediterranean to Central Asia (Croston and Williams, 1981). Following Eig (1929), this species is divided into two subspecies, *eu-triuncialis* and *orientalis*. Ssp. *eu-triuncialis* includes two varieties, *typica* and *constantinopolitana*, whereas ssp. *orientalis* consists of three varieties, *assyriaca*, *persica* and *anathera*.

By comparing the morphological and physiological characters of alloplasmic wheat lines, Mukai *et al.* (1978) found that two accessions of *Ae. triuncialis* ssp. *eu-triuncialis* have genetically different cytoplasms. One of them has the almost identical cytoplasm to that of *Ae. umbellulata* and the other to that of *Ae. caudata*. Restriction endonuclease analysis of the ctDNA supported these results (Ogihara and Tsunewaki, 1982, 1983).

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Together these observations indicate a possible diphyletic origin of *Ae. triuncialis* from the reciprocal crosses between *Ae. caudata* and *Ae. umbellulata* (Ogihara and Tsunewaki, 1982).

The present investigation aimed to clarify (a) the extent of ctDNA variation within *Ae. triuncialis*, (b) the geographical distribution of the accessions having different chloroplast genomes, and (c) the distribution of each chloroplast genome type among different taxa.

MATERIALS AND METHODS

The accessions used and their taxonomy, collection site and source are shown in table 1. These were provided by M. Tanaka and S. Sakamoto, Plant Germplasm Institute, Kyoto University; Y. Mukai, Osaka Kyoiku University; and the late Y. Nakai, Laboratory of Genetics, Kyoto University. As a control, two alloplasmic common wheats having the cytoplasm of *Ae. caudata* or *Ae. umbellulata* were used.

Intact chloroplasts were isolated from the homogenate of mature or seedling leaves and purified by the 10, 40 and 75 per cent Percoll or the 15, 30 and 60 per cent sucrose discontinuous gradient centrifugation. The final pellet was resuspended in TE buffer (50 mM Tris, 20 mM EDTA, pH 8.0) and lysed in 0.2 per cent sodium lauryl

Table 1 The origin of 26 *Ae. triuncialis* accessions used in the present investigation and their chloroplast genome types

Accession No.	Ssp. and var.*	Collection site	Source†	Chloroplast genome type‡
01	<i>eu-t. typ.</i>	Afghanistan; 28 km NW of Pul-i-khumri	KUSE 2505	2a
02	<i>orie. assy.</i>	Afghanistan; 20–35 km W of Maimana	KUSE 2517	3
03	<i>orie. pers.</i>	Afghanistan; 75 km W of Maimana	KUSE 2521	2a
04	<i>eu-t. cons.</i>	Azerbaijan; 34 km N of Baku	BEC 2924	2b
05	<i>eu-t. cons.</i>	Azerbaijan; 34 km N of Baku	BEC 2925	2b
06	<i>eu-t. cons.</i>	Azerbaijan; 34 km N of Baku	BEC 2926	2b
07	<i>eu-t. cons.</i>	Azerbaijan; 34 km N of Baku	BEC 2927	2b
08	<i>eu-t. cons.</i>	Azerbaijan; 34 km N of Baku	BEC 2928	2b
09	<i>eu-t. typ.</i>	Bulgaria; Albena-1	M356-1	3
10	<i>eu-t. typ.</i>	Bulgaria; Albena-2	M356-2	3
11	<i>eu-t. typ.</i>	Bulgaria; Albena-3	M356-3	3
12	<i>eu-t. typ.</i>	Bulgaria; Baltik-1	M356-4	3
13	<i>eu-t. typ.</i>	Bulgaria; Baltik-2	M356-5	3
14	<i>eu-t. typ.</i>	Cyprus; Cyprus Island	BMUK 6909	2a
15	<i>eu-t. cons.</i>	Georgia; NW of Tbilisi	BEC 2960	2a
16	<i>eu-t. typ.</i>	Greece; suburbs of Portaria	BMUK 6904	3
17	<i>eu-t. cons.</i>	Greece	N7050	2a
18	<i>eu-t. typ.</i>	Iran; 2 km N of Karaj	KUSE 2548	3
19	<i>orie. assy.</i>	Iraq; 16.4 km NW from Almidgadiya	BEM 4951	2a
20	<i>eu-t. typ.</i>	Rumania	N7045	2a
21	<i>eu-t. typ.</i>	Rumania	N7046	2a
22	<i>eu-t. typ.</i>	Spain; Carboneras	702	2a
23	<i>eu-t. typ.</i>	Spain; Canameres	710	2a
24	<i>eu-t. typ.</i>	Spain; near Almanza	734	2a
25	<i>eu-t. typ.</i>	Turkey; 24 km E of Mecitozii	BMUK 6853	2a
26	<i>eu-t. typ.</i>	Turkey; 27 km NW of Kirikkale	BMUK 6864	2a

* Full name: *eu-t.* = ssp. *eu-triuncialis*, *orie.* = ssp. *orientalis*, *typ.* = var. *typica*, *cons.* = var. *constantinopolitana*, *assy.* = var. *assyriaca*, *pers.* = var. *persica*.

† KUSE: Kyoto University Scientific Expedition to the Karakoram and Hindukush, 1955 (Kihara *et al.*, 1965). BMUK: Botanical Mission of the Kyoto University to the Eastern Mediterranean, 1959 (Yamashita and Tanaka, 1961). BEC: Botanical Expedition of Kyoto University to Caucasus, 1966. BEM: Botanical Expedition of Kyoto University to Mesopotamia, 1970.

‡ Chloroplast genome type 2a and 3 are identical to that of *Ae. caudata* and *Ae. umbellulata*, respectively.

sarcosinate solution, containing 200 µg/ml protenase K. CtDNA was prepared according to Kolodner and Tewari (1975).

The ctDNA was digested with four restriction endonucleases, *Bam*HI, *Hind*III, *Sma*I and *Xho*I, each of which recognises a specific six-base-pair sequence. These endonucleases were chosen because it had been established previously that they could distinguish the ctDNA of *Ae. caudata* from that of *Ae. umbellulata* (Ogihara and Tsunewaki, 1982, 1983). The methods of electrophoresing the digested ctDNA and analysing the restriction fragment pattern are the same as previously described (Ogihara and Tsunewaki, 1982).

RESULTS AND DISCUSSION

(a) CtDNA variation in *Ae. triuncialis*

As shown in fig. 1, the *Bam*HI-, *Hind*III-, *Sma*I- and *Xho*I-digests of ctDNA from 26 *Ae. triuncialis*

accessions produced three, two, three and three different restriction fragment patterns, respectively. These patterns are schematically drawn in fig. 2. These results demonstrate the presence of ctDNA variation in this species.

Ogihara and Tsunewaki (1982, 1983) recognised 11 major types plus five subtypes among the chloroplast genomes of 33 *Triticum* and *Aegilops* species (43 accessions in total), from the results of restriction endonuclease analysis of their ctDNA with eight endonucleases, including *Bam*HI, *Hind*III, *Sma*I and *Xho*I. Comparing the present results with theirs, 13 and eight accessions of *Ae. triuncialis* have type 2 and 3 chloroplast genomes of Ogihara and Tsunewaki, respectively, and the remaining five accessions are of a new type. The present and their results show that type 2 chloroplast genome is in *Ae. caudata* and type 3 in *Ae. umbellulata*, which are the two parental species of *Ae. triuncialis*. The new type chloroplast genome is unexpected given the known origin of this species.

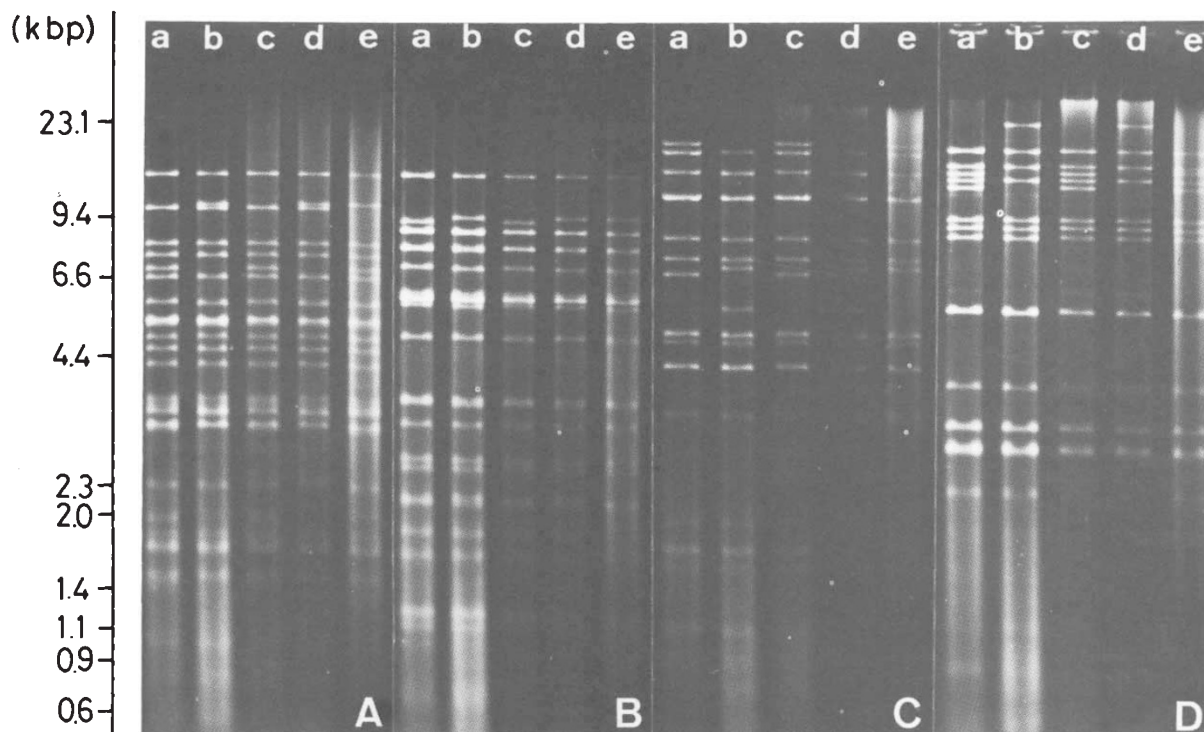


Figure 1 Restriction fragment patterns of ctDNA from three chloroplast genome types of *Ae. triuncialis* digested by (A) *Bam*HI, (B) *Hind*III, (C) *Sma*I and (D) *Xho*I. Lane a and b: Alloplasmic common wheat having cytoplasm of *Ae. caudata* and *Ae. umbellulata*, respectively. Lane c, d and e: Accession no. 01, 02 and 04 of *Ae. triuncialis*, representing chloroplast genome type 2a, 3 and 2b, respectively.

(b) Physical maps of the three chloroplast genomes found in *Ae. triuncialis*

By reference to the physical map of common wheat ctDNA given by Bowman *et al.* (1981), Ogihara (pers. comm.) has constructed the physical maps of 16 chloroplast genomes identified in *Triticum* and *Aegilops*, and has located the restriction sites of 11 endonucleases together with deletion/insertion sites.

By comparing the present results given in figs 1 and 2 to Ogihara's results, the physical map of the new chloroplast genome was constructed. This is shown in fig. 3, in which differences of type 2 (= *Ae. caudata*) and type 3 (= *Ae. umbellulata*) chloroplast genomes from the new one are also indicated. The new chloroplast genome shows only one difference, namely, a 0.3 kbp insertion from the *Ae. caudata* chloroplast genome, whereas it shows four restriction site differences from that of *Ae. umbellulata*, i.e., a gain of two *Bam*HI and one *Xho*I site and the loss of a *Sma*I site. Apparently, this chloroplast genome is more closely related to the *Ae. caudata* than to the *Ae. umbellulata* chloro-

plast genome. Thus, the chloroplast genome type newly found in five accessions of *Ae. triuncialis* is considered to be a subtype of chloroplast genome type 2, and is named type 2b. Accordingly, chloroplast genome of *Ae. caudata* will be renamed type 2a.

(c) Distribution of the three chloroplast genomes in *Ae. triuncialis*

Distribution in different taxa: Chloroplast genome types of all *Ae. triuncialis* accessions are indicated in the last column of table 1. Table 2 summarises the distribution of the three chloroplast genome types in different taxa of *Ae. triuncialis*. Both type 2a and 3 chloroplast genomes are found in the two subspecies, *eu-triuncialis* and *orientalis*. Type 2b chloroplast genome is found only in ssp. *eu-triuncialis* var. *constantinopolitana*.

Geographical distribution: fig. 4 shows the collection sites of *Ae. triuncialis* accessions having different chloroplast genomes, together with the distribution areas of this species and its two

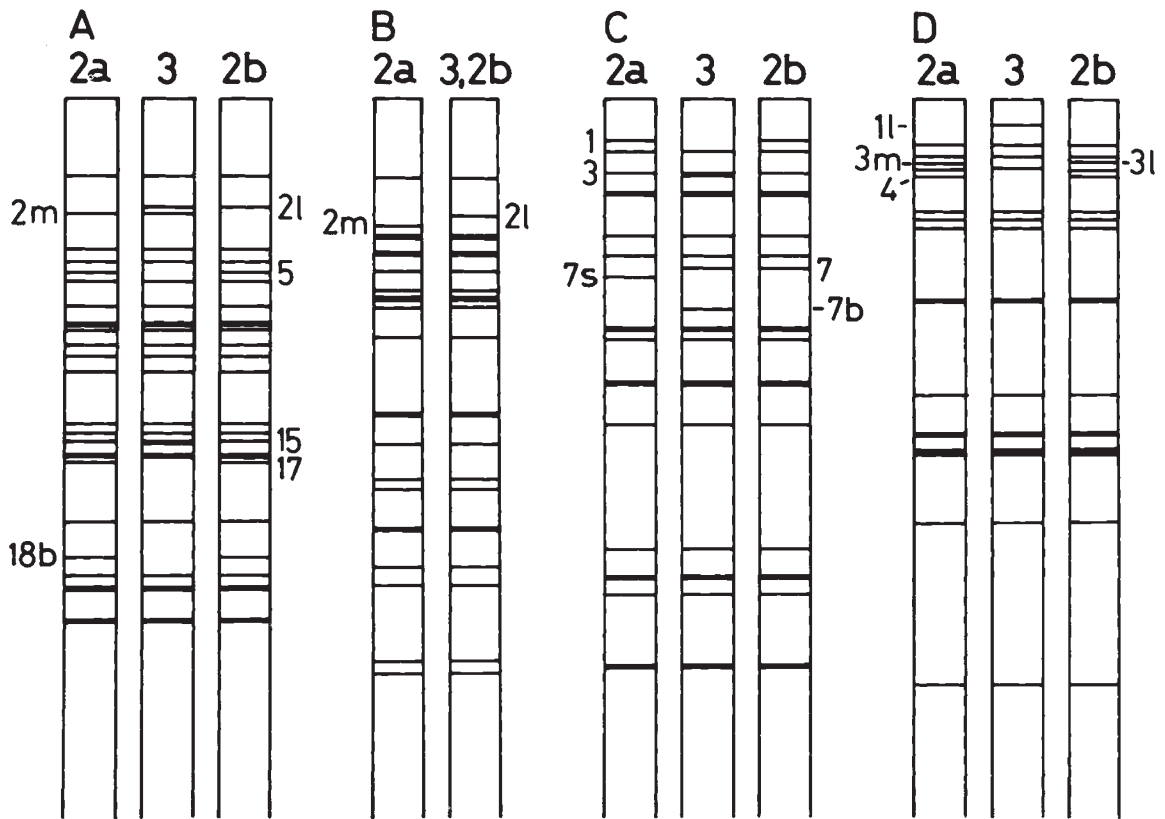


Figure 2 Schematic representation of (A) *Bam*HI, (B) *Hind*III, (C) *Sma*I and (D) *Xho*I restriction patterns of ctDNA from three chloroplast genome types, 2a, 2b and 3. Variable fragments are indicated in the respective lanes.

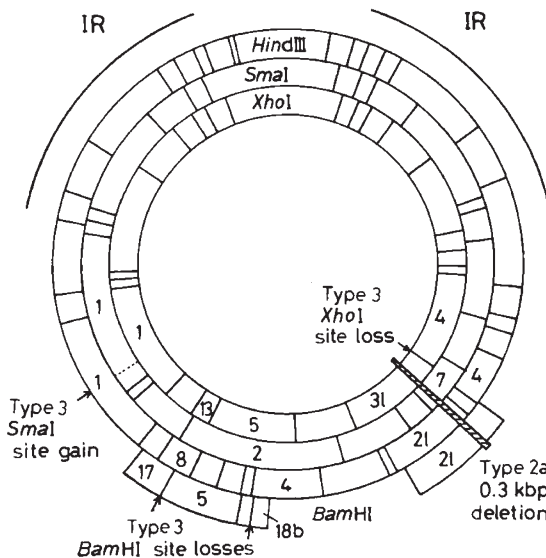


Figure 3 Physical map of the type 2b ctDNA, differences of which from those of type 2a and 3 ctDNA are also indicated.

parents. Type 2a and type 3 chloroplast genome-carriers occur widely in the distribution area of *Ae. triuncialis*. Although type 2b chloroplast genome-carrier was found only in Azerbaijan, Transcaucasus, the possibility that it is present in other areas cannot be excluded because of the small sample sizes we studied.

Table 2 Numbers of *Ae. triuncialis* accessions having different chloroplast genomes

Subspecies and variety	Chloroplast genome			
	2a	2b	3	Total
<i>Ssp. eu-triuncialis</i>				
var. <i>typica</i>	9	0	7	16
var. <i>constantinopolitana</i>	2	5	0	7
subtotal	11	5	7	23
<i>Ssp. orientalis</i>				
var. <i>assyriaca</i>	1	0	1	2
var. <i>persica</i>	1	0	0	1
subtotal	2	0	1	3
Total	13	5	8	26

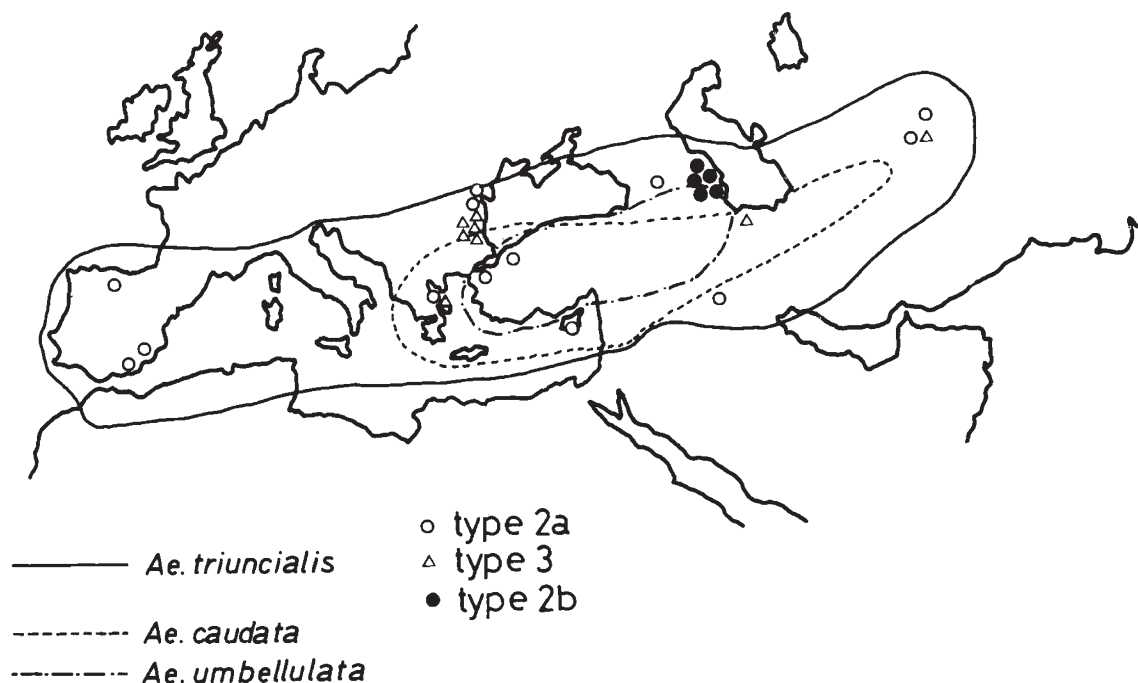


Figure 4 Geographical distribution of *Ae. caudata*, *Ae. umbellulata* and *Ae. triuncialis*, and collection sites of *Ae. triuncialis* accessions having the three chloroplast genome types.

Summarising all the results mentioned above, the following conclusions are drawn; (a) the type 2a chloroplast genome was derived from the cross, *Ae. caudata* × *Ae. umbellulata*, while the type 3 chloroplast genome from the reciprocal cross, *Ae. umbellulata* × *Ae. caudata*, and (b) the type 2b chloroplast genome originated from the type 2a chloroplast genome by a 0.3 kbp insertion. This could have occurred in *Ae. caudata* before or in *Ae. triuncialis* after the origin of the latter species. A further investigation on the intraspecific variation of chloroplast genome in *Ae. caudata* is necessary for deciding which is the actual case.

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