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The molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops*

7. Restriction endonuclease analysis of mitochondrial DNAs from polyploid wheats and their ancestral species *

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Summary. Many related species and strains of common wheat were compared by matching differences among their mitochondrial genomes with their "parent" nuclear genomes. We examined three species of *Aegilops*, section Sitopsis (*Ae. bicornis*, *Ae. sharonensis*, and *Ae. speltoides*), emmer wheat (*Triticum dicoccoides*, *T. dicoccum*, and *T. durum*), common wheat (*T. spelta*, *T. aestivum*, and *T. compactum*), and timopheevi wheat (*T. araraticum*, *T. timopheevi*, and *T. zhukovskyi*). A single source of the cytoplasm was used in all the species, except *Ae. speltoides* (two sources), *T. araraticum* (two), and *T. aestivum* (three). Following restriction endonuclease analyses, the mitochondrial genomes were found to comprise seven types, and a dendrogram showing their genetic relatedness was constructed, based upon the percentage of common restriction fragments. MtDNAs from *T. dicoccum*, *T. durum*, *T. aestivum*, and *T. compactum* yielded identical restriction fragment patterns; these differed from *T. dicoccoides* and *T. spelta* mtDNAs in only 2.3% of their fragments. The fragment patterns of *T. timopheevi* and *T. zhukovskyi* were identical, and these differed from *T. araraticum* mtDNA by only one fragment. In both the emmer-dinkel and timopheevi groups, mitochondrial genome differentiation is evident, suggesting a diphyletic origin of each group. MtDNAs from four accessions of the Sitopsis species of *Aegilops* differ greatly from one another, but those of *Ae. bicornis*, *Ae. sharonensis*, and *Ae. searsii*, belonging to the same subsection Emarginata, are relatively similar. MtDNAs of timopheevi species are identical, or nearly so, to those of *Ae. speltoides* accession (09), suggesting that the latter was the cytoplasm donor to the former, polyploid group. The origin of this polyploid group seems to be rather recent in that the diploid and polyploid species possess nearly identical mitochondrial genomes. We cannot determine, with precision, the cytoplasm donor to the emmer-dinkel group. However, our results do suggest that mitochondrial DNAs show larger evolutionary divergence than do the ctDNAs from these same strains.

Key words: Polyploid wheat – *Aegilops* (Sitopsis) – Mitochondrial genome – Restriction endonuclease analysis – Phylogeny

Introduction

Mitochondria are semiautonomous organelles containing their own DNA. In higher plants, the molecular organization of mitochondrial genomes is clarified by restriction mapping of entire mitochondrial DNA molecules, and their physical maps have been constructed in several species, e.g., *Brassica campestris* (Palmer and Shields 1984), *B. oleracea* (Chetrit et al. 1984), *B. nigra*, and *Raphanus sativus* (Palmer and Herbon 1986), *Spinacia oleracea* (Stern and Palmer 1986), *Zea mays* (Lonsdale et al. 1984), and *Triticum aestivum* (Quetier et al. 1985). These maps reveal a general feature of mitochondrial genomes of higher plants, namely, multipartity of DNA molecules generated by intramolecular recombination between repeated sequences located in the "master" DNA molecule.

However, less is known about the evolutionary history of plant mitochondrial genomes than is known of

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animal mitochondrial genomes (e.g., Brown 1983) and plant chloroplast genomes (e.g., Palmer 1987). One reason we know less of plant mtDNA is that variability is so great as to preclude meaningful comparisons between distantly related species. Attempts to circumvent this difficulty and to search for clues to a molecular basis of differentiation and evolution of plant mitochondrial genomes have taken the form of studying closely related species, even different strains of the same species (Sederoff et al. 1981; Holwerda et al. 1986; Pring et al. 1987; Palmer 1988).

In wheat (*Triticum*) and its related genus, *Aegilops*, several phylogenetic studies of cytoplasms have been carried out, including analyses of the genetic characteristics of whole cytoplasm and chloroplast DNA. We have abundant information about the genetic differences among the cytoplasms of most species in these two genera (Tsunewaki 1980, 1989; Ogihara and Tsunewaki 1988). Their mitochondrial genomes, however, are rarely studied from the phylogenetic and evolutionary point of view. Only Vedel et al. (1978, 1981) have reported differences of restriction patterns among mtDNAs of polyploid wheats and their ancestral diploid species. With restriction endonuclease analyses of mtDNAs, Terachi and Tsunewaki (1986) demonstrated mitochondrial genome differentiation among *Aegilops* species with identical chloroplast genomes. Breiman (1987) reported extensive intraspecific variation among different accessions of *Ae. speltoides*, using Southern hybridization. What is needed is a thorough and systematic investigation of mtDNA diversity in order to understand differentiation and evolution of mitochondrial genomes in these genera.

This paper reports our attempts to determine the extent of mitochondrial genome diversity among species carrying B, G, or S and S-related nuclear genomes, and to identify the cytoplasmic donors of polyploid wheats. Restriction fragment patterns of mtDNA of representative polyploid wheats have been compared to those of their putative diploid ancestors. Our results are the first to assign the donor of mtDNA to the timopheevi group, and to show intragroup variation of the mtDNA within the emmer-dinkel group of wheat.

Materials and methods

Plant materials

Six euplasmic wheat lines and ten alloplasmic lines of common wheat were used as sources of mtDNAs (Table 1). The alloplasmic lines, with the exception of *Ae. speltoides* cytoplasm (code

Table 1. Lines used as sources of mitochondrial DNA

a. Euplasmic lines

Group	Species	n	Nuclear[a] genome	Source[b]	Abbr.
Emmer	*T. durum* cv Stwart	14	AB	K	Stw
Dinkel	*T. aestivum* cv Chinese Spring	21	ABD	K	CS
Dinkel	*T. aestivum* cv Jones Fife	21	ABD	K	JF
Dinkel	*T. aestivum* cv Shirogane-komugi	21	ABD	K	Srg
Dinkel	*T. compactum* cv No. 44	21	ABD	K	Cmp
Dinkel	*T. spelta* var. *duhamelianum*	21	ABD	K	Splt

b. Alloplasmic lines

Cytoplasm donor						Nucleus donor
Section (or group)	Species	n	Nuclear[a] genome	Source[b]	Code no.	
Sitopsis	*Ae. speltoides*	7	S	K	08	CS
Sitopsis	*Ae. speltoides*	7	S	P	09	CS/Splt
Sitopsis	*Ae. sharonensis*	7	S^l	K	10	CS
Sitopsis	*Ae. bicornis*	7	S^b	M	12	CS
Emmer	*T. dicoccoides* var. *spontaneonigrum*	14	AB	K	21	CS
Emmer	*T. dicoccum* cv Hokudai	14	AB	K	22	CS
Timopheevi	*T. araraticum*	14	AG	K	23	Splt
Timopheevi	*T. araraticum*	14	AG	M	24	Splt
Timopheevi	*T. timopheevi*	14	AG	K	25	Splt
Timopheevi	*T. zhukovskyi*	21	AAG	M	51	Splt

[a] After Kihara (ref. Lilienfeld 1951)

[b] K – Kyoto University, Japan; M – S. S. Maan, North Dakota State University, USA; P – I. Panayotov, Institute for Wheat and Sunflower, Bulgaria

no. 09), were propagated by natural open-pollination in our experimental field. Seeds of alloplasmic lines with *Ae. speltoides* cytoplasm were kindly provided by Dr. H. Tsujimoto, Kihara Institute for Biological Research, Yokohama City University, Japan.

Isolation of mitochondria and purification of mtDNA

Mitochondria were isolated from etiolated leaves, according to Bonen and Gray (1980). MtDNA was purified from mitochondrial lysates by CsCl/EtBr centrifugation, after Terachi and Tsunewaki (1986).

Restriction endonuclease analysis of mtDNA

The restriction enzymes, *Bam*HI, *Hin*dIII, *Pst*I, *Pvu*II, and *Xho*I, all being six-base cutters, were used under the conditions specified by the manufacturer (Takara Shuzo Co. Ltd., Japan). Digested mtDNA was electrophoresed in agarose gels (0.8% or 1.0%), the bands were identified by UV light following EtBr staining (0.5 μg/ml) and photographed. Upon enlarging the photographs, the distance of each fragment from the electrophoretic origin was measured, from which its molecular size was estimated by comparing it with marker DNA fragments. Genetic similarities among the mitochondrial genomes were evaluated by the percentage of their common restriction fragments. Details of these calculations are given in below.

Table 2. Relationships among cytoplasmic, chloroplast, and mitochondrial genome types

Plasma type[a]	Ct genome type[b]	Mt genome type	Species (accession or cultivar)
S[b]	1b	Ib1	*Ae. bicornis* (12)
S[1]	1c	Ic	*Ae. sharonensis* (10)
G	5	Va	*Ae. speltoides* (09)
G	5	Va	*T. timopheevi* (25)
G	5	Va	*T. zhukovskyi* (51)
G	5	Vb	*T. araraticum* (23)
G	5	Vb	*T. araraticum* (24)
B	7	VIIa	*T. durum* (Stw)
B	7	VIIa	*T. dicoccum* (22)
B	7	VIIa	*T. compactum* (Cmp)
B	7	VIIa	*T. aestivum* (CS, JF, Srg)
B	7	VIIb	*T. dicoccoides spont.* (21)
B	7	VIIb	*T. spelta* (Splt)
S	8	VIII	*Ae. speltoides* (08)

[a] Tsunewaki and Tsujimoto (1983)
[b] Ogihara and Tsunewaki (1983)

Results

Restriction fragment patterns of mtDNAs isolated from polyploid wheats and Aegilops species in the section Sitopsis

All mtDNAs tested produced complicated restriction patterns, including large numbers of fragments and apparent non-stoichiometry in some of them. Close examination of these patterns reveal the following facts. First, restriction patterns of mtDNAs from four *Aegilops* accessions, i.e., *Ae. speltoides* (code nos. 08 and 09), *Ae. sharonensis* (10), and *Ae. bicornis* (12), can be distinguished by use of all five enzymes (Fig. 1). Second, mtDNAs of two emmer wheats, *T. dicoccum* (22) and *T. durum* (Stw), yield restriction patterns identical with those of *T. compactum* (Cmp) and three *T. aestivum* cultivars (CS, JF, and Srg), and mtDNA of *T. dicoccoides* var. *spontaneonigrum* (21) yields identical patterns to those of *T. spelta* (Splt) (Figs. 1 and 2). Similarly, restriction patterns of *Ae. speltoides* (09), *T. timopheevi* (25), and *T. zhukovskyi* (51) mtDNAs are identical. Finally, although mtDNAs of *T. dicoccoides* var. *spontaneonigrum* (21) and *T. spelta* (Splt) produce similar restriction patterns to those of *T. dicoccum* (22), *T. durum* (Stw), and three *T. aestivum* cultivars (CS, JF, and Srg), mtDNAs of the former clearly differ from the latter in *Hin*dIII, *Pst*I, and *Pvu*II patterns (Fig. 1 b–d, respectively). For example, a *Hin*dIII fragment present in the former (12.4 kbp in size, marked with • in Fig. 1 b) is not found in the latter. In the same way, *T. araraticum* (23 and 24) patterns differ from those of *Ae. speltoides* (09), *T. timopheevi* (25), and *T. zhukovskyi* (51) in that a *Hin*dIII fragment (6.7 kbp in size, marked with ◂ in Figs. 1 b and 2 b) is present only in the former.

According to these restriction fragment patterns, mtDNAs from 16 different cytoplasms can be classified into seven different types, designated as shown in Table 2. The cytoplasmic and chloroplast genome types are also shown in this table.

Genetic similarity among different mitochondrial genome types as revealed by restriction fragment pattern

As an index of the genetic similarity between a pair of mitochondrial genomes, the percentage of common restriction fragments is adopted. This parameter is expressed by $2A/B \times 100$, where A is the number of fragments found in common between the restriction fragment patterns of two mtDNAs, and B is the sum of the numbers of fragments observed in them. Both A and B are pooled for all five restriction patterns. Comparable data are always taken from the same gel. This procedure, however, ignores the stoichiometry of the fragments. The percentages of common restriction fragments, between all possible pairs of seven mitochondrial genomes, are given in Table 3. The highest value, 99.7%, is obtained between type Va mitochondrial genomes of *Ae. speltoides* (09), *T. timopheevi* (25), and *T. zhukovskyi* (51), and type Vb of the two *T. araraticum* lines (23 and 24). A value of 97.7% is observed between type VIIa of six emmer-dinkel wheat lines and type VIIb of *T. dicoccoides* var. *spontaneonigrum* (21) and *T. spelta* (Splt). At the other extreme, the low values of 54%–55% are found between the type Ic mitochondrial genome of *Ae.*

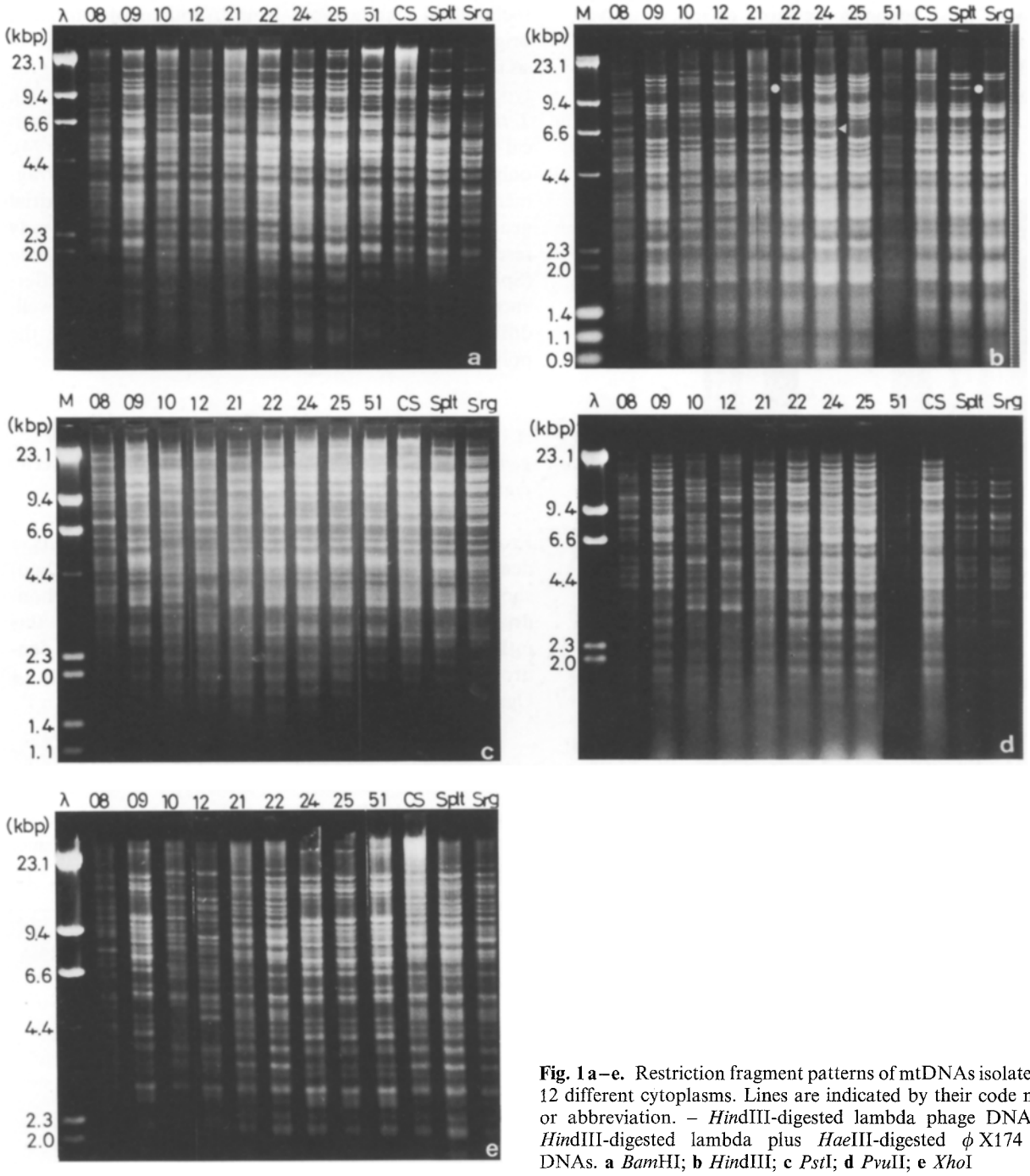

Fig. 1 a–e. Restriction fragment patterns of mtDNAs isolated from 12 different cytoplasms. Lines are indicated by their code number or abbreviation. – *Hin*dIII-digested lambda phage DNA. M – *Hin*dIII-digested lambda plus *Hae*III-digested ϕX174 phage DNAs. **a** *Bam*HI; **b** *Hin*dIII; **c** *Pst*I; **d** *Pvu*II; **e** *Xho*I

sharonensis (10) and type Va or type Vb mitochondrial genomes of timopheevi group wheats, including *Ae. speltoides* (09) (Table 3).

Discussion

An overall picture of mitochondrial genome differentiation in polyploid wheats and Aegilops Sitopsis

Mitochondrial DNAs from *Triticum* and *Aegilops* species, carrying B, G, or S and S-related nuclear genomes, are compared by overlap of restriction fragment patterns. In total, nearly 300 restriction fragments are scored in each mitochondrial genome pair. The percentage of common fragments is used as an indicator of genetic similarity (Table 3). We use this simple parameter because of the complex features of mitochondrial genomes, i.e., their organization and behavior do not allow adaptation to any of the established statistical methods developed for animal mtDNA and plant ctDNA (among others, Nei and Li 1979; Engels 1981). However, since large

fractions of these genomes are more or less conserved during speciation, and since we know so much about these related species, we feel that this parameter is of real significance as an index of similarity (Borck and Walbot 1982). Using this approach we have identified seven mitochondrial genome types, from 16 sources of cytoplasm originating from 12 polyploid wheat lines and four Sitopsis species (Table 2). It should be noted, however, that the type Va mitochondrial genome of *Ae. speltoides* (09), *T. timopheevi* (25), and *T. zhukovskyi* (51) is nearly identical to the type Vb genome of *T. araraticum* (23 and 24); only one fragment difference is observed among 309 fragments compared. Similarly, the type VIIa mitochondrial genome, found in six emmer-dinkel wheat lines, closely resembles type VIIb of *T. dicoccoides* (21) and *T. spelta* (Splt), with only seven of 307 fragments exhibiting differences (Table 2). Aside from these two, there are five well-differentiated mitochondrial genome types among the polyploid wheats and Sitopsis species studied here.

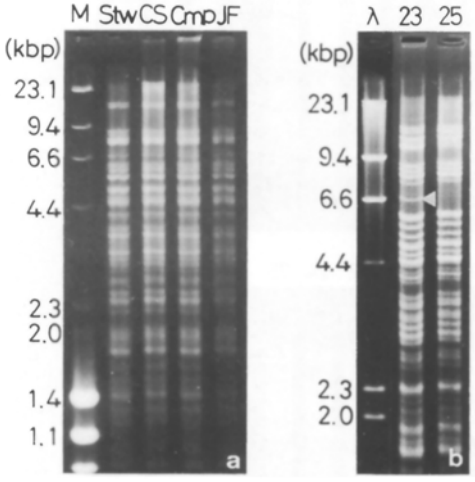

Fig. 2 a and b. *Hin*dIII restriction fragment patterns of mtDNA isolated from six cytoplasms. – *Hin*dIII-digested lambda phage DNA. M – *Hin*dIII-digested lambda plus *Hae*III-digested ϕ X174 phage DNAs. **a** – *T. durum* (Stw), *T. asestivum* cv Chinese Spring (CS), *T. compactum* (Cmp), and *T. aestivum* cv Jones Fife; **b** – *T. araraticum* (23) and *T. timopheevi* (25)

A comparison between mitochondrial and chloroplast genome differentiation during speciation of Sitopsis species and polyploid wheats

From the percentages of common mtDNA fragments, a dendrogram is constructed (using the UPGM method) showing genetic relatedness among the seven mitochondrial genome types (Fig. 3). In the same figure, this mtDNA dendrogram is compared with a ctDNA dendrogram drawn by the same method from the data of Ogihara and Tsunewaki (1988).

Table 3. Numbers of fragments shared in common between two mtDNAs isolated from polyploid wheats and *Aegilops* species of the section Sitopsis

Mt genome types compared[a]	No. common fragments						Total fragments comp. (B)	% Common fragments (2A/B)
	*Bam*HI	*Hin*dIII	*Pst*I	*Pvu*II	*Xho*I	Total (A)		
Ib1: Ic	26	27	30	18	20	121	292	82.9
Ib1: Va	18	16	26	12	17	89	303	58.7
Ib1: Vb	18	16	26	12	17	89	304	58.6
Ib1: VIIa	21	15	22	11	17	86	302	57.0
Ib1: VIIb	21	15	23	11	18	88	303	58.1
Ib1: VIII	18	20	21	14	19	92	296	62.2
Ic: Va	19	15	23	10	14	81	297	54.5
Ic: Vb	19	15	23	10	14	81	298	54.4
Ic: VIIa	23	14	23	11	16	87	296	58.8
Ic: VIIb	23	14	24	11	17	89	297	59.9
Ic: VIII	21	20	19	14	16	90	290	62.1
Va: Vb	31	28	38	27	30	154	309	99.7
Va: VIIa	22	18	27	18	23	108	307	70.4
Va: VIIb	22	19	27	17	24	109	308	70.8
Va: VIII	20	18	21	17	19	95	301	63.1
Vb: VIIa	22	19	27	18	23	109	308	70.8
Vb: VIIb	22	20	27	17	24	110	309	71.2
Vb: VIII	20	19	21	17	19	96	302	63.6
VIIa: VIIb	32	27	35	21	35	150	307	97.7
VIIa: VIII	25	16	21	11	20	93	300	62.0
VIIb: VIII	25	17	21	11	20	94	301	62.5

[a] Refer to Table 2 for the mitochondrial genome type of each species

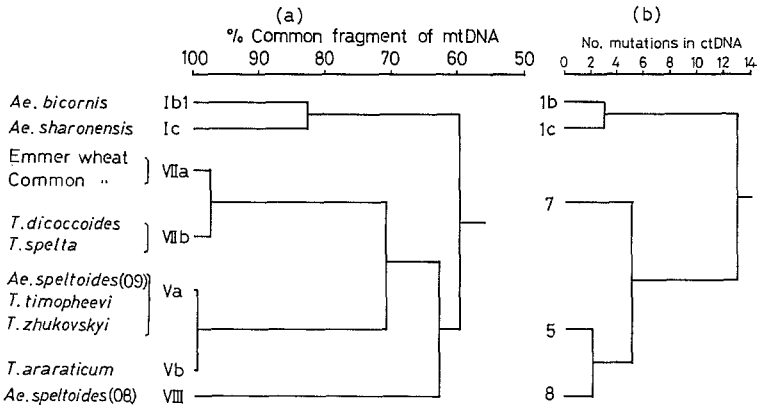

Fig. 3a and b. Dendrograms showing genetic relationships among mitochondrial (**a**) and chloroplast (**b**) genomes of polyploid wheats and Sitopsis species, based on the percentages of common fragments and numbers of mutations, respectively

In essence, the two dendrograms are alike, an indication that mitochondrial and chloroplast genomes have diverged in parallel during speciation. However, two differences must be pointed out. First, some differentiation of mitochondrial genomes appears to have occurred without parallel changes of chloroplast genomes. This observation supports others (Holwerda et al. 1986; Pring et al. 1987) that evolutionary change occurs more rapidly within mitochondrial genomes than within chloroplast genomes. These evolutionary rate differences may reflect more frequent structural changes (deletions/insertions, duplications, and inversions) in mtDNA than in ctDNA (Palmer 1988), and/or differences in genome size and relative amounts of noncoding sequence.

Another difference between the two dendrograms is the relationships among higher orders of the clusters. The branching points are close to one another and cannot be convincingly ordered in the dendrogram of mitochondrial genomes, whereas they are well ordered in the dendrogram of chloroplast genomes. This may be due to the increased inaccuracy in identifying fragment size differences among mtDNAs of somewhat distantly related species. Of course, this points out a limitation of the restriction fragment method for comparing mitochondrial genomes of distantly related species.

At the same time, the restriction fragment analysis of mtDNA seems well suited for phylogenetic studies of closely related taxa. CtDNA comparisons are useful for extending these phylogenetic studies to more distantly related species, as pointed out by Terachi and Tsunewaki (1986).

Mitochondrial genome differentiation within the section Sitopsis of Aegilops

The Sitopsis section is divided into two subsections, Emarginata and Truncata. Four species, *Ae. bicornis, Ae. longissima, Ae. searsii,* and *Ae. sharonensis* belong to the former, and *Ae. speltoides* to the latter. MtDNA of *Ae. longissima* is not included in this report.

Mitochondrial genomes of *Ae. bicornis* (12), type Ib1, and type Ic of *Ae. sharonensis* (10), were shown to be closely related (82.9% identity). According to Siregar et al. (1988), mtDNA of *Ae. searsii* is similar to mtDNAs of *Ae. bicornis* (12, 91% identical) and *Ae. sharonensis* (10, 79% identical). These facts indicate that a large part of the mitochondrial genome has been conserved during the speciation of *Ae. bicornis, Ae. sharonensis,* and *Ae. searsii,* three species belonging to the same subsection, Emarginata. The mitochondrial genomes of two *Ae. speltoides* accessions (08 and 09), of the other subsection, Truncata, differ greatly from those of *Ae. bicornis* (12) and *Ae. sharonensis* (10, Table 2).

The percentage of common fragments is low even between mitochondrial genomes of the two accessions of *Ae. speltoides* (63% identical). Our preliminary experiments indicate an alloplasmic line with a third *Ae. speltoides* cytoplasm possesses a type Vb mitochondrial genome, whereas a fourth *Ae. speltoides* cytoplasm has a type VIII genome. Since types Va and Vb are quite similar, we conclude that there are at least two well-differentiated mitochondrial genomes (types Va and Vb versus type VIII) in *Ae. speltoides;* these are associated with G and S plasma types, respectively (Tsunewaki 1980).

Breiman (1987) has reported extensive intraspecific mitochondrial genome diversity among different accessions of *Ae. speltoides,* as determined by Southern blot hybridization of total DNA by known mtDNA sequences. It appears that the intraspecific variation among mitochondrial genomes is not this high in other species of these two genera, but the data base is insufficient. For example, mtDNAs from two *T. araraticum* lines or three *T. aestivum* cultivars showed no differences among their restriction fragment patterns. According to Breiman (1987), no differences in hybridization profiles were observed in two *Ae. sharonensis* lines and four *Ae. searsii* lines. In addition, mtDNAs from six *Ae. squarrosa* lines, belonging to different varieties, showed few differences in their *Bam*HI and *Hin*dIII pat-

terns (Terachi et al. 1985). These results show much more variability among mitochondrial genomes of *Ae. speltoides* than is found in other species. The reasons for this remain to be investigated.

Possible phylogenetic differentiation in both the emmer-dinkel and timopheevi groups

Differentiation among mitochondrial genomes isolated from cytoplasms of the emmer-dinkel group (type VII a versus VII b) is in contrast to identical chloroplast genomes taken from the same cytoplasms. For example, a 12.4-kbp *Hin*dIII fragment, present in mtDNAs of *T. dicoccoides* (21) and *T. spelta* (Splt), is absent in mtDNAs of the other six lines of emmer-dinkel wheat. Interestingly, this difference is found in both the tetraploid and the hexaploid cytoplasms. Two explanations are possible. The mtDNA structural change occurred independently in emmer and dinkel wheats, or it occurred first in emmer, and then was transmitted to dinkel wheat. According to N. Mori (personal communication), the type VII a mitochondrial genome, found in six emmer-dinkel wheat lines, is also present in *T. dicoccoides*, a wild emmer wheat. This suggests that the differentiation of the mitochondrial genome into VII a and VII b types occurred while the mitochondria reside in the wild emmer wheat. Later, both types were transferred into the cultivated emmer wheat. *T. spelta*, with hulled grains, and dinkel wheat, with naked grains, appear to have originated diphyletically from the emmer wheats carrying the VII a- and VII b-type mitochondrial genomes, respectively. Their diphyletic origin had already been suggested from comparative gene analyses on hybrid necrosis (Tsunewaki 1968).

A minor but clear differentiation of mitochondrial genomes is evident within the timopheevi group, for example, the Va type, in *T. timopheevi* and *T. zhukovskyi*, and the V b type, in *T. araraticum* (wild timopheevi), are clearly different. As mentioned earlier, both Va and V b mitochondrial genomes are found in *Ae. speltoides*. This indicates that differentiation of precursor mitochondrial genomes into Va and V b types occurred within cytoplasms of diploid ancestors. The observed, minor difference in mitochondrial genomes in this group, including 2x, 4x, and 6x species, suggests a rather recent origin of the timopheevi group.

Cytoplasm donors to polyploid wheats revealed by restriction fragment analysis of mtDNA

According to nuclear genome constitution, polyploid wheats are classified into three groups, emmer (AABB), dinkel (AABBDD), and timopheevi (AAGG and AAAAGG). The A and D genomes originated from einkorn wheat (Kihara 1924) and *Ae. squarrosa* (Kihara 1944; McFadden and Sears 1946), respectively. The origin of B and G genomes is still debated. Which diploid *Aegilops* species donated these genomes to polyploid wheats is not certain. Nearly all of the diploid *Aegilops* species (section Sitopsis) have been nominated as the donor. Analyses of the genetic characteristics of the cytoplasm (Tsunewaki 1980) and of ctDNA restriction patterns (Tsunewaki and Ogihara 1983) of polyploid wheats and their putative ancestor species reveal that the cytoplasms of polyploid wheats are greatly different from those of contemporary einkorn and *Ae. squarrosa*. Restriction fragment patterns of mtDNAs from einkorn and *Ae. squarrosa* also differ from those of polyploid wheats (T. Terachi and K. Tsunewaki, unpublished data). Thus, B and G nuclear genome donors to polyploid wheats appear also to be the cytoplasm donors to them. By analyzing *Eco*RI restriction fragment patterns of mtDNAs from *T. aestivum*, *Ae. speltoides*, *Ae. sharonensis*, and *T. urartu*, Vedel et al. (1981) found that all of these diploid species have mtDNAs that are different from that of *T. aestivum*. They concluded that none of them was the immediate cytoplasm donor to the latter.

Our results support their conclusion. None of the diploid species studied here carries mitochondrial genome identical to those of emmer-dinkel wheats. On the other hand, the mitochondrial genomes of *T. timopheevi* (25) and *T. zhukovskyi* (51) are identical to those of *Ae. speltoides* (09), but not to the other Sitopsis species. These results support our previous conclusion (Tsunewaki and Ogihara 1983), drawn from analyses of ctDNA restriction fragments, namely, that the latter donated the cytoplasm and the G genome to the timopheevi group of wheat. This is in accord with the findings of Kimber and Athwal (1972) that the S genome within a lower pairing form of *Ae. speltoides* shows complete chromosome pairing with the AG genomes of timopheevi wheat.

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