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

5. Mitochondrial genome diversity among *Aegilops* species having identical chloroplast genomes*

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Summary. Restriction fragment patterns of mtDNA isolated from the cytoplasm of three groups of *Aegilops* species (or accessions) which are known to carry the identical chloroplast genome but distinctly different cytoplasmic genomes (plasmons) have been analysed using five restriction endonucleases. Two to four different mitochondrial genomes are found in each group, between which the percent common restriction fragments amounts to 86–97%, whereas the same parameter obtained between mitochondrial genomes of the different groups ranges from 34 to 42%. Mitochondrial genome diversity is far more extensive than the chloroplast genome diversity, and the former provides a useful key for the phylogenetic relationships between cytoplasms of closely related species or even different accessions of the same species. The mitochondrial and chloroplast genome differentiation most certainly accounts for the plasmon variability known in this genus.

Key words: *Aegilops* – Mitochondrial DNA – Restriction endonuclease analysis – Mitochondrial genome diversity – Plasmon differentiation

Introduction

Cytoplasmic inheritance is a well known phenomenon in higher plants. Variegation, male sterility, growth anomalies and susceptibilities to some pathotoxins and herbicides are examples. The material basis of this phenomenon is believed to reside in the organellar DNAs in chloroplasts and mitochondria. In fact, in addition to rRNA and tRNA genes constituting their own translation systems, chloroplast DNA codes for a large number of polypeptides which serve as subunits of chloroplast proteins and enzymes (e.g. Dyer 1985); and mitochondrial DNA in higher plants encodes cytochrome *c* oxidase subunits I (Isaac et al. 1985) and II (Fox and Leaver 1981), apocytochrome *b* (Dowson et al. 1984) and the α subunit of the F_1 ATPase (Hack and Leaver 1983). However, we do not know yet the molecular mechanisms determining the expression of the aforementioned characters, except for the case of atrazine resistance in *Amaranthus hybridus* (Hirschberg and McIntosh 1983) and in a few other plants. Thus, basic studies on organellar genomes and their interaction with nuclear genomes are much needed; the present comparative analysis of organellar DNAs isolated from closely related plant species is a useful beginning approach.

In wheat (*Triticum*) and its related genus *Aegilops*, genetic diversity of cytoplasmic genomes (plasmons) among species has been extensively studied by two different means. One is to compare alloplasmic lines of common wheat, i.e., wheat lines having the same common wheat nucleus but different cytoplasms introduced from numerous *Triticum* and *Aegilops* species by repeated backcrosses (Kihara 1951; Fukasawa 1959; Maan 1975; Panayotov and Gotsov 1976; Tsunewaki 1980; Tsunewaki and Tsujimoto 1983). The second is restriction endonuclease analyses of organellar DNAs, particularly, of chloroplast DNA (Vedel et al. 1978; Ogihara and Tsunewaki 1982, 1983; Tsunewaki and Ogihara 1983; Bowman et al. 1983; Terachi et al. 1984). From these studies, we have a large catalogue of information of genetic differences among cytoplasmic and chloroplast genomes, and of the phylogenetic relationships among them.

However, our knowledge of mitochondrial genomes – structure and function, variation and phylogeny – in *Triticum* and *Aegilops* is still meager although Vedel et al. (1978) began

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the pioneering work almost ten years ago. We have discovered discrepancies between cytoplasmic and chloroplast genome diversifications in these genera (Tsunewaki 1980; Ogihara and Tsunewaki 1982, 1983; Tsunewaki and Tsujimoto 1983; Terachi et al. 1984) which form the basis of our first step toward analysing mitochondrial genome diversity. The lines tested were those among which we observed no ctDNA variation but clear cytoplasmic variation. Within such lines, mitochondrial DNA differences were studied by the restriction endonuclease method.

The results still can not be used to assign known cytoplasmic functions to specific mtDNA variations. However, the results do provide new information on mitochondrial genome diversity evolved during speciation in *Aegilops*. The results also demonstrate the usefulness of mtDNA variability as a fine-tuned "molecular clock" with which to measure time in the phylogenetic history of higher plants.

Materials and methods

Plant materials

In the present investigation, alloplasmic lines of common wheat were the source of mtDNAs of nine *Aegilops* species (11 accessions in total), as shown in Table 1. Each alloplasmic line is indicated hereafter with the names of its cytoplasm (in parentheses) hyphenated with nucleus donor. All lines, except (*mutica*-P)-CS, were chosen because of their normal growth vigor and high seed fertility. Their open-pollinated seeds were used. Rows of the male sterile (*mutica*-P)-CS line were placed between rows of normal CS, which allows seed-set on the male sterile line by natural out-crossing with neighboring plants. Alloplasmic lines of common wheat, with *Aegilops* cytoplasm, were used because their seeds and fresh etiolated leaves could be harvested in larger quantity.

About 150 gm seeds of each line were soaked 24 h in running water before sowing in wooden flats filled with sterilized soil. The flats were kept in a greenhouse and covered with cartons for 10 to 12 days until leaf harvest. In most cases, about 500 gm etiolated leaves were harvested per line.

Isolation of intact mitochondria

The procedures used for the isolation of intact mitochondria are essentially the same as those of Bonen and Gray (1980), though etiolated leaves were used instead of viable embryos. In most cases, the collected leaves were divided into two parts (about 250 gm each) and cut into 1–2 cm long pieces, and homogenised five times, 3 s each, using a mixer with 1 l of the following homogenisation buffer; 0.44 M mannitol, 50 mM Tris-Cl (pH 8.0), 3 mM EDTA, 1 mM 2-mercaptoethanol and 0.1% (w/v) BSA. The homogenate was filtered through four layers of cheesecloth and two layers of Miracloth (Calbiochem), prior to centrifugation for 10 min at 3,500 rpm using a Hitachi RPR-12 rotor. The supernatant was centrifuged for 20 min at 11,000 rpm using the same rotor to collect crude mitochondria. The pellet was resuspended in 15 ml A buffer, that is the homogenisation buffer in which mannitol was replaced with sucrose of the same concentration. The suspension was homogenised using a Teflon homogeniser, and the homogenate was incubated with DNase I (40 μg/ml at the final concentration, Warthington) and 0.2 ml $MgCl_2$ (1.0 M) for 25 min at room temperature in order to remove the contaminating nuclear and chloroplast DNAs. After the incubation, the homogenate was diluted with 3 volumes of B buffer (the same as A buffer, except for 20 mM, instead of 3 mM, EDTA) and centrifuged for 15 min using a Hitachi RPR-20 rotor. This procedure was repeated. The resultant pellet was resuspended in 15 ml A buffer, and homogenised again using a Teflon homogeniser. The homogenate was loaded on the top of a discontinuous sucrose gradient (1.15, 1.30 and 1.45 M each, instead of 0.44 M sucrose in A buffer), and centrifuged for 1 h at 22,000 rpm using a Hitachi RPS-27-2 rotor. After centrifugation, intact mitochondria were recovered from the 1.30–1.45 M interface with a glass pipette, gently diluted with

Table 1. Alloplasmic lines of common wheat used as sources of mtDNA

Alloplasmic line[a]	Cytoplasm donor					
	Code no.	Species	Nuclear gemome	Plasma[b] type	Chloroplast[c] genome type	Source[d]
(*bicornis*)-CS[e]	12	*Ae. bicornis*	S^b	S^b	1b	M
(*kotschyi*)-CS	33	*Ae. kotschyi*	C^uS^v	S^v	1b	K
(*variabilis*)-CS	34	*Ae. variabilis*	C^uS^v	S^v	1b	K
(*mutica*-M)-CS	13	*Ae. mutica*	Mt	Mt	4	M
(*mutica*-P)-CS	14	*Ae. mutica*	Mt	Mt^2	4	P
(*umbellulata*)-JF	03	*Ae. umbellulata*	C^u	C^u	3	K
(*triuncialis*)-CS	26	*Ae. triuncialis*	C^uC	C^u	3	K
(*biuncialis*)-CS	29	*Ae. biuncialis*	C^uM^b	C^u	3	K
(*columnaris*)-CS	30	*Ae. columnaris*	C^uM^c	C^u	3	K
(*triaristata* 4x)-CS	32	*Ae. triaristata*	C^uM^t	C^u	3	K
(*triaristata* 6x)-JF	54	*Ae. triaristata*	$C^uM^tM^{t2}$	C^u	3	K

[a] Indicated with the names of the cytoplasm (in parentheses) hyphenated with nucleus donor
[b] After Tsunewaki and Tsujimoto (1983)
[c] After Ogihara and Tsunewaki (1983)
[d] K: Laboratory of Genetics, Kyoto Univ., Japan; M: S.S. Maan, North Dakota State Univ., USA; P: I. Panayotov, Institute for Wheat and Sunflower, Bulgaria
[e] CS and JF: *Triticum aestivum* cv. 'Chinese Spring' and 'Jones Fife', respectively

2 volumes of C buffer, made of 0.44 M sucrose, 50 mM Tris-Cl (pH 8.0) and 3 mM EDTA, and finally pelleted by centrifugation for 15 min at 15,000 rpm using a RPR-20 rotor. Pelleted intact mitochondria were lysed in 3 ml TE buffer made of 50 mM Tris-Cl (pH 8.0), 20 mM EDTA and 2% (w/v) sodium N-lauroyl sarcosinate. The lysate was treated with Proteinase K (Merck, 200 μg/ml at the final concentration) for at least 1 h at 37 °C. The lysates from the two parts of starting material were put together and stored at −80 °C till use.

Purification of mtDNA

MtDNA was purified from the mitochondrial lysate described above. Sterilised CsCl and ethidium bromide (1 gm/ml and 200 μg/ml, respectively, at the final concentration) were added to the lysate, and the mixture was centrifuged for 40–44 h at 37,000 rpm using a Hitachi RP-65T rotor or 12 h at 45,000 rpm using a Hitachi RPV-65T rotor. After this, the DNA fraction that fluoresced under long wave UV light, was recovered by puncturing the tube with a syringe. Ethidium bromide and CsCl were removed from this fraction, and mtDNA was precipitated with ethanol. Finally, mtDNA was dissolved into an appropriate volume of DNA buffer made of 10 mM Tris-Cl (pH 7.9), 10 mM KCl and 0.4 mM EDTA. Usually, about 500 gm starting material yielded mtDNA sufficient for ten electrophoretic runs, although the yield varied depending upon the plant materials used.

Restriction endonuclease analysis of mtDNA

All mtDNA samples were digested with the following five enzymes; *Bam*HI, *Hin*dIII, *Pst*I, *Pvu*II and *Xho*I. The digestion was carried out according to the directions given by the supplier, Takara Shuzo Co. Ltd. or Nippon Gene Co. Ltd. Electrophoresis of the digests was performed at 1 V/cm for 40 h or 2 V/cm for 20 h, using 0.8% (for *Bam*HI and *Hin*dIII digests) or 1.0% (for *Pst*I, *Pvu*II and *Xho*I digests) agarose slab gel made with TAE buffer (40 mM Tris, 20 mM sodium acetate and 2 mM EDTA, after Maniatis et al. 1982). DNA fragments stained with ethidium bromide (0.5 μg/ml) were made visible by long wave UV light illumination, and then photographed. Restriction fragment patterns of different mtDNA samples were examined using enlarged photographs. The molecular sizes of individual DNA fragments were estimated from their mobilities by comparison with known fragment sizes of *Hin*dIII-digested λ phage DNA.

Results

Restriction fragment patterns of mtDNAs isolated from alloplasmic wheat lines with Aegilops *cytoplasms*

a) MtDNAs isolated from lines with type 1 b and type 4 chloroplast genome. Restriction patterns of mtDNAs isolated from the cytoplasms of *Ae. bicornis* (code no. 12) and *Ae. kotschyi* (33), both of which have type 1 b chloroplast genomes, and those of two *Ae. mutica* lines, i.e., *mutica*-M (13) and *mutica*-P (14), both of which have type 4 chloroplast genomes, were compared with each other, as shown in Fig. 1. All of the restriction patterns were complex, with large numbers of fragments and non-stoichiometric copy numbers of some fragments. Although the patterns of four mtDNAs were different from each other, mtDNA of *Ae. bicornis* gave rise to patterns similar to those of *Ae. kotschyi,* whereas the patterns of the two *Ae. mutica* accessions were similar.

The distances between the electrophoretic origin and each fragment were measured, from which molecular sizes were estimated (data not shown). The restriction patterns of *Ae. variabilis* (34) mtDNA are not shown, but were identical to those of *Ae. kotschyi* mtDNA, with all five restriction enzymes.

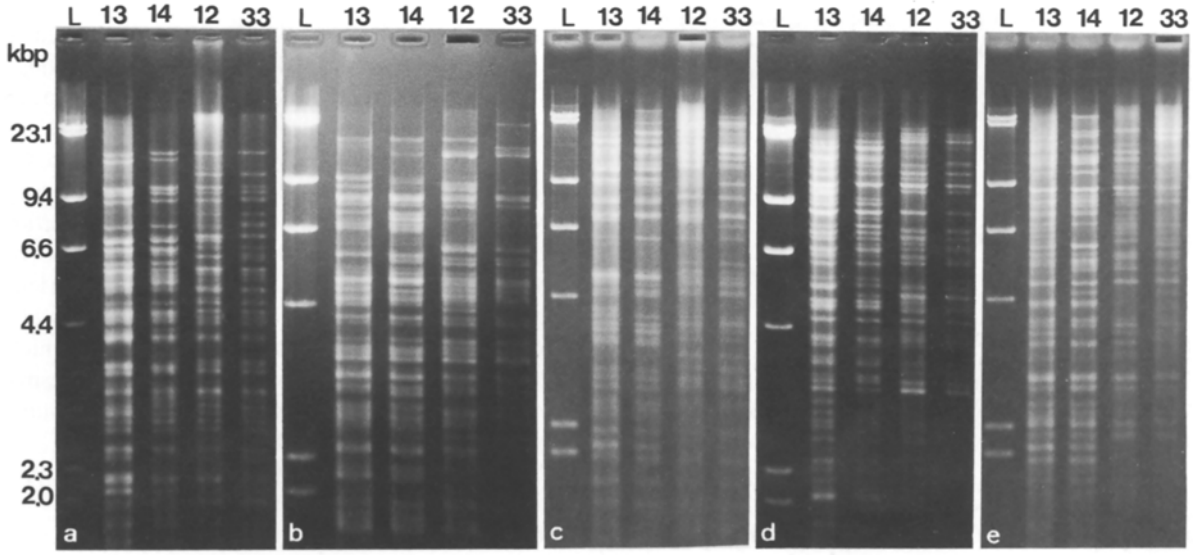

Fig. 1a–e. Restriction fragment patterns of mtDNA isolated from *Ae. mutica*-M (13), *Ae. mutica*-P (14), *Ae. bicornis* (12) and *Ae. kotschyi* (33) cytoplasms. L: *Hin*dIII-digested lambda phage DNA used as a molecular size marker. **a** *Bam*HI: **b** *Hin*dIII; **c** *Pst*I; **d** *Pvu*II; **e** *Xho*I

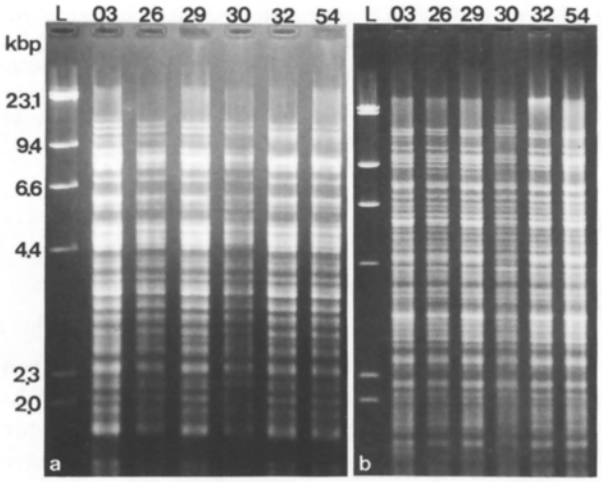

Fig. 2 a, b. Restriction fragment patterns of mtDNAs isolated from six C^u type cytoplasms. Species are indicated by their code number. L: *Hin*dIII-digested lambda phage DNA used as a molecular size marker. **a** *Bam*HI; **b** *Hin*dIII

b) MtDNAs isolated from the cytoplasms with type 3 chloroplast genomes. Restriction fragment patterns of mtDNAs isolated from six cytoplasms of the C^u plasma type, with type 3 chloroplast genomes, i.e., those of *Ae. umbellulata* (code no. 03), *Ae. triuncialis* (26), *Ae. biuncialis* (29), *Ae. columnaris* (30) and 4x and 6x forms of *Ae. triaristata* (32 and 54, respectively) were compared. Although all five enzymes were used, only the *Bam*HI and *Hin*dIII patterns are shown (Fig. 2). The molecular sizes of individual fragments were estimated, but the data are not shown. As is clear from Fig. 2, mtDNA of *Ae. triuncialis* produced patterns identical to those of *Ae. biuncialis.* Similarly, mtDNAs from 4x and 6x forms of *Ae. triaristata* gave rise to identical patterns. Thus, mtDNAs isolated from the six C^u type cytoplasms can be classified into the following four groups; (1) *Ae. umbellulata,* (2) *Ae. triuncialis* and *Ae. biuncialis,* (3) *Ae. columnaris,* and (4) 4x and 6x forms of *Ae. triaristata.*

Similarity of the mitochondrial genomes between related species

Due to the unique structural and behavioural features of plant mitochondrial DNA (e.g. Palmer and Shields 1984), it is impossible to make meaningful comparisons between plant mtDNAs using any established statistical parameters developed for animal mtDNA and plant ctDNA (Nei and Li 1979; Engels 1981; others). However, it is possible to compare common restriction fragments among mtDNAs of related plant species. 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 found in them. Both A and B are pooled for all restriction fragment patterns obtained from five enzyme digests. This parameter ignores the similarity in copy number of each fragment.

MtDNA fragments of *Ae. bicornis, Ae. kotschyi* and two lines of *Ae. mutica* were electrophoresed in the same gel. Similarly, mtDNAs from the six C^u cytoplasms were studied in the same gel. Therefore, percentages of common fragments were calculated from same gels only, except for comparison between *Ae. umbellulata* and *Ae. bicornis,* and for *Ae. umbellulata* and *Ae. mutica*-M. The percentages of common fragments for 25 pairs of 11 cytoplasms are shown in Table 2. The results clearly indicate that mitochondrial genomes from the cytoplasms with identical chloroplast genomes are much more closely related than those from cytoplasms with different chloroplast genomes are. The percent common restriction fragments between *Ae. bicornis* and *Ae. kotschyi* was 88.9%, between two *Ae. mutica* lines 85.8%, and between any two C^u type cytoplasms, higher than 90%. The percentage was lower between other species, e.g. 41.7% between *Ae. bicornis* and *Ae. mutica*-M and 38.5% between *Ae. kotschyi* and *Ae. mutica*-P. Based on these data, the mitochondrial genomes described here are designated as shown in the first column of Table 3.

Discussion

Relationships among the plasma types, chloroplast genomes and mitochondrial genomes in Triticum *and* Aegilops

Based upon their phenotypic effects, in 12 common wheats, the cytoplasms of 31 *Triticum* and *Aegilops* species fall into 13 types (Tsunewaki and Tsujimoto 1983), while their chloroplast genomes comprise 11 major and five subtypes, which are differentiated by their restriction endonuclease patterns (Ogihara and Tsunewaki 1982, 1983). By grouping the properties revealed by both methods, these cytoplasms fall into 16 groups (Tsunewaki and Tsujimoto 1983). In the present investigation, mtDNAs isolated from three groups (11 sources) of *Aegilops* cytoplasm, within each of which the chloroplast genome was invariant and the plasma type variant, were compared for restriction endonuclease fragment patterns.

The first group includes *Ae. bicornis* (plasma type S^b), *Ae. kotschyi* (S^v) and *Ae. variabilis* (S^v), all having the same 1b type chloroplast genome. The S^v cytoplasm of *Ae. kotschyi* and *Ae. variabilis* is characterised by induction of haploid parthenogenesis in the common wheat strain, 'Salmon', and complete male sterility

Table 2. Number of the fragments shared in common between two mtDNAs from closely related *Aegilops* species

MtDNAs[a] compared	No. common fragments						Total no. frag. compared (B)	% Common frag. (2A/B)
	*Bam*HI	*Hin*dIII	*Pst*I	*Pvu*II	*Xho*I	Total (A)		
(a) Within-group comparison								
12: 33	33	41	31	33	26	164	369	88.9
12: 34	33	41	31	33	26	164	369	88.9
33: 34	39	44	34	41	29	187	374	100.0
13: 14	33	31	34	37	32	167	389	85.8
03: 26	49	46	40	44	43	221	455	97.1
03: 29	49	46	40	44	43	221	455	97.1
03: 30	49	46	37	41	40	212	458	92.3
03: 32	50	44	40	45	43	221	455	97.1
03: 54	50	44	40	45	43	221	455	97.1
26: 29	50	46	40	46	45	227	454	100.0
26: 30	47	46	37	43	39	211	457	92.3
26: 32	49	44	40	44	43	219	454	96.5
26: 54	49	44	40	44	43	219	454	96.5
29: 30	47	46	37	43	39	211	457	92.3
29: 32	49	44	40	44	43	219	454	96.5
29: 54	49	44	40	44	43	219	454	96.5
30: 32	48	44	37	41	40	209	457	91.5
30: 54	48	44	37	41	40	209	457	91.5
32: 54	50	45	41	46	45	227	454	100.0
(b) Between-group comparison								
12: 13	16	18	17	18	10	79	379	41.7
12: 14	16	15	16	18	9	74	376	39.4
33: 13	16	19	16	17	11	79	382	41.4
33: 14	15	14	15	20	9	73	379	38.5
03: 12[b]	16	15	12	13	8	64	377	34.0
03: 13[b]	15	18	14	16	18	81	390	41.5

[a] The sources of mtDNAs are given in their code numbers which are shown in Table 1. MtDNAs of the same "group" means those isolated from the cytoplasms known to carry the same chloroplast genome

[b] Only the fragments larger than 2 kbp were compared because two DNAs were electrophoresed in different gels

Table 3. Relationships among the cytoplasm (plasma types), chloroplast and mitochondrial genomes in the nine *Aegilops* species studied in the present investigation

Mitochondrial genome type	Chloroplast genome type					
	1b		3		4	
	Plasma type	Species	Plasma type	Species	Plasma type	Species
Ib1	S^b	*Ae. bicornis*				
Ib2	S^v	*Ae. kotschyi*, *Ae. variabilis*				
IIIa			C^u	*Ae. umbellulata*		
IIIb			C^u	*Ae. triuncialis*, *Ae. biuncialis*		
IIIc			C^u	*Ae. triaristata* (4x and 6x forms)		
IIId			C^u	*Ae. columnaris*		
IVa					Mt	*Ae. mutica* M
IVb					Mt^2	*Ae. mutica* P

Note: Chloroplast genome types and plasma types are cited from Ogihara and Tsunewaki (1982) and Tsunewaki (1980), respectively

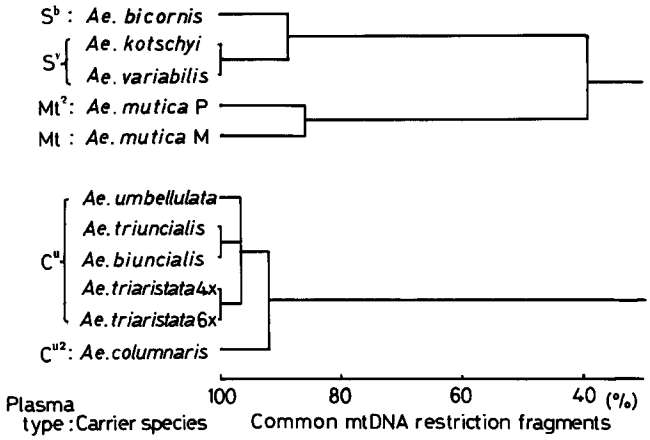

Fig. 3. The dendrogram showing genetic relationships between mitochondrial genomes of *Aegilops* species based on the percentage common fragments

in three of the 12 common wheats used, whereas the S^b cytoplasm has essentially no effect upon the phenotypes of any common wheat (Tsunewaki 1980; Tsunewaki and Tsujimoto 1983). The present result revealed that the mitochondrial genomes of *Ae. kotschyi* and *Ae. variabilis* are identical, while that of *Ae. bicornis* is somewhat different from them, the percentage common restriction fragments being 88.9%.

The second group includes the cytoplasms of two *Ae. mutica* accessions, one carrying Mt plasma type, the other Mt^2. The Mt cytoplasm induces complete male sterility in three of 12 common wheats tested, delays heading for 4.3 to 15.3 days (10.6 days on the average), and increases dry matter, plant height and some other related characters. On the contrary, the Mt^2 cytoplasm causes complete male sterility in all 12 common wheats, and slightly depresses growth vigor but with no specific effect on heading (Tsunewaki 1980; Tsunewaki and Tsujimoto 1983). However, their chloroplast genomes show identical restriction fragment patterns, with eight endonucleases (Ogihara and Tsunewaki 1982, 1983; Terachi et al. 1984). In the present investigation, mtDNAs isolated from the Mt and Mt^2 cytoplasms produced distinctly different restriction fragment patterns, the percentage common fragments being 85.8%. It appears that the phenotypic differences observed between the two plasma types can be associated to their mitochondrial genome differences.

The third group includes six cytoplasms of *Ae. umbellulata, Ae. triuncialis, Ae. biuncialis, Ae. columnaris* and 4x and 6x accessions of *Ae. triaristata,* all of which have the same C^u plasma type (Tsunewaki 1980). In addition, the ctDNAs from these cytoplasms yield identical restriction fragment patterns, indicating that their cytoplasms are identical or very similar (Ogihara and Tsunewaki 1982; Terachi et al. 1984). However, alloplasmic lines of some common wheats, e.g., cv. 'Chinese Spring' and 'Norin 26', with *Ae. columnaris* cytoplasm expresses less variegation than do the same nuclear genomes with other cytoplasms of C^u plasma type. As a consequence, the former alloplasmic lines show greater vigor of growth, resulting in taller plants and greater mass than the latter. Based on these facts, Mukai et al. (1978) classified the *columnaris* cytoplasm into C^{u2}, a subtype of C^u plasma type. The present results show that at least four different mitochondrial genomes exist among the species having C^u type cytoplasm, among which the mitochondrial genome of *Ae. columnaris* is the most differentiated. Here again, the best explanation for cytoplasmic differentiation is mitochondrial genome variability.

Using a similar method, Borck and Walbot (1982) determined that the percentage common fragments between each pairwise combination of mtDNAs isolated from four cytoplasmic types, C, N, S and T, of maize (*Zea mays*) ranged from 53 to 67%. Comparing these values with 89%, 86% and more than 90% between *Ae. bicornis* and *Ae. kotschyi,* between *Ae. mutica*-M and *Ae. mutica*-P, and between six C^u plasma type-carriers, respectively, it would seem that mitochondrial genome divergence is less extensive among the three *Aegilops* groups than among the four sources of *Zea mays* cytoplasm.

Based on the percent common mtDNA restriction fragments given in Table 2, a cluster analysis was carried out to depict the phylogenetic relationship among the mitochondrial genomes described above, using the UPGMA method of Sneath and Sokal (1963). The results are given in Fig. 3. This schema clearly shows that mitochondrial genome diversification is completely paralleled by plasmon diversification, and that the comparative restriction endonuclease analysis of mtDNAs from related species is a valid criterion for clarifying phylogenetic relationships.

High-speed diversification of mitochondrial genomes during speciation

From the present results the 4x and 6x forms of *Ae. triaristata* which have the nuclear genome constitution, $C^uC^uM^tM^t$ and $C^uC^uM^tM^tM^{t2}M^{t2}$, respectively, were shown to have identical mitochondrial genomes, comparing *Bam*HI, *Hin*dIII, *Pst*I, *Pvu*II and *Xho*I restriction patterns. Similarly, *Ae. kotschyi* and *Ae. variabilis,* having $C^uC^uS^vS^v$ nuclear genomes, have identical mtDNAs, and *Ae. triuncialis* (nuclear genome C^uC^uCC) and *Ae. biuncialis* ($C^uC^uM^bM^b$) also have identical mtDNA genomes. These facts show certain conservatism of the mitochondrial genome during speciation.

On the contrary, evidence that mitochondrial genome differentiation is faster than chloroplast genome differentiation is shown by differences between *Ae. bicornis* (nuclear genome S^bS^b) and *Ae. kotschyi* (including *Ae. variabilis*, $C^uC^uS^vS^v$), all with the type 1 b chloroplast genome; between two accessions of *Ae. mutica* (MtMt) possessing the type 4 chloroplast genome, and between *Ae. umbellulata* (C^uC^u), *Ae. triuncialis* (including *Ae. biuncialis*) and *Ae. triaristata* (4x and 6x forms), all with the same type 3 chloroplast genome.

In this genus, chloroplast genome diversity has provided useful information of interspecific relationships (Vedel et al. 1978; Ogihara and Tsunewaki 1982, 1983; Tsunewaki and Ogihara 1983; Terachi et al. 1984). On the other hand, mitochondrial genome variability is so extensive among the species with different chloroplast genomes that phylogenetic relationships can not be inferred from them. However, mtDNA restriction patterns are useful in clarifying phylogenetic relationships between the species, or even between different accessions of the same species having the same chloroplast genome. In a conclusion, a comparative study of the organellar DNAs, by the restriction endonuclease analysis, provides a wide spectrum of information of phylogenetic relationships among related species, in that ctDNA and mtDNA divergence are analogs of slow and fast phylogenetic time clocks.

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