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Mitochondrial DNA diversity in the genera of *Triticum* and *Aegilops* revealed by Southern blot hybridization

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Summary. Southern blot hybridization of total DNA to defined mitochondrial DNA sequences provides a sensitive assay for mtDNA variation in the genera of *Triticum* and *Aegilops*. A clear distinction between cytoplasms of tetraploid species sharing the "AG" haploid genome is reported for the first time. The *Sitopsis* section of the genus *Aegilops* showed the most extensive intra- and inter-specific variation, whereas no variation could be detected among the cytoplasms of polyploid *Triticum* species (wheats) sharing the AB haploid genome. Extensive cytoplasmic intraspecific diversity was revealed in *Ae. speltoides*.

Key words: Mitochondrial DNA – Total DNA – *Triticum* – *Aegilops*

Introduction

Although the mitochondrial genes are relatively few in number, they play indispensable roles in the key processes of respiration and photosynthesis. Moreover, the organelles demonstrate different kinds of genetic variability. One of these is DNA sequence heterogeneity, which can be measured directly at the molecular level by using restriction endonucleases. Studies on animal mitochondrial DNA (mtDNA) have shown that mtDNA diversity within a species and genotypic differences between local groups are very high, the substitution rates for mitochondrial protein coding gene being 10% higher than for nuclear genes (Brown et al. 1981). It seems that the overall genetic diversity and population subdivsion may be greater for mitochondrial than for nuclear genes in animals.

Restriction site polymorphism in chloroplast DNA from several plants has been analysed and the detected diversity was much lower than expected and much lower than the diversity of animal mtDNA populations (Blanks and Birky 1985; Clegg et al. 1984).

Information on plant mitochondrial DNA variation is very scanty except in *Zea* where extensive surveys of mtDNA heterogeneity and variation have been conducted (Levings and Pring 1977; Borck and Walbot 1982; Kemble et al. 1983; McNay et al. 1983). The studies were based on the restriction pattern and presence or absence of episomes related to S1 and S2, which characterise the S male-sterile group (Pring and Levings 1978; Kemble et al. 1983; Weissinger et al. 1982). These surveys identified 18 groups of races among 93 entries of Latin American races (Weissinger et al. 1982) and many variants among the Mexican races (Kemble et al. 1983).

In the *Triticum* and *Aegilops* species, a classical genetic approach contributed extensively in the identification of cytoplasms. A detailed classification of *Triticum* and *Aegilops* cytoplasms was achieved by constructing nuclear cytoplasmic hybrids in which the hexaploid wheat nucleus was combined by many backcross generations with the cytoplasm of several of its close relatives from the genera *Triticum* and *Aegilops*, resulting in the respective alloplasmic lines. This approach has revealed considerable genetic diversity among *Triticum* and *Aegilops* species and has indicated that certain phenotypic effects are associated with specific cytoplasmic types. The differences and similarities of the phenotypic effects have been used as a basis to classify the cytoplasms of *Triticum* and *Aegilops* species and to study their relationships (Tsunewaki et al. 1980).

The pattern of heterogeneity in *Triticum* and *Aegilops* chloroplast DNA was analysed in detail and found to provide a useful tool in the classification of cytoplasm but it was not possible to attach any phenotypic significance to the ctDNA variation (Bowman et al. 1983; Ogihara and Tsunewaki 1982).

Analysis of mtDNA may provide a most sensitive tool for assessing cytoplasmic diversity, therefore, the level of heterogeneity in the mitochondrial genomes from species and accessions of *Triticum* and *Aegilops* was examined.

Materials and methods

1 Plant material

The species, cultivars and populations used in this survey were as follows: Hexaploid wheat *T. aestivum;* tetraploid wheats, *T. dicoccoides, T. timopheevii* and *T. araraticum;* diploid wheats, *T. monococcum, T. boeticum;* diploid wild wheats, *Ae. bicornis, Ae. longissima,* (accession numbers 03, 04, 07, 08, 09, 10, 11, 14, 15), *Ae. searsii, Ae. sharonensis, Ae. speltoides* (accessions 01, 03, 04, 05, 07, 08, 20, 21, 26). Accession numbers and plant material were provided by Professor M. Feldman, Weizmann Institute of Science, Rehovot, Israel. One *Ae. longissima* and one *Ae. speltoides* were obtained from Dr. D. M. Lonsdale, Plant Breeding Institute, Cambridge, U.K.

2 Preparation of total DNA

Total DNA was prepared according to Abbott et al. (1985). 0.1–1 g of coleoptiles and leaves of 5 day-old etiolated seedlings were ground in liquid nitrogen. The powder was mixed with 6 volumes of DNA isolation buffer (100 mM NaCl, 100 mM Tris-HCl, pH 8.5, 50 mM EDTA, 0.2% SDS, 0.1 mg/ml proteinase K). After incubation, the lysate was extracted with phenol, phenol/chloroform (1 : 1) and chloroform repeatedly, until a clear aqueous phase was obtained. The aqueous phase was ethanol precipitated by addition of sodium acetate to a final concentration of 0.3 M and 2½ volumes of cold ethanol. The precipitate was resuspended in 300 µl TE buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA) and treated with boiled RNase A, 100 µg/ml, and 60 units/ml RNase T1 at 37 °C for 2 h. The aqueous phase was re-extracted with phenol, phenol/chloroform (1 : 1) and chloroform/isoamyl alcohol (24 : 1) after which the DNA was precipitated by addition of 0.3 M sodium acetate and 2½ volumes of cold ethanol. The precipitate was vacuum dried and resuspended in 100 µl TE buffer.

3 Mitochondrial DNA preparation

Mitochondria were isolated and lysed according to Galun et al. (1982) except for the following modifications: etiolated 5 day-old coleoptiles and leaves were collected at 4 °C and homogenised with buffer H (0.44 M sucrose, 0.05 M Tris-HCl, pH 7.5, 3 mM EDTA, 1 mM mercaptoethanol, 0.2% BSA in a mortar and pestle. After differential centrifugation the enriched mitochondrial fraction was run through a discontinuous sucrose gradient with the following molarities: 1.45 M, 1.3 M, 1.15 M. The purified mitochondria were collected at the 1.3/1.45 interface.

After lysis the mtDNA was purified on a CsCl gradient (1.7 g/ml) run to equilibrium in a Ti65 rotor on a Beckman L55 ultracentrifuge for 40 h at 40,000 rpm. The gradient was fractionated by pumping it through a hole pierced in the bottom of the tube and collecting the fragments in the wells of a microtiter plate. Aliquots taken from the wells were run on a 1% agarose gel to determine those fractions containing mtDNA, which were subsequently recovered and the CsCl was dialysed out at 4 °C for 48 h against 1 mM Tris-HCl, pH 8, 0.1 mM EDTA.

4 Restriction endonuclease digestion and Southern blot hybridisation

Samples of mtDNA (0.5–1 µg) or total DNA (10 µg) were digested for 6 or 16 h, respectively. The enzyme concentrations used for total DNA were 30–40 units/10 µg DNA. The restricted DNA was fractionated on 0.8% agarose slab gels and blotted according to Southern (1975). Prehybridisation and hybridisation were carried out with constant shaking at 65 °C in 10× Denhardt's 3× SSC, (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS, 100 µg/ml denaturated and sheared salmon sperm DNA. After 2–4 h prehybridisation the freshly boiled nick translated probe was added and allowed to hybridise for 16–24 h. After hybridisation the nitrocellulose sheets were rinsed once at 65 °C in 3× SSC, 0.2% SDS for 15 min and the unhybridised material was removed by two 30 min washes in 0.3× SSC, 0.2% SDS at 65 °C. A final 30 min wash in 0.3× SSC was performed in order to remove the SDS. The sheets were dried and exposed to X-ray film at −80 °C using intensifying screens.

5 Recombinant DNA clones

The plasmid pBN6601 is a 3.9 kb Bam HI-EcoRI maize mtDNA fragment containing the cytochrome oxidase subunit I gene (*COX I*) and its flanking regions (Isaac et al. 1985). The plasmid pZmEI is a 2.4 kb maize mtDNA fragment subcloned into pBR322 (Fox and Leaver 1981) and contains the cytochrome oxidase subunit II gene (*COX II*). These clones were a gracious gift from Dr. C. J. Leaver, Department of Botany, University of Edinburgh, Scotland. The cosmid clones 4G7 and 4C12 represent contiguous 40kb mtDNA fragments from a *Triticum aestivum* mtDNA cosmid library prepared in the pHC79 cosmid vector (unpublished results) according to Hohn and Collins (1980), Lonsdale et al. (1981), and Lonsdale et al. (1984). The L4C is an M13 clone of 900 bp Hind III-Pst I fragment of *T. aestivum* mitochondrial cytochrome oxidase subunit I including 240 bp of the flanking region upstream the 660 bp 5′ terminus of the coding sequence and refered to as the 5′ wheat *COX I* clone or L4C (received from Dr. L. Bonen, Department of Biochemistry, Ottawa University, Canada, manuscript in preparation).

The DNA was labelled by nick translation using αP^{32}dCTP or dATP (Amersham, 3,000 cm/m mole) (Rigby et al. 1977). The labelled DNA was separated from incorporated nucleotides by chromatography on a 1 ml Sephadex G-50 column.

Results

1 Detection of specific mtDNA sequences in total DNA preparation

The mitochondrial genes of maize and wheat exhibit a high degree of sequence homology (Boer et al. 1985; Bonen et al. 1984). Therefore it is possible to use heterologous genes from maize as probes for wheat mtDNA sequences on Southern blots of wheat total DNA. This approach facilitates the screening of wild wheat cytoplasms where the amount of plant material available was scarce and from which mtDNA could not be prepared easily.

Probing restriction digests of *Triticum aestivum* mtDNAs isolated from dark grown coleoptiles with the recombinant plasmid pBN6601 (maize mitochondrial *COX I* gene) and the cosmid 4G7 from the wheat mitochondrial cosmid library, demonstrated that the mtDNA and total DNA samples had identical hybridization profiles (Figs. 1, 2, 3). This demonstrates

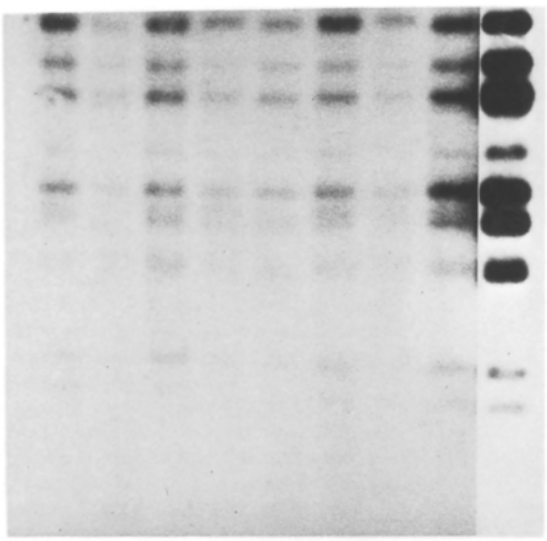

Fig. 1. Autoradiographs of Southern blot hybridizations of *Triticum* total and mitochondrial DNA digested with Bam HI and hybridized with the nick translated 4G7 cosmid. Slot designations: 1, 2 = *T. aestivum;* 3, 4 = *T. durum;* 5, 6 = *T. dicoccum;* 7, 8 = *T. dicoccoides;* 9 = *T. aestivum.* Slots 1–8: total DNA; slot 9: mtDNA

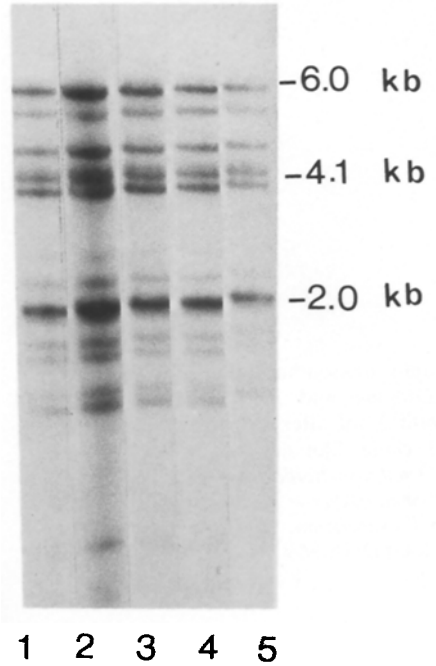

Fig. 2. Autoradiographs of Southern blot hybridization of total and mtDNA from *Triticum* species digested with Bam HI and hybridized with the nick translated 4C12 cosmid. Slot designations: 1 = *T. aestivum*; 2 = *T. dicoccoides*; 3 = *T. dicoccum*; 4 = *T. durum*; 5 = *T. aestivum.* Slots 1–4: total DNA; slot 5: mtDNA

that for these probes, promiscous DNA transfer (Stern and Lonsdale 1982; Timmis and Scott 1985) of these sequences to the nuclear genome had not occurred or was below the level of detection. Also, it demonstrates the absence of sequences homologous to chloroplast DNA within the probes (Lonsdale 1985).

2 Variation of mitochondrial DNA in polyploid wheats

Total DNA was extracted from 8 cultivars of *T. aestivum,* 5 cultivars of *T. durum,* 5 cultivars of *T. dicoccum,* and 10 cultivars of *T. dicoccoides.* The DNA was prepared from 5 day-old etiolated coleoptiles, each sample represented by seeds collected from a single plant. The restricted DNA was separated by electrophoresis on agarose gel and the Southern blots (Southern 1975) were hybridized to cosmids 4G7 and 4C12, each representing a contiguous fragment of about 40Kb from a wheat mtDNA cosmid library. The size of the nine and twelve Bam HI fragments detected by the 4G7 and 4C12 clones, respectively, were identical for all the species sharing the "AB" haploid genome (Figs. 1 and 2). Southern blots of *T. aestivum, T. durum, T. dicoccum* and *T. dicoccoides* DNA restricted with EcoRI, Pst I and Xho I and hybridized with pBN6601 (*COX I*) and pZmE1 (*COX II*) (Fox and Leaver 1981; Isaac et al. 1985), displayed identical hybridization patterns (data not shown).

The mitochondrial genome of the hexaploid and tetraploid *Triticum* species sharing the "AB" genome appear to be very stable, apparently being unaffected by the nuclear genotype (Figs. 1 and 2).

The *Triticum* species sharing the "AG" haploid genome were represented in this study by *T. araraticum* and *T. timopheevii.* These two species share a common 2.4 kb Bam HI fragment but a novel fragment of 8 kb is apparent on the *T. araraticum* after hybridization of the Southern blots with the pBN6601 probe (Fig. 3, lanes 7 and 8). Recent results show an intraspecific variation occurring among the *T. araraticum* cytoplasms (unpublished results).

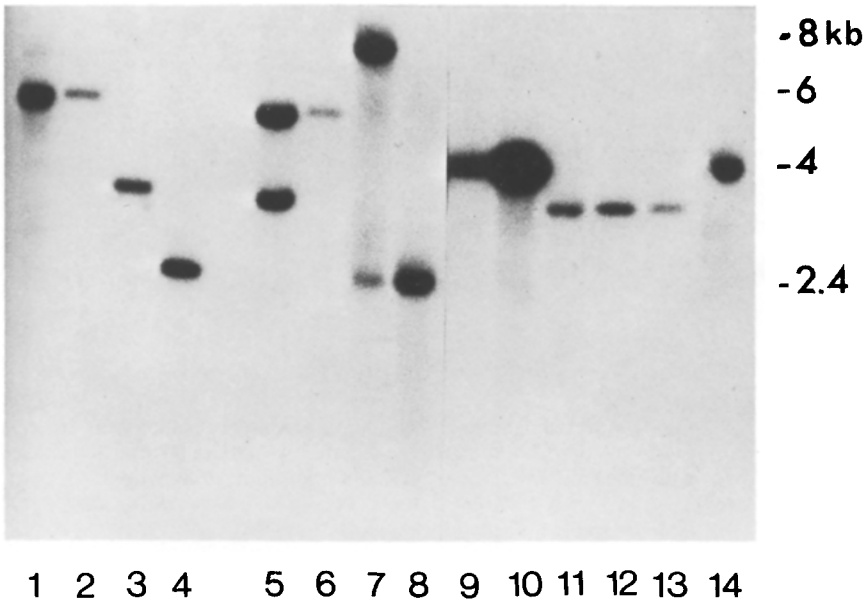

Fig. 3. Autoradiographs of Southern blots of *Triticum* and *Aegilops* total DNA and mtDNA digested with Bam HI and hybridized with the nick translated pBN6601 clone. Slot designations: 1 = *Ae. searsii*; 2 = *Ae. sharonensis*; 3 = *Ae. speltoides* (01); 4 = *Ae. speltoides* (Cambridge); 5 = *Ae. longissima*; 6 = *Ae. bicornis*; 7 = *T. araraticum*; 8 = *T. timopheevii*; 9 = *T. aestivum* (Lakhish); 10 = *T. aestivum*; 11 = *T. monococcum*; 12 = *T. boeticum* (02); 13 = *T. boeticum* (03); 14 = *T. dicoccoides*. Slot 10: mtDNA; all other slots: total DNA

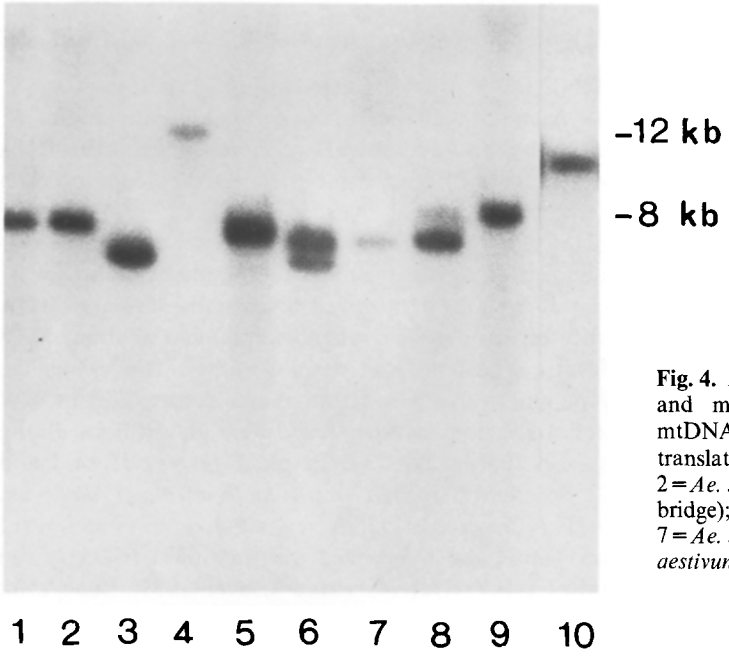

Fig. 4. Autoradiographs of Southern blots from total DNA and mtDNA of *Triticum* and *Aegilops* total DNA and mtDNA digested with XhoI after hybridization to the nick translated pBN6601 clone. Slot designations: 1 = *Ae. searsii;* 2 = *Ae. sharonensis;* 3 = *Ae. speltoides;* 4 = *Ae. speltoides* (Cambridge); 5 = *Ae. longissima* (01); 6 = *Ae. longissima* (Cambridge); 7 = *Ae. bicornis;* 8 = *T. araraticum;* 9 = *T. timopheevii;* 10 = *T. aestivum.* Slots 1–9: total DNA; slot 10: mtDNA

Digestion by the Xho I endonuclease and hybridization with the pBN6601 probe revealed 7.6 Kb and 8.6 Kb fragments for *T. araraticum* and *T. timopheevii* respectively (Fig. 4, lanes 8 and 9). This is the first report on unique restriction fragments of mitochondrial DNA which can distinguish between the cytoplasms of the closely related species *T. timopheevii* and *T. araraticum.*

T. monococcum and *T. boeticum* were represented in this study by five accessions. The two species share a 3.2 kb Bam HI fragment hybridizing to the pBN6601 probe, being specific for the diploids *Triticum* species having the "A" haploid genome (Fig. 3, lanes 11, 12, 13) but not present in the polyploids sharing the "A" genome (Fig. 3, lanes 9, 10, 14).

Extensive variation in hybridization profiles were found among species belonging to the Sitopsis section. The two species showing a common pattern were *Ae. sharonensis* and *Ae. searsii* which were only recently recognized as separate species (Feldman and Kislev 1977). Hybridization of the Bam HI and Xho I Southern blots with the pBN6601 exhibited similar fragments

Table 1. Fragments detected on Southern blots of *Triticum* and *Aegilops* spp. by hybridization of total DNA digests to the radiolabelled plasmid containing the maize mt *COX I* gene. Fragment sizes are presented in kb

Species	Bam HI fragments	XhoI fragments
T. aestivum	4.0	12
T. durum	4.0	12
T. dicoccoides	4.0	12
T. dicoccum	4.0	12
T. timopheevii	2.4	8.6
T. araraticum	8, 24	7.6, 8.6
T. monococcum	3.2	–
T. boeticum	3.2	–
Ae. searsii	6.0	8.5
Ae. sharonensis	6.0	8.5
Ae. bicornis	5.8	8.0
Ae. longissima	5.8, 3.5	8.6, 7.2, or[a] 8.5
Ae. speltoides	2.5 or[a] 3.5	7.5 or[a] 15

[a] In species where interspecific variation was observed the size of the fragments is given for the two species tested

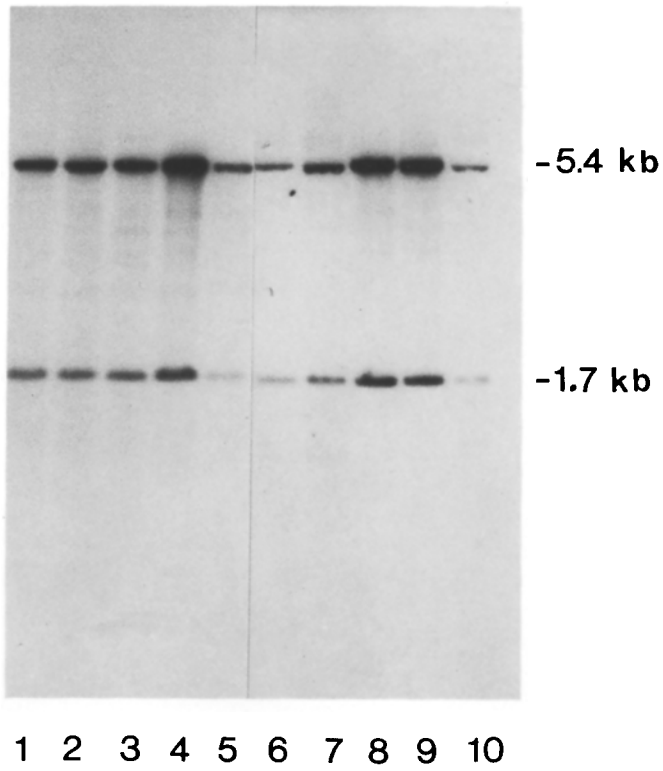

Fig. 5. Autoradiograph of Southern blot hybridization of total DNA from accessions of *Ae. longissima* restricted with Bam HI and hybridized with the L4C clone (5′ *COX I* wheat mtDNA). Slot designations: 1=03-2; 2=04; 3=09; 4=10; 5=12; 6=07-1; 7=08-1; 8=11; 9=14; 10=15

of 6 kb and 8.5 kb respectively (Figs. 3 and 4, lanes 1 and 2).

Two *Ae. sharonensis* and four *Ae. searsii* DNA preparations digested with PstI and EcoRI endonucleases and hybridized with the 4G7 cosmid and the pZmE1 clone demonstrated no differences in their hybridization profiles (data not shown). *Ae. bicornis* total DNA restricted with Bam HI and Xho I showed fragments of 5.8 and 8 kb, respectively, after hybridization with the pBN6601 clone (Fig. 3, lane 6; Fig. 4, lane 7; Table 1).

Two accessions of *Ae. longissima* displayed different profiles after digestion with Xho I and hybridization with the radioactively labelled pBN6601 clone; a 8.5 kb or 7.5 kb and 8.5 kb fragments were observed (Fig. 4, lanes 5 and 6). *Ae. speltoides* accessions displayed fragments of 7.5 kb or 15 kb and 3.5 kb or 2.5 kb following Xho I and Bam HI restriction and hybridization with the pBN6601 clone (Figs. 3 and 4, lanes 3 and 4; Table 1). Since these two species have demonstrated intraspecific diversity, more accessions were tested by hybridizing Bam HI restricted DNA with the L4C (5′ wheat *COX I*) clone. The 10 accessions of *Ae. longissima* collected from Israel exhibited a common pattern of 5.4 kb and 1.7 kb Bam HI fragments (Fig. 5) and substoichiometric fragments of 3.5 kb and other lengths could be detected in several accessions (e.g. Fig. 5, lanes 3 and 4).

The hybridization profile of 10 accessions of *Ae. speltoides* revealed quantitative and qualitative variation occurring among cytoplasms of this species (Fig. 6, Table 2). The most prevalent fragments after hybridization with the 5′ wheat *COX I,* were a 2.4 kb and 3.3 kb Bam HI fragment (Fig. 6). In addition to the major fragments detected after exposure of the autoradiograph for 24 h, six fragments were easily detected after a four day exposure (Table 2). The occurrence and intensity of the eight fragments varied among the accessions tested (Fig. 6, Table 2). Accession 08 represented by three individual plants revealed a similar hybridization pattern (Fig. 6, lanes 3, 4, 5): the 3.3 kb, 2.7 kb, and 2.4 kb being the major fragments differing in their relative intensity whereas the fragments 5.35 kb, 5.0 kb, 4.86 kb and 1.6 kb were clearly detected only after longer exposures. Another major group consists of four accessions (07-2, 20, 21, 26) which hybridized predominantly to the 2.4 kb, moderately to the 5.35, 5.0 and 4.86 kb and faintly to the 1.6 kb fragment (Table 2, Fig. 6). In several accessions a limited hybridization profile was observed. Accession 04-3 hybridized to the 2.4 kb fragment and accession 01-1 to the 3.3 kb fragment. Intraspecific variation in the mtDNA organization as revealed by hybridization with the 5′ *COX I* wheat clone appears to be more prominant in the *Ae. speltoides* than in the other diploid species tested.

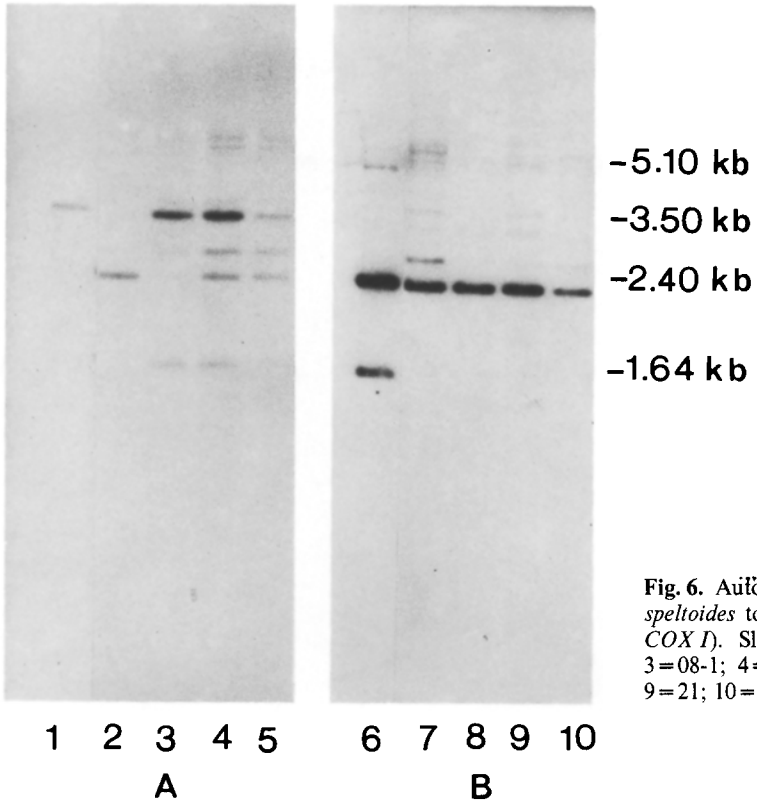

Fig. 6. Autoradiographs of Southern blots hybridization of *Ae. speltoides* total DNA hybridized with the L4C clone (5′ wheat *COX I*). Slot designations: *Panel A*, 1–5: 1=01-1; 2=04-3; 3=08-1; 4=08-12; 5=08-13. *Panel B*, 6=03-5; 7=07; 8=20; 9=21; 10=26

Table 2. Hybridization pattern of Bam HI digested *Ae. speltoides* total DNA and hybridized with the radioactively labelled L4C (5′ *T. aestivum COX I*) clone

Ae. speltoides accession no.	Fragment size in kb							
	5.35	5.1	4.86	3.56	3.3	2.73	2.4	1.64
08-1	+	+	+	+	+ + +	+	+	+ +
08-12	+ +	+ +	+ +	+	+ + +	+ +	+ +	+ +
08-13	+ +	+ +	+ +	+	+ + +	+ +	+ +	+
03-5	+ +	+ +	+ +	+	+	+	+ + +	+ + +
07-2	+ +	+ +	+ +	+	+	+ +	+ + +	+
20	+	+	+	+ +	+	+	+ + +	+
21	+ +	+ +	+ +	+ +	+ +	+ +	+ + +	+
26	+ +	+ +	+ +	+	+ +	+ +	+ + +	+
04-3	–	–	+	–	–	+	+ + +	+
01-1	–	–	–	–	+ + +	–	–	–

+: Faintly detected after a 4 day exposure
+ +: Moderately detected after a 24 h exposure
+ + +: Predominant fragment easily detected after a 24 h exposure of autoradiography

Discussion

Hybridization of total cellular DNA to defined chloroplast and mitochondrial DNA sequences facilitates the detection and characterization of the organellar genomes without the complication of isolating purified mtDNA from small amounts of tissue. Thus, total DNA can provide a suitable source for identifying mitochondrial genes in cases where plant material is scarce. No sequences were detected in the total DNA preparations of *T. aestivum* which were not detected in mitochondrial DNA, strongly suggesting that sequences related to the COX I probes are not present in the nuclear genome (Timmis and Scott 1985) or that sequences homologous to the chloroplast genome were absent from the probes used (Lonsdale 1985). In spite

of the vast polymorphism occurring in natural populations and individuals of *T. dicoccoides* (Nevo et al. 1982) no variation in the mitochondrial DNA was demonstrated. Geographic distribution or cultivation of the tetraploid wheats sharing the haploid "AB" genome did not affect mitochondrial DNA organization. The mitochondrial genome of the hexaploid and the tetraploid *Triticum* species tested in this survey appear to be very stable, apparently being unaffected by the nuclear genotype (Figs. 1 and 2). The similarity of the hybridization profiles suggests a common origin of the cytoplasm for all the polyploid wheats sharing the "AB" genome. Restriction fragments with different mobilities are described for each of the two tetraploids sharing the "AG" haploid genome, indicating the existence of variant cytoplasms. The cytoplasm of *T. timopheevii* and *T. araraticum* were indistinguishable by either the chloroplast DNA or by the phenotypic classification (Bowman et al. 1983; Tsunewaki et al. 1980).

Extensive variation in hybridization profiles were found among species belonging to the Sitopsis section: The *Ae. sharonensis* and *Ae. searsii* being the less variable and *Ae. speltoides* the most diverse (Figs. 3 and 6). Diversity among cytoplasms of the diploid *Ae. longissima* and *Ae. speltoides* was previously revealed by analysing chloroplast DNA restriction fragment patterns and the phenotypic effect produced in alloplasmic lines; The results on the mitochondrial DNA diversity correlate well with the latter results (Bowman et al. 1983; Ogihara and Tsunewaki 1982; Tsunewaki 1980).

The observed diversity of the cytoplasms of the diploid wild wheats may be due to nuclear selection of particular mitochondrial genotypes. Such effects have been demonstrated in maize and yeast systems. A strong influence of the nuclear genotype on mtDNA organization was detected in the maize cms-S (cytoplasmic male sterility S) cytoplasm. Evidence was provided for the influence of the nuclear genotype on replication (or degradation) S_1 and S_2 plasmids and also in determinating the frequency and type of cms-S reversion event (Laughnan et al. 1981). In yeast, a nuclear gene has been described which is essential for keeping mitochondrial DNA integrity (Labouesse et al. 1985).

Intraspecific variation in mtDNA organization has been demonstrated here by analysing 11 accessions of *Ae. speltoides* collected from Israel and Turkey. A few predominant patterns emerged but a wide variation could be easily detected (Fig. 6). Since the purified mtDNA was not analysed in these samples, the possibility of cross hybridization between mitochondrial probes and non-mitochondrial sequences cannot be ruled out.

The intraspecific variation occurring in the *Ae. speltoides* species are quantitative and qualitative (Table 2). These results fit the multipartite model described for the mitochondrial genome of Brassica and maize (Palmer and Shields 1984; Lonsdale et al. 1984), as well as the restriction map of wheat (Quetier et al. 1985).

The entire sequence complexity of these mitochondrial genomes can be organized as a single circular DNA molecule which has been termed the "master circle". Recombination between repeated sequences present on the master chromosome leads to formation of smaller subgenomic circles which exhibit a complex restriction profile of submolar and supramolar amounts of mitochondrial DNA fragments. Evidence for homologous recombinations between repeated sequences containing the 18S and 5S ribosomal RNA genes was reported to occur in wheat mitochondria (Falconet et al. 1984).

Recently it has been observed that mitochondrial sequences which exist as a single copy in one cytoplasm can be duplicated and become recombinantionally active in related cytoplasms (Lonsdale 1986). The possibility that the *COX I* gene in *Ae. speltoides* is located near a repeated sequence involved in recombination, therefore causing the large variation among the accessions, will be tested.

Evaluation of the full extent of variations occurring in mtDNA as a marker of cytoplasmic diversity will be a major contribution for evolution and genetic studies as well as for practical breeding purposes. This study provides sensitive and simple procedures to undertake such an evaluation.

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