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Variability and uniformity of mitochondrial DNA in populations of putative diploid ancestors of common wheat

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Summary. By using restriction endonuclease digestion patterns, the degree of intraspecific polymorphism of mitochondrial DNA in four diploid species of wheat and *Aegilops, Ae. speltoides, Ae. longissima, Ae. squarrosa,* and *Triticum monococcum,* was assessed. The outbreeding *Ae. speltoides* was found to possess the highest degree of variability, the mean number of nucleotide substitutions among conspecific individuals being 0.027 substitutions per nucleotide site. A very low degree of mtDNA variation was detected among *Ae. longissima* accessions, with most of the enzyme-probe combinations exhibiting uniform hybridization patterns. The mean number of substitutions among *Ae. longissima* individuals was 0.001 substitutions per nucleotide site. The domesticated diploid wheat *T. monococcum* var. *monococcum* and its conspecific variant *T. monococcum* var. *boeoticum* seem to lack mitochondrial DNA variability altogether. Thus, the restriction fragment pattern can be used as a characteristic identifier of the *T. monococcum* cytoplasmic genome. Similarly, *Ae. squarrosa* accessions were found to be genetically uniform. A higher degree of variation among accessions is observed when noncoding sequences are used as probes then when adjacent coding regions are used. Thus, while noncoding regions may contain regulatory functions, they are subject to less stringent functional constraints than protein-coding regions. Intraspecific variation in mitochondrial DNA correlates perfectly with the nuclear variability detected by using protein electrophoretic characters. This correlation indicates that both types of variation are selectively neutral and are affected only by the effective population size.

Key words: *Aegilops* – *Triticum* – Mitochondrial DNA – Intraspecific polymorphism

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Introduction

Restriction endonuclease digestion patterns of organelle DNA and, in particular, the high levels of within- and between-population variability in animal mitochondrial (mt) DNA sequences have been used for discerning phylogenetic relationships among groups of organisms and for assessing the degree of intraspecific variability (e.g., Palmer and Zamir 1982; Tsunewaki and Ogihara 1983; Clegg et al. 1984; Palmer 1985; Wilson et al. 1985; Avise 1986). Plant mitochondrial genomes are different from animal mtDNA in that they change rapidly in size and structure but slowly in primary sequence (Palmer and Hebron 1988). Although mtDNA restriction pattern variability has been used as a measure of phylogenetic relationship in several plant species, the large size, complexity, and frequent rearrangements of their mitochondrial genomes precludes direct interpretation of restriction enzyme patterns from total genomic DNA (Sederoff et al. 1981; Sederoff 1987). To overcome these limitations, restriction patterns that are identified by specific mitochondrial gene probes hybridized to Southern blots have been used (Bland et al. 1985; McLean and Hanson 1986; Chowdhury and Smith 1988; Graur et al. 1989).

Studies on plant mtDNA intraspecific variation are relatively limited, being confined mainly to maize and its relatives (Levings and Pring 1977; Pring and Levings 1978; Kemble et al. 1983; McNay et al. 1983; Sisco et al. 1985; Pring et al. 1980, 1987). Other species include barley (Holwerda et al. 1986), *Brassica* (Palmer 1988), *Solanum* (McLean and Hanson 1986), pearl-millet (Chowdhury and Smith 1988), oats (Rines et al. 1988), and rice (Chowdhury et al. 1988).

In this study, we assess the degree of intraspecific polymorphism in four diploid species of wheat and *Aegilops: Ae. speltoides, Ae. longissima, Ae. squarrosa,*

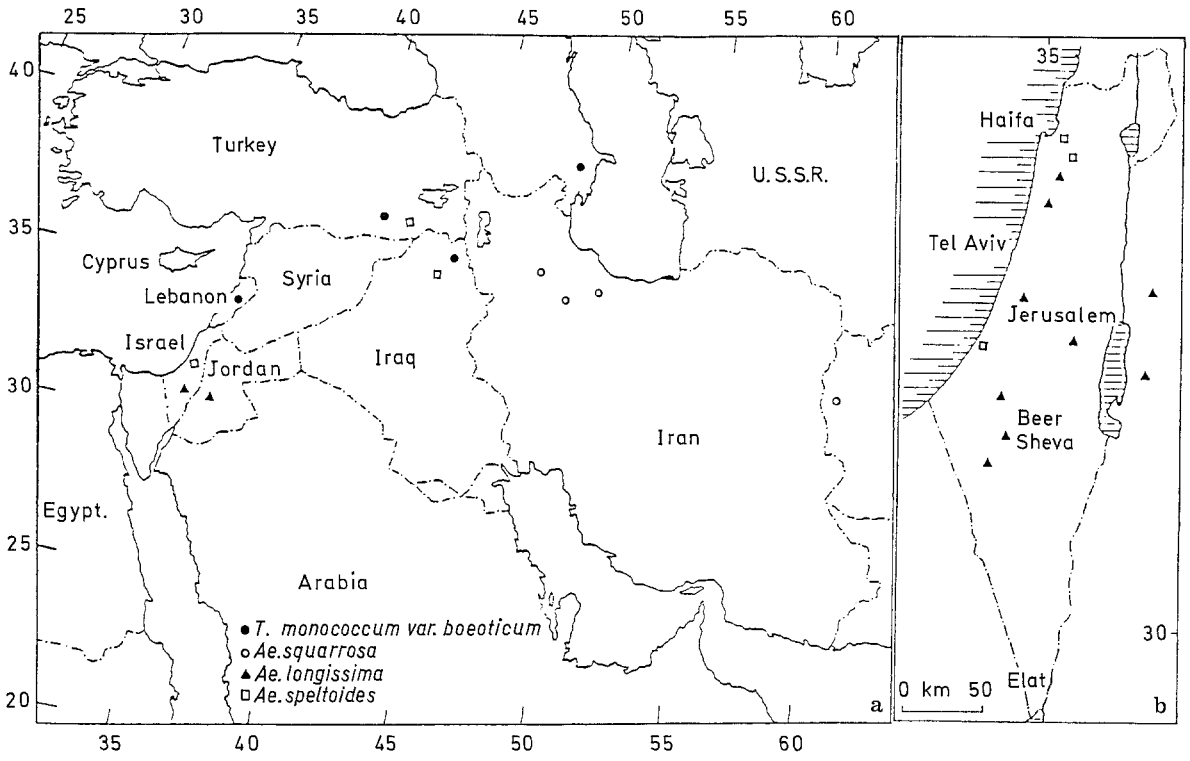

Fig. 1. **a** Main collection sites of *Triticum monococcum* var. *boeoticum, Aegilops speltoides, Ae. longissima,* and *Ae. squarrosa.* **b** Detailed collection sites of *Ae. longissima* and *Ae. speltoides* from Jordan and Israel

and *Triticum monococcum*. Two varieties of *T. monococcum* were studied: *T. monococcum* var. *monococcum* and *T. monococcum* var. *boeticum*. We also evaluate the sensitivity of several probes that are frequently used as markers for cytoplasmic identification.

Materials and methods

Plant material

Except for the cultivated varieties, all diploid species are euplasmic lines collected from the designated geographical locations (Fig. 1). *T. monococcum* var. *monoccum:* 01, 04 (cultivated varieties); *T. monococcum* var. *boeticum:* 01 (Lebanon); 02 (Turkey); 03 (Iraq); 04 (Azerbaijan); *Ae. squarrosa:* 01; 04 (Afghanistan); 08, 11, 12, 13, 22, 25, 27, 28, 29 (Iran); *Ae. longissima:* 01, 02, 03, 04, 05, 07, 11, 14, 15 (Israel); 08, 09, 10 (Jordan); *Ae. speltoides:* 01, 03, 04, 07, 08, 09 (Israel); 12, 26, 47 (Turkey); 20, 21 (Iraq). The *Triticum* and *Aegilops* lines used for mtDNA and total DNA extractions were provided by M. Feldman from the Department of Plant Genetics, Weizman Institute of Science, Rehovot, Israel. When multiple samples were taken from a single plant, a numeral was appended to the accession number.

Recombinant DNA clones

The following mitochondrial recombinant DNA clones were used. The maize mitochondrial *atp α* gene is a *Hin*dIII 4.2-kb fragment cloned in a pUC vector (Braun and Levings 1985). The maize mitochondrial *atp 9* gene is an *Xba*I 2.2-kb fragment cloned in a pUC vector (Dewey et al. 1985b). The maize mitochondrial *atp 6* gene is a 2.7-kb *Hin*dIII fragment cloned in a pUC vector (Dewey et al. 1985a). The 5′ *COX* I probe is an M13 clone of a 900-bp *Hin*dIII-*Pst*I fragment of the *T. aestivum* mitochondrial cytochrome oxidase subunit I gene, including 240 bp of the 5′ flanking region. The 5′ *COX II* probe is an M13 clone of a 230-bp *Taq*I fragment of the *T. aestivum* mitochondrial 5′ cytochrome oxidase subunit II gene (Bonen et al. 1984). The 3′ *cob* probe is a 770 bp *Bam*HI-*Hin*dII fragment of the *T. aestivum* mitochondrial 3′ apocytochrome b gene (Boer et al. 1985). L14a is a *T. aestivum Taq*I fragment of 160 bp located upstream of the apocytochrome gene (Boer et al. 1985). MH3 is a 180-bp *Hin*dIII-*Msp*I fragment located 50 bp upstream of the mitochondrial wheat *COX I* gene.

The chloroplast clones were *Pst*I fragments cloned in pBR322 and representing the whole *T. aestivum* chloroplast genome (Bowman and Dyer 1986). Clones P3, P4, P5, P6, P7, P8, P9, and P10 were nick-translated and used as a mixture for probing the *Ae. speltoides* total DNA.

The maize clones were obtained from C. J. Leaver, Department of Botany, Oxford University, UK. The wheat mtDNA clones were received from L. Bonen, Department of Biochemistry, Ottawa University, Canada. The wheat chloroplast DNA clones were obtained from C. Bowman, The Institute of Plant Science Research, Norwich, UK.

Total DNA and mtDNA extractions, restriction endonuclease digestion, transfer of DNA fragments from agarose gels to nitrocellulose sheets, labelling of recombinant plasmids by nick-translation, and filter hybridization were performed as in Breiman (1987). For the probe clones in M13 vector, second-strand labelling by the Klenow large fragment of DNA polymerase was used. In cases where DNA was used for several hybridizations, the DNA transfer was carried out on nylon membranes (Gene Screen Plus; NEN Research Products, DuPont). The transfer,

Table 1. *Ae. speltoides* DNA fragments hybridizing with mtDNA probes

Probe	Accession no.	Fragment size (kb)			
		*BamH*I	*Bcl*I	*EcoR I*	*Hin*dIII
5′ *COX I*	01-1	3.3	3.7	15	9.4
	04-3	2.4	3.1	10.6	3.9
	08-1, 08-12, 08-13	1.8, 2.4, 2.7, 3.1	4.0	10.6	8.1
	03-5	1.9, 2.6, 5.1	3.7	22.8	3.0
	07-2, 20, 21, 26	2.6, 2.9	3.8	7.8	3.9
	03-12	–	4.6	22.8	3.0
5′ *COX II*	03-5	2.7, 3.6, 4.1	–	–	–
	07-2, 20, 21, 26	2.7, 3.6, 4.1, 5.1	–	–	–
3′ *cob*	01-1, 01-5, 03-5, 03-10, 03-12, 04-4	2.5	9.4, 4.4	1.9	–
	05-6, 07-2, 08-1, 20	2.5	11.5, 4.4	1.9	–
atp α	01-1, 01-5	3.9, 2.8, 2.0, 0.9	4.3	–	–
	07-2, 09, 20, 21, 26, 27	3.9, 2.8, 2.0, 0.9	9.2, 13.9	–	–
	12	3.9, 2.8, 2.0, 0.9	8.5	–	–
atp 6	01-1, 01-5	3.8, 2.7	3.9, 4.2	–	–
	07-2, 09, 20, 21, 26, 27	3.8, 2.7	3.3	–	–
	12	3.8, 2.7	3.7	–	–
atp 9	03-4, 03-10	2.5	2.9	–	–
	03-11, 03-12, 03-13, 04-3	2.5	2.9, 3.1	–	–
	04-5, 05-6	2.5	–	–	–
MH3	01-1, 01-5	1.9	–	–	–
	03-4, 03-10, 03-11, 03-12, 05-6, 07-2	1.9, 3.0	–	–	–
	03-13, 04-3, 08-1, 08-12, 08-13	1.9, 3.0, 3.2	–	–	–
	12, 20, 21, 26	3.0	–	–	–
L14a	01-1, 01-5, 09, 47	5.6	–	–	–
	03-4, 03-10, 03-11, 03-12	3.1, 3.6	–	–	–
	03-13, 04-3	3.1, 3.3	–	–	–
	04-5, 12	3.1	–	–	–
	05-6, 20, 21, 26	3.1, 5.6	–	–	–
	08-1, 08-12, 08-13	3.1, 3.3, 4.3	–	–	–
	04-3	3.1, 3.3, 7.0	–	–	–

removal of the bound DNA for rehybridization, and hybridization of the Gene Screen Plus blots were performed according to the manufacturer's protocols.

Measures of genetic polymorphism

To estimate the number of nucleotide substitutions between two conspecifiv individuals from the proportion of shared restriction enzyme fragments, we used Nei and Li's (1979) method. The arithmetic mean number of nucleotide substitutions for all pairwise comparisons within a species was used to estimate the degree of intraspecific polymorphism.

Results

Restriction fragment patern of Ae. speltoides DNA hybridized with coding and noncoding mtDNA sequences

Ae. speltoides (SS) is an outbreeding member of the Sitopsis section, and has been suggested to be one of the putative donors of the B genome of *T. aestivum* (Riley et al. 1958; Rees and Walters 1965; Jaaska 1978; Kerby and Kuspira 1987; but see Graur et al. 1989). Preliminary observations on the high level of polymorphism observed in this species (Breiman 1987) as well as the interest in identifying the origin of the B genome by restriction endonuclease profiles of mtDNA have prompted us to analyze 12 accessions of *Ae. speltoides*. Since mitochondrial genes of maize and wheat exhibit a high degree of sequence similarity (Bonen et al. 1984; Boer et al. 1985; Bonhomme et al. 1989), it is possible to used heterologous genes from maize and wheat as probes for mtDNA sequences on Southern blots of total DNA (Breiman 1987; Graur et al. 1989).

The hybridization profile of the DNA extracted from *Ae. speltoides* revealed quantitative and qualitative differences among accessions and among individuals within single accessions (Table 1, Fig. 2). The hybridization pattern depends on the enzyme-probe combination. Nine *Ae. speltoides* accessions revealed a similar hybridization pattern when the DNA was digested with *Bam*HI and hybridized with the *atp α* clone, whereas three separate

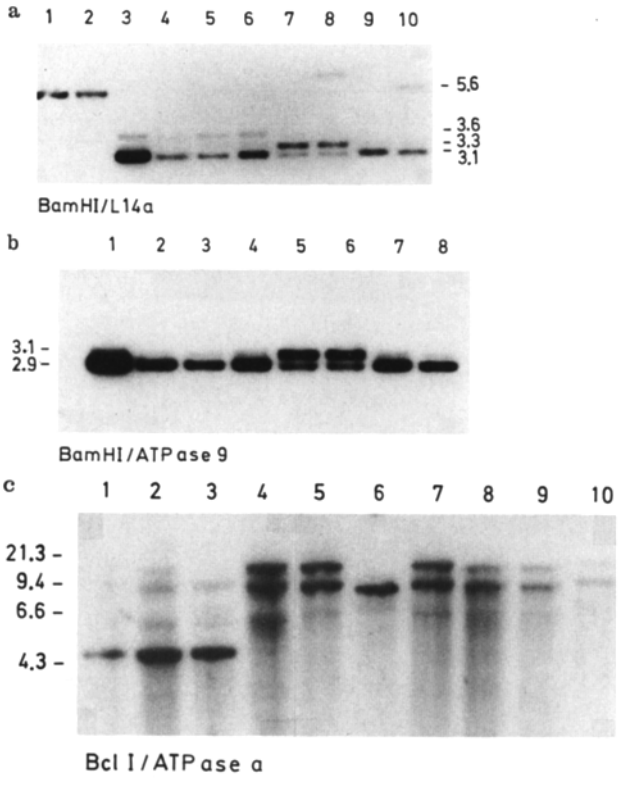

Fig. 2. **a** Autoradiograph of Southern blots from total DNA of *Aegilops speltoides* digested with *Bam*HI and hybridized with L14a. *Slot designations: 1*=01-1, *2*=01-5, *3*=03-4, *4*=03-10, *5*=03-11, *6*=03-12, *7*=03-13, *8*=04-3, *9*=04-5, *10*=05-6. **b** Autoradiograph of Southern blots from total DNA of *Ae. speltoides* digested with *Bam*HI and hybridized with *atp 9*. *Slot designations: 1*=03-13, *2*=3-10, *3*=03-11, *4*=03-12, *5*=03-13, *6*=04-3, *7*=04-5, *8*=05-6. **c** Autoradiograph of Southern blots from total DNA of *Ae. speltoides* digested with *Bcl*I and hybridized with *atp α*. *Slot designations: 1*=01, *2*=01-1, *3*=01-5, *4*=07-2, *5*=09, *6*=12, *7*=20, *8*=21, *9*=26, *10*=47

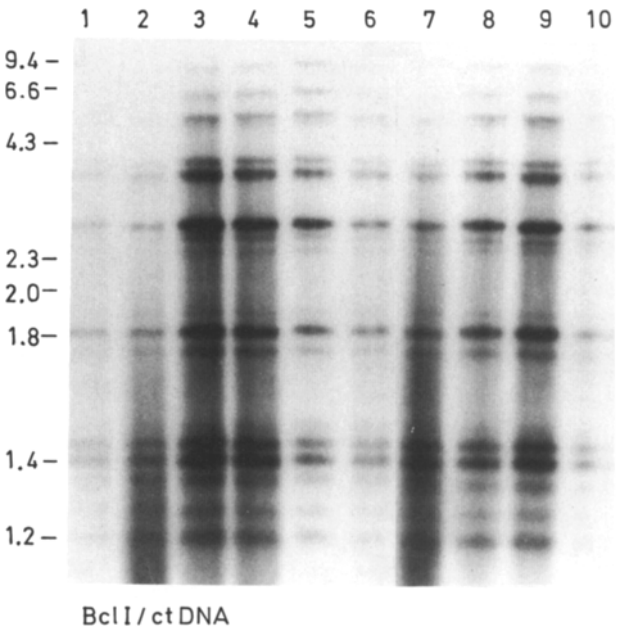

Fig. 3. Autoradiograph of Southern blots of total DNA from *Aegilops speltoides* digested with *Bcl*I and hybridized with a mix of wheat *Pst*I chloroplast DNA clones (see 'Materials and methods'). *Slot designations: 1*=01, *2*=01-1, *3*=01-5, *4*=07-2, *5*=08-1, *6*=09, *7*=20, *8*=21, *9*=26, *10*=47

hybridization patterns were observed when the DNA was digested with *Bcl*I and hybridized with the same probe (Table 1). Hybridization of Southern blots with noncoding mtDNA sequences revealed more intraspecific variation than that in protein-coding sequences (Table 1; Fig. 2a, b). However, fragments hybridized with the *atp α* probe were also found to be variable, although they corresponded to a protein-coding sequence. The mean number of nucleotide substitutions among *Ae. speltoides* individuals in our sample was 0.027 substitutions per nucleotide site.

In an attempt to correlate variation to ecotype, the hybridization pattern of plants collected from similar geographical locations was compared. We observed no correlation between geographical location and hybridization pattern. For instance, accessions 07 and 08, both collected in Haifa (Israel), revealed diverse patterns when digested with *Bam*HI or *Bcl*I and hybridized with 5′ *COX I*. Diverse patterns were also produced by other restriction endonuclease/probe combinations. In contrast, accessions collected in Turkey (26) and Haifa (07) revealed similar hybridization patterns when digested with *Bcl*I or *Bam*HI and hybridized with *atp α*, *atp 6*, 5′ *COX II*, and 5′ *COX I* (Table 1).

In order to rule out the possibility that seed contamination may have contributed to the extreme variation observed in the *Ae. speltoides*, the DNA from chloroplasts (cpDNA) of several accessions that have shown mtDNA variability was analyzed (Fig. 3). The total DNA extracted from *Ae. speltoides* accessions was hybridized with wheat cpDNA clones representing the major proportion of the chloroplast genome. An identical pattern of hybridization was obtained for all the accessions, indicating a much larger degree of heterogeneity in the mtDNA than in the cpDNA. The stability of the mtDNA restriction endonuclease pattern of an individual plant was assessed by comparing the patterns obtained from seedlings originating in seeds of the same plants (01–05) sown and collected in 1976 and in 1984. Identical profiles were obtained by hybridization of the DNA digested with *Bam*HI, *Bcl*I, and *Hin*dIII to the 5′ *COX I* probe (Fig. 4). It was therefore concluded that the mtDNA variation is stable. Reciprocal crosses performed between plants with diverse hybridization patterns demonstrated exclusive maternal inheritance of mtDNA, eliminating the possibility of biparental contribution to the variation (data not shown).

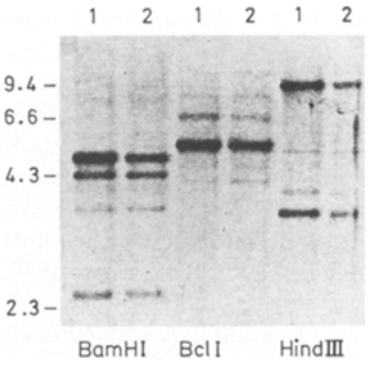

Fig. 4. Autoradiograph of Southern blots of total DNA from *Aegilops speltoides* 01-5 digested with *Bcl*I, *Bam*HI, and *Hin*dIII and hybridized with 5′ *COX I*. The seedlings from which the total DNA was prepared were grown in 1976 (*lane 1*) and 1984 (*lane 2*) from seeds of the same plant

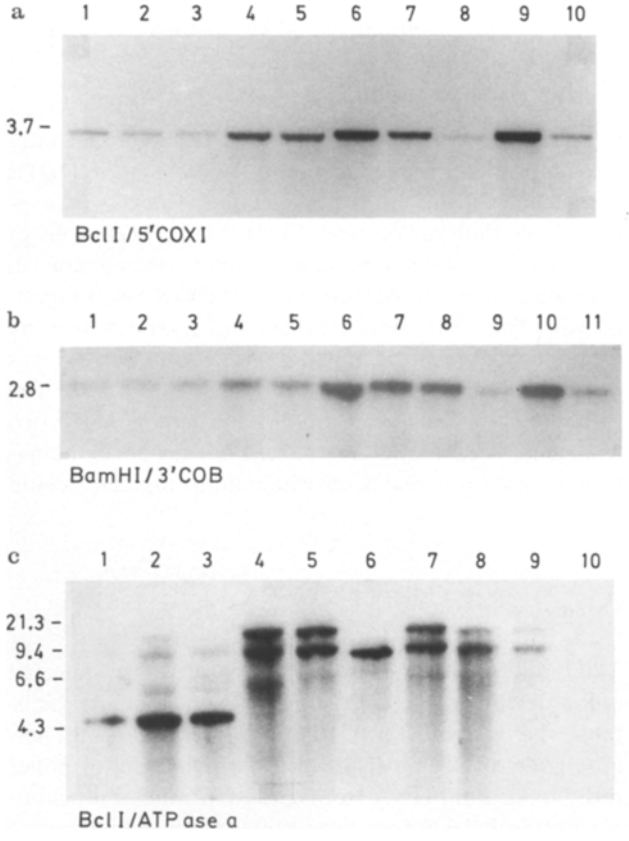

Fig. 5. **a** Autoradiograph of Southern blots of total DNA from *Aegilops longissima* digested with *Bcl*I and hybridized with 5′ *COX I*. *Slot designations: 1*=03-2, *2*=04, *3*=04-4, *4*=07-1, *5*=08-1, *6*=10, *7*=11, *8*=12, *9*=14, *10*=15. **b** Autoradiograph of Southern blots of total DNA of *Ae. longissima* digested with *BamH*I and hybridized with 3′ *cob*. *Slot designations: 1*=03-2, *2*=*04*, *3*=04-4, *4*=07-1, *5*=08-1, *6*=09, *7*=10, *8*=11, *9*=12, *10*=14, *11*=15. **c** Autoradiograph of Southern blot of total DNA of *Ae. longissima* digested with *Bcl*I and hybridized with *atp α*. *Slot designations: 1*=01, *2*=01-4, *3*=02-1, *4*=02-3, *5*=02-7, *6*=02-36, *7*=03-2, *8*=04, *9*=05-1, *10*=07-1, *11*=09, *12*=10

Table 2. *Ae. longissima* DNA fragments hybridizing with mtDNA probes

Probe	Accession no.	Fragment size (kb)		
		*Bam*HI	*Bcl*I	*Hin*dIII
5′ *COX* III	All accessions	3.7	2.1, 0.9	4.5
5′ *COX I*	All accessions	5.4, 1.8	3.7	2.8
3′ *cob*	All accessions	2.8	1.0	0.9
atp 6	All accessions	1.4	4.9	2.8
atp 9	All accessions	2.5	6.2	8.0
atp α	01-4	–	6.1, 4.0, 2.4	–
	02-36	–	6.3, 5.3, 4.2	–
	05-1	–	5.3, 4.2	–
	02-7, 02-3, 02-1, 03-2, 04, 07-1, 09-10	–	5.3, 4.2	–

The four *Bam*HI fragments hybridizing to *atp α* (Table 1) indicate that two copies of the gene are present in the *Ae. speltoides* mitochondrial genome. Assuming a high degree of similarity between the coding sequences of *T. aestivum* and *Ae. speltoides*, the four bands detected on the *T. aestivum* Southern blot are assumed to be caused by the cutting of the two *atp α* copies by *Bam*HI (Bonhomme et al. 1989). From the single bands present on the Southern blot, it may be deduced that *atp 9* and *COX I* are present as single copies in the *Ae. speltoides* mitochondrial genome (Table 1).

Restriction fragment analysis of Ae. longissima DNA hybridized with wheat mtDNA coding sequences

Ae. longissima (S′S″) has also been suggested to be one of the donors of the B genome (Hirai and Tsunewaki 1981; Ogihara and Tsunewaki 1982; Kerby and Kuspira 1987; but see Graur et al. 1989). Of the 12 accessions used in this study, 9 were collected in Israel from the Upper Galillee to the Negev and from the Mediterranean coast to the Dead Sea (Fig. 1).

A very low degree of mtDNA variation was detected among the accessions (Table 2), with most of the enzyme-probe combinations exhibiting uniform hybridization patterns (Fig. 5). Digestion of the DNA with *Bcl*I and hybridization with the *atp α* probe distinguished several types of mtDNA sequences (Fig. 5c, Table 2). The mean number of substitutions among *Ae. longissima* individuals in our sample was 0.001 substitutions per nucleotide site, or about 1/25 of the value in *Ae. speltoides*.

Restriction fragment pattern of T. monococcum var. monococcum and T. monococcum var. boeoticum DNA hybridized with wheat mtDNA coding sequences

The domesticated diploid wheat *T. monococcum* var. *monococcum* (AA) is still cultivated in mountainous areas

Table 3. *T. monococcum* var. *monococcum* and var. *boeoticum* DNA fragments hybridizing with mtDNA probes

Probe	Fragment size (kb)		
	*Bam*HI	*Hin*dIII	*Eco*RII
5′ *COX I*	4.0, 2.0	9	6.5, 4.6
5′ *cob*	6.0, 3.0	4.0, 1.8	5.3, 1.9
5′ *COX* II	3.6, 3.4, 1.8	3.4	8.9
atp 6	6.5, 4.6, 3.7, 3.4, 2.9	4.8, 2.7, 1.2, 0.8	7.6, 4.1, 2.2, 0.8
atp 9	4.5, 3.7, 3.4, 2.9	9.0, 8.2, 2.7	7.9, 6.8, 5.1
atp α	4.7, 3.3, 2.3, 1.0	6.6, 5.9, 4.6, 2.9	4.4, 3.1, 2.0, 1.0

Table 4. *Ae. squarrosa* DNA fragments hybridizing with wheat or maize mtDNA probes. Numbers in parentheses denote faint bands

Probe	Fragment size (kb)			
	*Bam*HI	*Hin*dIII	*Eco*RI	*Sal*I
5′ *COX I*	3.7 (12.4)	5.6	11.8	–
5′ *cob*	5.9, 3.2	4.6, 4.3	1.8	15
5′ *COX II*	4.1, 3.7	14, 6.0	2.5, 1.0	16, 3.9
atp α	7.7, 2.4	11.2 (5.3, 4.3)	4.2	12 (18.4)
atp 6	2.9 (2.1, 3.4, 2.6)	1.4, 1.1	9.9	14
atp 9	2.8	8.6 (11, 3.5)	4.9, 2.9	–

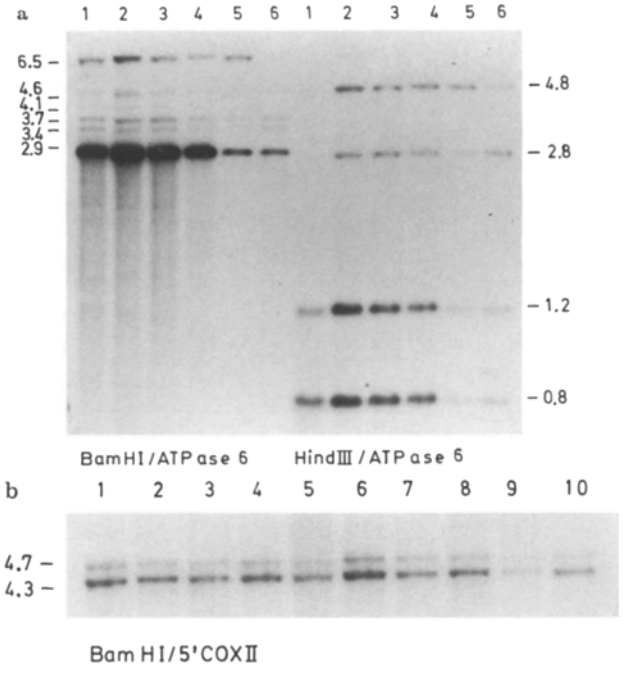

Fig. 6. a Autoradiograph of Southern blot of total DNA of *Triticum monococcum* var. *monococcum* and var. *boeoticum* digested with *Bam*HI and *Hin*dIII and hybridized with *atp α*. *Slot designations: T. monococcum* var. *boeoticum*, *1*=01-7, *2*=02-2, *3*=03, *4*=04; *T. monococcum* var. *monococcum*, *5*=01, *6*=04. **b** Autoradiograph of Southern blot of total DNA of *Aegilops squarrosa* digested with *Bam*HI and hybridized with 5′ *COX II*. *Slot designations: 1*=01, *2*=04, *3*=08, *4*=11, *5*=12, *6*=13, *7*=22, *8*=25, *9*=27, *10*=28

of southeast Europe and Turkey (Harlan and Zohary 1966). *T. monococcum* var. *boeticum* (AA) is widely distributed throughout western Mediterranean countries and is represented in this study by accessions from Lebanon, Turkey, Iran, and Azerbaijan. On the basis of cytogenetic studies, it is believed that *T. monococcum* is the source of the A genome of *T. aestivum*. Recent evidence points to *T. monococcum* var. *urartu* as the most likely donor of the A genome (Kerby and Kuspira 1987; Graur et al. 1989; Smith-Huerta et al. 1989).

T. monococcum seems to lack mtDNA variability altogether, and no polymorphism among the accessions could be detected by Southern blot hybridization (Table 3, Fig. 6a). In fact, the fragment sizes revealed on the Southern blots, hybridized with the coding sequences, can be used as characteristic identifiers of the *T. monococcum* cytoplasmic genome.

Restriction fragment pattern of Ae. squarrosa (ex T. tauschii) DNA hybridized with wheat mtDNA coding sequences

On the basis of meiotic chromosome behavior, chromosome morphology, and C-banding, *Ae. squarrosa* (DD) is accepted as the donor of the D genome (Riley and Chapman 1960; Gill and Kimber 1974). Eleven accessions of *Ae. squarrosa* originating mainly from various regions in Iran were analyzed. Although the total DNA was digested with five restriction enzymes and hybridized to six different probes, an identical hybridization pattern was obtained for all accessions (Table 4, Fig. 6b). The protein-coding sequences could be located on one or two fragments, depending on the enzyme-probe combination, except in several cases where faint fragments could be seen.

Discussion

The high degree of sequence conservation detected among mtDNA coding regions of higher plants facilitates the study of mtDNA variation by using heterologous mtDNA probes on Southern blots of either mtDNA or total DNA from species in which mitochondrial genes have not yet been isolated. The advantage of using total DNA is that minute amounts of plant material are sufficient for DNA extraction and large numbers of accessions can be screened. However, using heterologous mtDNA probes on Southern blots of total DNA cannot exclude the hybridization of the probes with nonmitochondrial DNA sequences. The uniform hybridization pattern obtained for *T. monococcum* and *Ae. squarrosa*, and the almost uniform pattern in *Ae. longissima*, support the assumption that the hybridization of the maize and wheat mtDNA coding sequences to

the Southern blots reveal *bona fide Aegilops* and *Triticum* mtDNA coding sequence (Tables 2–4). Therefore, those probes can be considered adequate tools for identifying the cytoplasms of these species.

Ae. speltoides is a species with a high degree of intraspecific variability (Table 1). A higher degree of variation among accessions is observed when noncoding sequences are used as probes. Thus, despite the fact that the 5′ regions of the apocytochrome b and cytochrome oxidase subunit I genes may contain regulatory functions, they are subjected to less stringent functional constraints than the protein-coding regions (Li and Graur 1991). The uniformity of the cpDNA pattern markedly contrasts with the variability observed in the mtDNA, indicating that the mtDNA in these species evolves at a higher rate than cpDNA. Since the classification of *Triticum-Aegilops* cytoplasms was performed by studying the restriction pattern of cpDNA, and by analysis of the cytoplasmic effects on alloplasmic lines or on single accessions of each species, this extensive intraspecific variation within the wild species could not be revealed (Tsunewaki 1980; Terachi and Tsunewaki 1986; Ogihara and Tsunewaki 1988). Nonetheless, some intraspecific variation at the cpDNA level has been demonstrated in several *Triticum* and *Aegilops* species (Bowman et al. 1983; Terachi et al. 1988).

The conclusions drawn by Ogihara and Tsunewaki (1988) from the similarity between chloroplast genome organization of *T. aestivum* (type 7) and *Ae. speltoides* (type 8) cannot be sustained by the studies on mtDNA organization of *Ae. speltoides* and *T. aestivum*. None of the *Ae. speltoides* accessions resembled the hybridization pattern of *T. aestivum* (Graur et al. 1989). However, the presence of the great variation can provide the basis for speculating that the cytoplasm of *T. aestivum* has been derived from an individual belonging to *Ae. speltoides*, which either does not exist in nature anymore or its type has not been included in the molecular studies performed to date.

Intraspecific variation in diploid species of wheat and *Aegilops* has also been studied by using protein electrophoretic characters (Asins and Carbonell 1986). The diploid species with the greatest intraspecific variability was *Ae. speltoides*, which is an outbreeding species, whereas *T. monococcum* and *Ae. squarrosa*, which are inbreeding species, were found to have very low intrapopulational genetic variability (Smith-Huerta et al. 1989). The low variability found in *T. monococcum* var. *boeoticum* and *Ae. squarrosa*, the intermediate variability in *Ae. longissima*, and the high variability in the *Ae. speltoides* nuclear genome (Asins and Carbonell 1986) correlate directly with the variation found in the mitochondrial genome of these species. Since the selective mechanisms involved in maintaining nuclear heterozygosity are supposed to be different from those maintaining variation in the maternally inherited mtDNA, it may be possible that both types of variation (nuclear and mitochondrial) are selectively neutral, and that they are affected only by the effective population size, which is higher in outbreeding species than in inbreeding ones (Nei and Graur 1984).

References

Asins MJ, Carbonell EA (1986) A comparative study on variability and phylogeny of *Triticum* species. Theor Appl Genet 72:551–558

Avise JC (1986) Mitochondrial DNA and the evolutionary genetics of higher animals. Philos Trans R Soc Lond Ser B 312:325–342

Bland MM, Matzinger DF, Levings CS III (1985) Comparison of the mitochondrial genome of *Nicotiana tabacum* with its progenitor species. Theor App Genet 69:535–541

Boer PH, McIntosh JE, Gray MW, Bonen L (1985) The wheat mitochondrial gene for apocytochrome b: absence of prokaryotic ribosome binding site. Nucleic Acids Res 13:2281–2292

Bonen L, Boer PM, Gray MW (1984) The wheat cytochrome oxidase subunit II has an intron insert and three amino acid changes relative to maize. EMBO J 3:2531–2536

Bonhomme S, Bind S, Bonen L (1989) Comparison of the wheat mitochondrial *atp 9* gene sequence with mitochondrial and chloroplast homologues from other plants. Plant Mol Biol 13:395–397

Bowman CM, Dyer TA (1986) The location and possible evolutionary significance of small disperse repeats in wheat DNA. Curr Genet 10:931–941

Bowman CM, Bonnard G, Dyer TA (1983) Chloroplast DNA variation between species of *Triticum* and *Aegilops*. Location of the variation on the chloroplast genome and its relevance to the inheritance and classification of the cytoplasm. Theor Appl Genet 65:247–262

Braun CJ, Levings CS III (1985) Nucleotide sequence of the F_1 ATPase gene from mitochondria. Plant Physiol 79:571–577

Breiman A (1987) Mitochondrial DNA diversity in the genera of *Triticum* and *Aegilops* revealed by Southern blot hybridization. Theor Appl Genet 73:563–570

Chowdhury MKU, Smith RL (1988) Mitochondrial DNA variation in pearl millet and related species. Theor Appl Genet 76:25–32

Chowdhury MKU, Schaeffer GW, Smith RL, Matthews BF (1988) Molecular analysis of organelle DNA of different subspecies of rice and the genomic stability of mtDNA in tissue-cultured cells of rice. Theor Appl Genet 76:533–539

Clegg MT, Brown AHD, Whitefeld PR (1984) Chloroplast DNA diversity in wild and cultivated barley: implications for genetic conservation. Genet Res 43:339–343

Dewey RE, Levings CS III, Timothy DM (1985a) Nucleotide sequence of ATPase subunit 6 gene of maize mitochondria. Plant Physiol 79:914–919

Dewey RE, Schuster AM, Levings CS III, Timothy DH (1985b) Nucleotide sequence of the ATPase proteolipid (subunit 9) gene of maize mitochondria. Proc Natl Acad Sci USA 82:1015–1019

Gill BS, Kimber G (1974) Giemsa C-banding and the evolution of wheat. Proc Natl Acad Sci USA 71:4086–4090

Graur D, Bogher M, Breiman A (1989) Restriction endonuclease profiles of mitochondrial DNA and the origin of the B genome of bread wheat *Triticum aestivum*. Heredity 62:335–342

Harlan JR, Zohary D (1966) Distribution of wild wheats and barley. Science 153:1074–1080

Hirai A, Tsunewaki K (1981) Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. VI. Fraction 1 protein of 49 cytoplasms. Genetics 99:487–498

Holwerda BC; Jana S, Crosby WL (1986) Chloroplast and mitochondrial DNA variation in *Hordeum vulgare* and *Hordeum spontaneum*. Genetics 114:1271–1291

Jaaska U (1978) NADP-dependent aromatic alcohol dehydrogenase in polyploid wheat and their diploid relatives. On the origin and phylogeny of polyploid wheats. Theor Appl Genet 53:209–217

Kemble RJ, Gunn RE, Flavell RB (1983) Mitochondrial DNA variation in races of maize indigenous to Mexico. Theor Appl Genet 65:129–144

Kerby R, Kuspira J (1987) The phylogeny of the polyploid wheat *Triticum aestivum* (bread wheat) and *Triticum turgidum* (macaroni wheat). Genome 29:722–737

Levings CS III, Pring DR (1977) Diversity of mitochondrial genomes among normal cytoplasms of maize. J Hered 68:350–354

Li W-H, Graur D (1991) Fundamentals of Molecular Evolution. Sinauer Associates, Sunderland, MA 284 pp

McLean PE, Hanson MR (1986) Mitochondrial DNA sequence divergence among *Lycopersicon* and related *Solanum* species. Genetics 112:649–667

McNay JW, Pring DR, Lonsdale DM (1983) Polymorphism of mitochondrial DNA 5′ region among normal cytoplasm of maize. Plant Mol Biol 12:177–189

Nei M, Graur D (1984) Extent of protein polymorphism and the neutral mutation theory. Evol Biol 17:73–18

Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Ogihara Y, Tsunewaki K (1982) Molecuilar basis fo the genetic diversity in the cytoplasm in *Triticum* and *Aegilops*. I. Diversity of the chloroplast genome and its lineage revealed by the restriction pattern of ctDNA. Jpn J Genet 57:371–396

Ogihara Y, Tsunewaki K (1988) Diversity and evolution of chloroplast DNA in *Triticum* and *Aegilops* as revealed by restriction fragment analysis. Theor Appl Genet 76:321–322

Palmer JD (1985) Comparative organization of chloroplast genomes. Annu Rev Genet 19:325–354

Palmer JD (1988) Intraspecific variation and multicircularity in *Brassica* mitochondrial DNAs. Genetics 118:341–351

Palmer JD, Zamir D (1982) Chloroplast DNA evolution and phylogenic relationships in *Lycopersicon*. Proc Natl Acad Sci USA 79:5006–5010

Palmer JD, Hebron LA (1988) Plant mitochondrial DNA evolves rapidly in structure but slowly in sequence. J Mol Evol 28:87–97

Pring DR, Levins CS III (1978) Heterogeneity of maize cytoplasmic genomes among male-sterile cytoplasms. Genetics 89:121–136

Pring DR, Conde MF, Levings CS III (1980) DNA heterogeneity within the C group of maize male sterile cytoplasms. Crop Sci 20:159–162

Pring DR, Lonsdale DM, Gracen VE, Smith AG (1987) Mitochondrial DNA duplication/deletion events and polymorphism of the C group of male-sterile cytoplasms. Theor Appl Genet 73:646–653

Rees H, Walters MR (1965) Nuclear DNA and the evolution of wheat. Heredity 20:73–82

Riley R, Chapman V (1960) The D genome of hexaploid wheat. Wheat Int Serv 2:18–19

Riley R, Unerou J, Chapman V (1958) Evidence on the origin of the B genome of wheat. J Hered 49:91–98

Rines HW, Gengenbach BG, Boylan KL, Storey KK (1988) Mitochondrial DNA diversity in oat cultivars and species. Crop Sci 28:171–176

Sederoff RR (1987) Molecular mechanism of mitochondrial genome evolution in higher plants. Am Nat 130:530–545

Sederoff RR, Levings CS III, Timothy DM, Hu WWL (1981) Evolution of DNA sequences organization in mitochondrial genomes of *Zea*. Proc Natl Acad Sci USA 38:5953–5957

Sisco PH, Gracen VE, Everett HL, Earle ED, Pring DR, McNay JW, Levings CS III (1985) Fertility restoration and mitochondrial nucleic acids distinguish at least five subgroups among cms-S cytoplasms of maize (*Zea mays* L.). Theor Appl Genet 71:5–15

Smith-Huerta NL, Huerta AJ, Banhart D, Waines JS (1989) Genetic diversity in wild diploid wheats *Triticum monococcum* var. *boeoticum* and *T. urartu* (Poaceae). Theor Appl Genet 78:260–264

Terachi T, Tsunewaki K (1986) The molecular basis of genetic diversity among *Aegilops* species having identical chloroplast genomes. Theor Appl Genet 73:175–181

Terachi T, Ogihara Y, Tsunewaki K (1988) The *rbcL* genes in wheat and several *Aegilops* species with divergent chloroplast genomes. In: Miller TE, Koebner RMD (eds) Proc. 7th Int Wheat Genetics Symp. Cambridge University Press, Cambridge, pp 789–795

Tsunewaki K (ed) (1980) Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. Japan Society for the Promotion of Science, Tokyo, 290 pp

Tsunewaki K, Ogihara Y (1983) The molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops*. II. On the origin of polyploid wheat cytoplasm as suggested by chloroplast DNA restriction fragment patterns. Genetics 104:155–171

Wilson AC, Cann RL, Carr SM, George M, Gyllenstein JB, Helm-Bychowski KM, Higuchi RG, Palumbi SR, Prager EM, Sage RD, Stoneking M (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. Biol J Linn Soc 26:375–400