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Sequence of the 18S-5S ribosomal gene region and the cytochrome oxidase II gene from mtDNA of *Zea diploperennis*

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Summary. The coding and flanking sequences of the 18S-5S ribosomal RNA genes and the cytochrome oxidase subunit II gene of *Zea diploperennis* mitochondrial DNA have been determined and compared to the corresponding sequences of normal maize (*Zea mays* L.) Both length and substitution mutations are found in the coding region of the 18S rRNA gene, whereas only one substitution mutation is found in the coding region of cytochrome oxidase II. Sequence divergence between maize and *Zea diploperennis* is about one-tenth of that between wheat and maize. The rate of nucleotide divergence by base substitution is less for plant mitochrondrial genes than for comparable genes in animal mitochondria.

Key words: mtDNA – DNA sequences – Ribosomal genes – Cytochrome oxidase II – *Zea diploperennis*

Introduction

Zea diploperennis Iltis, Doebley and Guzman is a diploid perennial teosinte first collected in 1977. Its discovery evoked great interest because of its potential importance in the evolution of modern maize (*Zea mays* L.) (Iltis et al. 1979). *Z. diploperennis* has been studied to assess its relationship to modern maize by morphology, cytology and isozymic variation (Doebley and Iltis 1980; Doebley 1983; Pasupuleti and Galinat 1982; Mastenbroek et al. 1981; Doebley et al. 1984; Smith et al. 1984). These data suggest that *Z. diploperennis* is a distinct species of *Zea* which warrants placement in a separate section of the genus (Iltis and Doebley 1980).

Restriction endonuclease digestion patterns of mitochondrial DNA (mtDNA) readily distinguish teosintes and modern maize (Timothy et al. 1979). These studies support the classification of the genus based on properties of the nuclear genomes. Restriction digest patterns of *Z. diploperennis* mitochondrial DNA (mtDNA) show more similarity to the mtDNA of Guatemala teosinte (*Zea luxurians*) and tetraploid perennial teosinte (*Zea perennis*) than to the other teosintes and maize. The mtDNA of several annual teosintes closely resembles that of modern maize (Timothy et al. 1979).

To investigate the divergence of mtDNA between maize and *Z. diploperennis* we have compared the nucleotide sequences of three genes and their flanking sequences in *Z. diploperennis* with the same genes from modern maize. Sequence comparisons have provided information on the extent and mechanisms of divergence of mtDNA within the genus *Zea*.

Materials and methods

Isolation and characterization of mtDNA

MtDNA was purified from etiolated seedlings of *Z. diploperennis* (progeny number 540 and 555, Timothy et al. 1983) and normal maize (B73 × Mo17) as previously described (Pring and Levings 1978). DNA preparations were digested with the restriction endonuclease *Bam*HI or *Eco*RI and fractionated by agarose gel electrophoresis (Spruill et al. 1980).

Cloning of mtDNA

Total mtDNA was digested with the restriction endonuclease *Bam*HI or *Eco*RI and ligated into *Escherichia coli* plasmid

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pUC9. The resulting plasmid molecules were used to transform *E. coli* strain JM83. Ampicillin resistant, lac$^-$ colonies were selected, replicated and fixed onto nitrocellulose filters (Maniatis et al. 1982). Filters were screened by molecular hybridization with 32-P labelled probes. Positive clones were detected by autoradiography. The *Bam*HI library was screened with clone ZmmtN542-2 (Chao et al. 1983), which contains the 18S and 5S rRNA genes of maize mitochondria. The *Eco*RI library was screened with the 2.4 kb fragment containing the cytochrome oxidase subunit II gene, *mox*I (Fox and Leaver 1981). The teosinte clones for the 18S-5S ribosomal gene complex and cytochrome oxidase subunit II gene obtained were designated Zdmt6 and ZdCOII respectively.

Restriction mapping, subcloning, and sequencing

A restriction map of Zdmt6 was generated using six restriction endonucleases. Subclones of Zdmt6 and ZdCOII were prepared by digesting with restriction enzymes and cloning into the vectors M13mp9, mp10 and mp11 (Messing et al. 1981). DNA sequences were determined by the dideoxynucleotide chain-termination method of Sanger et al. (1977). Universal primers (15 and 17 bp, PL Biochemicals) were used for sequencing of both Zdmt6 and ZdCOII subclones. In addition, specific oligonucleotides, prepared with the Applied Biosystems 380A DNA synthesizer, were used to prime synthesis on large (2.2 and 3.3 kb) subclones of Zdmt6. Synthesized primers were detritylated while attached to the solid support, deprotected for 8–12 h in 28% ammonium hydroxide, evaporated to dryness under vacuum and resuspended in 50 µl water. Then 25 µl of 7.5 *M* ammonium acetate and 150 µl cold 95% ethanol were added and the primer precipitated for 45 min at −20 °C. The precipitate was collected by centrifugation for 10 min at 12,800 *g*, the supernatant removed, and remaining ethanol evaporated. The pellet was resuspended in 100 µl water and purified on a Sephadex (G25 fine) spin column (Maniatis et al. 1982) equilibrated with distilled water. Primers were used for sequencing without further purification.

Results

Estimation of relative genetic distance from restriction patterns

The *Bam*HI digestion pattern of *Z. diploperennis* mtDNA is distinct from that of normal maize (Timothy et al. 1983). About 45 different bands are distinguished. Two different accessions of *Z. diploperennis* have been studied. Seed lot numbers 540 and 530 are from the same accession but differ by one band. Both of these mtDNAs differ appreciably from two seed lots (525 and 509) of a second accession. Interestingly, both accessions are from the same original collection (Guzman 1978).

Restriction fragment polymorphisms may estimate the number of nucleotide substitutions between mtDNAs. The number of substitutions is roughly calculated from the number of bands which differ between two digestion patterns. This estimate is imprecise when distinct restriction fragments are overlooked because they have similar electrophoretic mobilities and when mutations are not randomly-distributed single-base substitutions. Nevertheless, general estimates of the amount of variation in mtDNA may be obtained from stained gels. The relative number of different fragments can be used to approximate genetic distance (Fig. 1) by using the average linkage cluster analysis (Sneath and Sokal 1973).

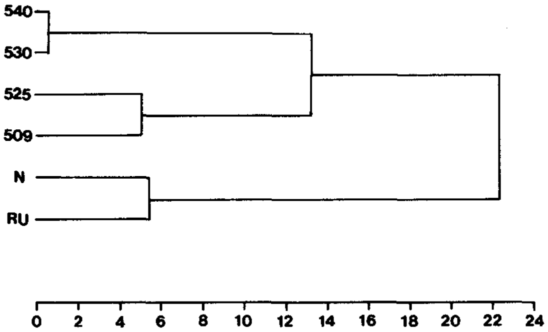

Fig. 1. Average linkage cluster analysis based on restriction fragment data from *Bam*HI and *Eco*RI digestion patterns (Timothy et al. 1983). *Scale* indicates average number of differences between individual accessions or groups

Mapping the 18S-5S rRNA region in Z. diploperennis

In maize, the 18S and 5S rRNA genes are separated by only 111 bp and are found on a 16 kb *Bam*HI fragment (Chao et al. 1983; Chao et al. 1984). In *Z. diploperennis*, *Z. luxurians* or *Z. perennis*, the 18S-5S region is contained on a 6 kb *Bam*HI fragment. This fragment was detected and isolated from a pUC9 *Bam*HI library of *Z. diploperennis* mtDNA using a maize fragment as a probe. Restriction mapping of four positive clones showed them to contain identical 6 kb fragments (Fig. 2). One of these teosinte clones, Zdmt6, was selected for further characterization.

Isolation and restriction mapping of the region containing cytochrome oxidase II from Z. diploperennis

Similarly, a clone containing the COII gene from *Z. diploperennis* was isolated from an *Eco*RI library using a COII gene from maize (*mox*I) as a probe (Fox and Leaver 1981). The teosinte clone, designated ZdCOII, was identical to the maize clone in size (2.4 kb) and in the location of *Eco*RI, *Hind*III and *Bam*HI restriction sites (data not shown).

Nucleotide sequence analysis of the 18S-5S region

M13 subclones of the Zdmt6 clones were sequenced by the dideoxynucleotide chain-termination technique. Thus 2,461 bases of the 6 kb clone were determined; this included 98 bp of the 5′ flanking region of the 18S gene, the entire 18S coding region, the 111 bp intergenic

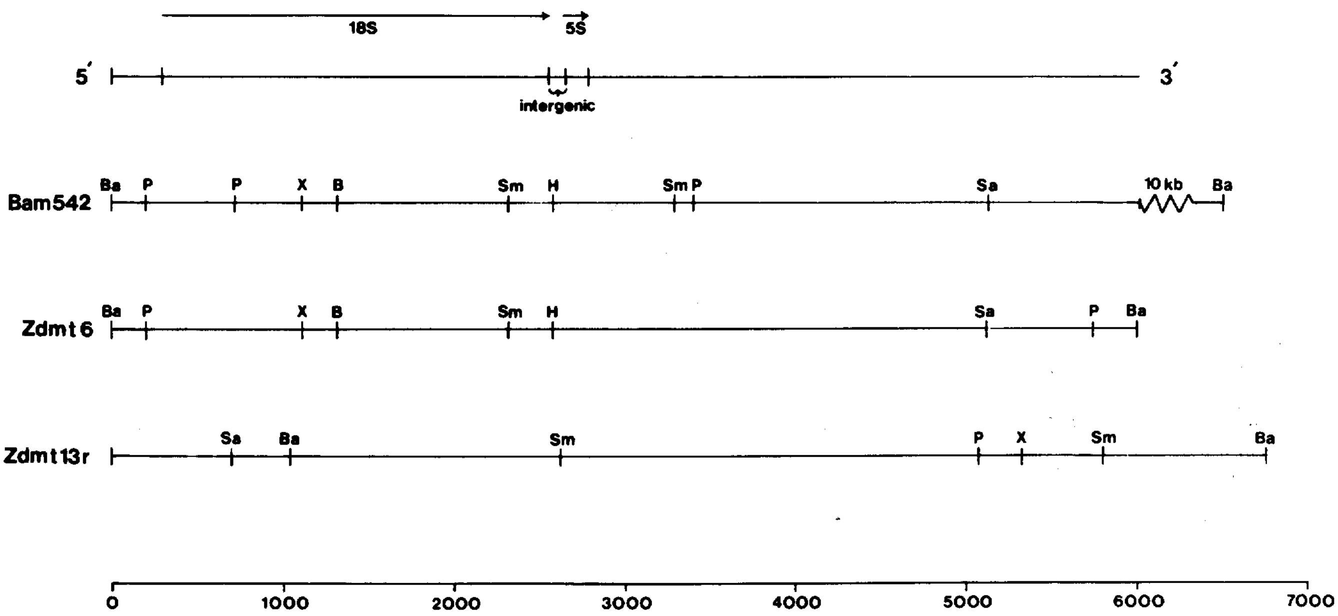

Fig. 2. Restriction maps of the 18S-5S rRNA gene region from the mitochondria of *Zea mays* (Bam542) and *Z. diploperennis* (Zdmt6). Restriction sites are designated as follows: *B, Bgl*II; *Ba, Bam*HI; *H, Hind*III; *P, Pst*I; *Sa, Sal*I; *Sm, Sma*I; *X, Xho*I. *Scale* given in base pairs

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Z. mays          5' - GGGGA TTTCT TTCAG CCAAC ATCCT GCCTG CC--- ----- ----T TAGTG GCCCC
Z. diploperennis               A                  A         AGC CTGCC TGCC

Z. mays               TCTCT GATAA GG--- ---AA ACGAA AAAAA AATGA CAATC TGGTC CTTTC AACTG - 3'
Z. diploperennis                    TTT TCA      C                            GA    A C
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Fig. 3. Sequence alignment of the 3′ flanking region of the 18S-5s rRNA genes of *Z. mays* and *Z. diploperennis. Dashes* indicate deleted nucleotides. Only nucleotides differing in the teosinte sequence are shown

region, the 5S gene, and 158 bp extending from the 3′ end of the 5S gene. The 18S gene from *Z. diploperennis* differs from maize by an insertion of a T dimer (position 138 of maize) which eliminates a *Pst*I site, a transition, G to A, at base 141 and a length mutation (four bases) at position 874 of the maize sequence. In the 3′ flanking region, several mutations, insertions, deletions and substitutions are apparent when compared to the maize sequence (Fig. 3). Teosinte sequences 5′ to the gene, in the intergenic region, and the 5S gene itself were identical to those of maize.

Sequence analysis of cytochrome oxidase subunit II

Here 1,802 bp of the ZdCOII clones were sequenced from M13 subclones. The sequenced region included 110 bp adjacent to the 5′ end of the COII gene, the first exon of the gene, a 794 bp intron, the second exon, and 115 bp 3′ to the structural gene. Differences between the maize and *Z. diploperennis* sequence occur in the 5′ flanking region and the second exon. The teosinte 5′ flank contains a 6 bp deletion relative to maize at position – 3 (Fig. 4) assuming the second ATG start codon to be functional (Dawson et al. 1984). In addition, there are 12 base substitutions with a ratio of transitions to transversions of 4/8. In the second exon of the teosinte clone, a single base change occurs at position 1,463. This change, a T to G transversion, is in the third position of a codon for leucine and does not alter the amino acid.

Discussion

Nucleotide changes between the 18S rDNA sequences of maize and *Z. diploperennis* occur in two regions characterized by rapid sequence evolution in many small subunit ribosomal genes (Woese et al. 1983; Chao et al. 1984). The TT insertion at maize position 138 and the G to A transition at position 141 are located in adjacent helical stem and loop segments respectively (Fig. 5) of a proposed secondary structure (Chao et al. 1984). The length of the stem remains constant; only the loop size is altered. This area is part of an 100 bp insertion found only in plant mtDNA. The functional significance of this insert in translation is unknown. The insert is itself located in a "universally variable" region of the small subunit RNA.

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Z. mays          5' - CACCC AATTC TGGAT CTGAA TATTG GTGAG TACTA TGTCT CATTC ACAAA TCTAT
Z. diploperennis               AA  TCC           A  ACA C   T    T

Z. mays               CCTTG TCTAT GCTAC TCACT CTCGG TTTGG TCCTA CTTCT GGTGC TGCCA - 3'
Z. diploperennis                                                        --- ---
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Fig. 4. Sequence alignment of 5′ flanking region of cytochrome oxidase subunit II of *Z. mays* and *Z. diploperennis*. Only nucleotides differing from those in maize are shown in the teosinte sequence. *Dashes* indicate nucleotides absent in the *Z. diploperennis* sequence

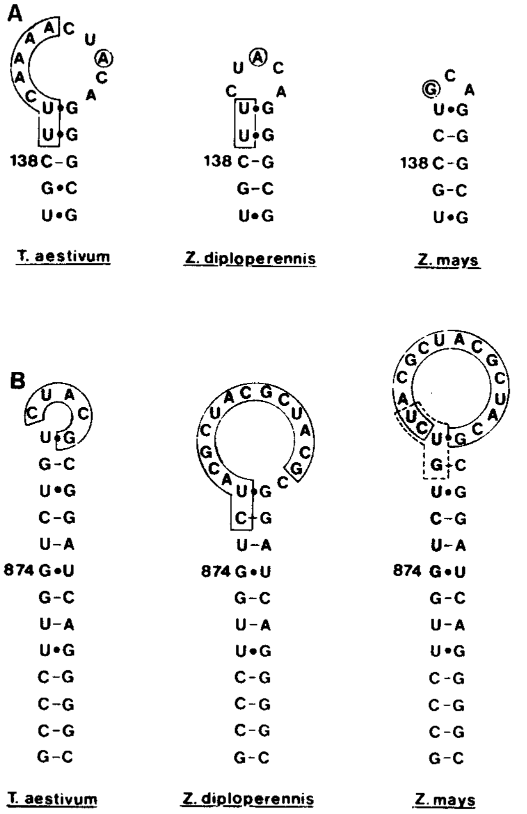

Fig. 5 A, B. Proposed secondary structures for the regions of the small subunit rRNA genes differing between *Z. diploperennis* mtDNA are compared with the equivalent regions in wheat. **A** nucleotides deleted in maize relative to wheat and *Z. diploperennis* are *boxed;* the A to G transition is *circled.* Number designation (*C-138*) is that of maize. **B** The 5 bp direct repeat common to all three sequences is *boxed.* The additional overlapping repeat unit GUCU is shown in maize by a *dotted box.* GUCU, starting at the base marked 874 (using the maize numbering system) is repeated in maize and wheat but not in *Z. diploperennis*

The length mutation at the maize position 874 alters the size of a loop (Fig. 5). In this region, a five bp repeat (CUACG) appears once in the wheat sequence (Spencer et al. 1984) and three times in the sequences of maize and *Z. diploperennis* (Chao et al. 1984). An additional repeat of four base pairs (GUCU) occurs in maize and wheat but not in *Z. diploperennis* (Fig. 5). In this region, the sequence varies between prokaryotes and eukaryotes, but compensating base changes have conserved the secondary structure.

Short direct tandem repeats have been documented in other systems, including the *lac*I gene of *E. coli.* (Farabaugh et al. 1978), the 16S-23S spacer of the chloroplast ribosomal genes (Takaiwa and Sugiura 1982), and the non-coding regions associated with the *rbc*L gene of maize and barley chloroplasts (Zurawski et al. 1984). During DNA replication, misalignment of sequences in either the template or the strand undergoing elongation can result in deletional or duplicational events, respectively. This can come about through mispairing, the formation of stem-loop structures or slippage during replication. Once a repeated sequence is established, the probability of subsequent length mutations increase due to misalignment of the repeats (Drake et al. 1983; Ripley and Glickman 1983).

An alternative mechanism thought to generate short direct repeats is the excision of transposable elements (Schwartz-Sommer et al. 1985; Saedler and Nevers 1985). Short repeats may be 'footprint' sequences that indicate visitation by a mobile element.

The 18S 5′ flanks of *Z. mays* and *Z. diploperennis* are notable for their sequence conservation. In addition to the sequence identity determined for the 98 bp immediately 5′ to the mature end of the 18S rRNA, sequence data for the region 400 bp further 5′ indicate continued conservation around the common *Pst*I site at maize position −490 (B. Gwynn, unpublished results; Dale et al. 1985). In wheat (Falconet et al. 1984), evidence exists for multiple copies of the ribosomal genes. Four specific sequence arrangements are found flanking the conserved 18S-5S gene region, with two different sequences detected 5′ to the 18S gene. Reciprocal crossing over within the 18S-5S complex is hypothesized to produce the four different *Sal*I restriction fragments observed, all of which include the ribosomal genes (Falconet et al. 1984). Depending upon which 5′ sequence is present, an entire initiator tRNA or only a 23 nucleotide segment from the 3′ end lies one base pair from the start of the 18S gene (Gray and Spencer 1983; Falconet et al. 1984). These variations in sequence 5′ to the 18S-5S complex suggest the possibility of different means of transcriptional initiation and subsequent processing for the maize and wheat small subunit RNA's, although sequences 5′ to the initiator tRNA in wheat may prove similar to those of maize.

Table 1. The number of base substitution mutations and length mutations between maize (*Zea mays*), *Zea diploperennis*, wheat (*Triticum aestivum*) and soybean (*Glycine max*) are compared for mitochondrial 18S-5S gene sequences. Soybean (*Glycine max*) is compared for mitochondrial 18S-5S gene sequences. The number of bases involved in deletions or insertions are presented as length mutations. The estimated minimum number of events to account for the length mutations is determined by counting adjacent bases deleted or inserted as one event. Where the length of two sequences being compared is different, the length of the maize sequence was used to calculate the percentage of change. Data presented in the table comes from Grabau 1985 for soybean, Chao et al. 1983, 1984 for maize and Spencer et al. 1981 and Spencer et al. 1984 for the wheat sequences. *Dashes* indicate that the sequence information was not available for comparison

	Zea diploperennis	Wheat	Soybean
Coding 18S			
Base substitutions	1	25	118
Length mutation (# bases changed)	6	48	165
Minimum events for length mutation	2	7	11
Length of sequence compared	1,966	1,955	1,990
% Change due to base substitutions	0.05	1.2	6.0
% Change due to length mutations	0.30	2.4	8.4
5′ Flanking			
Bases changed	0	–	–
Length compared	98	–	–
% Change	0	–	–
Intergenic 18S-5S			
Bases changed	0	–	–
Length compared	111	–	–
% change	0	–	–
5S Sequence			
Base substitutions	0	3	–
Length mutation (# bases changed)	0	4	–
Minimum events for length mutation	0	1	–
Length of sequence compared	126	122	–
% Change due to base substitutions	0	2.4	–
% Change due to length mutation	0	3.2	–
3′ Flanking			
Base substitutions	7	–	–
Length mutation (# bases changed)	18	–	–
Minimum events for length mutation	2	–	–
Length of sequence compared	94	–	–
% Change due to base substitutions	7.4	–	–
% Change due to length mutation	19	–	–

There is a preference for transversions (V) over transitions (I) in the cytochrome oxidase subunit II regions (9 V/4 I). A bias of 2 V/1 I would be expected on a purely random basis, but many studies have found a transitional bias. In animal mitochondria, substitution events are strongly biased in favour of transitions (Brown et al. 1982; Aquadro and Greenberg 1983), although a recent study (Cann et al. 1984) has identified a bias toward transversions in human mtDNA. The authors suggest this may result from the nature of the restriction sites in which the transversional bias is detected. A small transitional bias is reported for chloroplast sequences, about 1.9 I/V for coding and 1.5 I/V for non-coding regions (Clegg et al. 1984).

In this study, the majority of sequence changes are confined to two areas: the 5′ flank of the COII gene and the 3′ flank of the 18S-5S region. The 5′ flank of the cytochrome oxidase subunit II gene contains 93% of the variation in this sequence (Fig. 4). Wheat (Bonen et al. 1984), rice (Kao et al. 1984) and *Oenothera* (Hiesel and Brennicke 1983) sequences, while displaying less than 25% similarity to maize, show sequence similarity with one another. Rice and wheat sequences are similar (76% for the first 110 bp). The 5′ sequence from *Oenothera* contains interrupted blocks of sequence similar to rice an wheat, suggesting that substitution and insertion/deletion events are responsible for the divergence between the grasses and *Oenothera*. The lack of similarity between the *Zea* species and the other grasses suggests a rearrangement has occurred after the divergence of the line leading to *Zea* and before the divergence of *Z. mays* and *Z. diploperennis*.

The second region displaying a high degree of variability is the 3′ sequence flanking the 18S-5S rRNA

Table 2. The number of base substitutions and length mutations are compared between maize (*Zea mays*) *Zea diploperennis,* wheat (*Triticum aestivum*), rice (*Oryza sativa*), primrose (*Oenothera berteriana*) and pea (*Pisum sativum*) for the mitochondrial gene coding for cytochrome oxidase II. The methods of calculation of percentage change follow those described in Table 1. Data shown in this table comes from Fox and Leaver 1981 for maize, Bonen et al 1984 for wheat, Hiesel and Brennicke 1983 for primrose, Kao et al. 1984 for rice and Moon et al. 1985 for pea. *Dashes* indicate that the sequence information was not available for comparison. *Asterisks* denote regions where the sequence similarity was very low, probably due to a rearrangement in the lineage leading to maize. In this region, rice wheat and primrose are similar to each other but show no similarity to maize

Coding					
Base substitutions	1	9	4	86	54
Length mutation (# of bases changed)	0	0	0	6	21
Minimum events for length mutation	0	0	0	1	7
Length of coding region	783	783	783	774	777
% Change due to base substitutions	0.13	1.1	0.51	11	6.9
% Change due to length mutation	0	0	0	0.77	0.27
Intron					
Base substitutions	0	7	7	–	–
Length of intron	794	1,216	1,265	0	0
Length mutation (# of bases changed)	0	422	473	794	794
Minimum events for length mutations	0	1	3	1	1
% Change due to base substitution	0	0.88	0.88	–	–
% Change due to length mutation	0	53	60	100	100
5′ Flanking					
Base substitutions	12	**	**	**	**
Length mutation (# of bases changed)	6	**	**	**	**
Minimum events for length mutation	1	**	**	**	**
Length of sequence compared	110	**	**	**	**
% Change due to substitutions	11	**	**	**	**
% Change due to length mutations	5.4	**	**	**	**
3′ Flanking					
Base substitutions	0	1	0	**	20
Length mutations	0	17	21	**	29
Minimum events for length mutation	0	1	3	**	5
Length of sequence being compared	115	115	115	**	115
% Change due to substitutions	0	0.87	0	**	17
% Change due to length mutations	0	15	18	**	25

gene complex. For this sequence, comparisons are available only for *Z. mays* and *Z. diploperennis* (Fig. 3). It was found that 80% of the divergence in the entire 18S-5S region occurs in this area and again results from both substitutions and length mutations. Substitutions occur twice as often as insertion/deletion events (8/4). Sequence beyond the 9 bp immediately 3′ to the 5S gene contains no further similarity, presumably due to a rearrangement.

The substitution mutation rate in plant mtDNA (Table 1; Table 2) is considerably less than that of animal mtDNA. Assuming a date for the divergence of the major groups of grasses of about 60 million years ago as suggested by Stebbins (Chao et al. 1984; Zurawski et al. 1984), the substitution rate for coding regions of the ribosomal genes and the cytochrome oxidase II gene is 0.019% per million years. The calculation for the cytochrome oxidase coding region alone is 0.014% per million years. Zurawski et al. (1984) estimate the substitution rate at 0.12% divergence per million years for the *rbc*L genes of barley and maize chloroplasts. In contrast, the rate of silent substitutions alone in the coding sequences of two genes studied from primate mitochondria has been estimated to be as high as 10% per million years (Brown et al. 1982).

Very few genes have been sequenced from plants where good estimates of times of divergence are available. The reliability of estimated rates of divergence will be somewhat uncertain until more data becomes available.

The mechanisms of divergence also vary between the genomes of plant mtDNA, animal mtDNA and ctDNA. Rearrangements are very frequent in plant mtDNA (Schardle et al. 1984), common in ctDNA (Palmer 1983; Palmer et al. 1985) and rare in animal mtDNA, where gene organization is highly conserved (Sederoff 1984). For all three types of genomes, substitution events are more frequent in the coding regions of structural genes than are length mutations (this paper; Cann and Wilson 1983; Zurawski et al. 1984). The reasons for the different modes of DNA evolution and rates of mutation in organelle genomes remain a mystery.

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References

Aquadro CF, Greenberg BD (1983) Human mitochondrial DNA variation and evolution: analysis of nucleotide sequences from seven individuals. Genetics 103:287–312

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

Brown WM, Prager EM, Wang A, Wilson AC (1982) Mitochondrial DNA sequences of primates: tempo and mode of evolution. J Mol Evol 18:225–239

Cann RL, Wilson AC (1983) Length mutations in human mitochondrial DNA. Genetics 104:699–711

Cann RL, Brown WM, Wilson AC (1984) Polymorphic sites and the mechanism of evolution in human mitochondrial DNA. Genetics 106:479–499

Chao S, Sederoff RR, Levings CS III (1983) Partial sequence analysis of the 5S and 18S rRNA gene region of the maize mitochondrial genome. Plant Physiol 71:190–193

Chao S, Sederoff RR, Levings CS III (1984) Nucleotide sequence and evolution of the 18S ribosomal RNA gene in maize mitochondria. Nucleic Acids Res 12:6629–6644

Clegg MT, Rawson JRY, Thomas K (1984) Chloroplast DNA variation in pearl millet and related species. Genetics 106:449–461

Dale RMK, McClure BA, Houchins JP (1985) A rapid single stranded strategy for producing a sequential series of overlapping clones for use in DNA sequencing: application to sequencing the corn mitochondrial 18S rDNA. Plasmid 13:31–40

Dawson AJ, Jones VP, Leaver CJ (1984) The apocytochrome b gene in maize mitochondria does not contain introns and is preceded by a potential ribosome binding site. EMBO J 3:2107–2113

Doebley JF (1983) The maize and teosinte male inflorescence: a numerical taxonomic study. Ann Mo Bot Gard 70:21–70

Doebley JF, Iltis HH (1980) Taxonomy of *Zea* (Gramineae). I. A subgeneric classification with key to taxa. Am J Bot 67:982–993

Doebley JF, Goodman MM, Stuber CW (1984) Isoenzymatic variation in *Zea* (Gramineae). Syst Bot 9:203–218

Drake JW, Glickman BW, Ripley LS (1983) Updating the theory of mutation. Am Sci 71:621–630

Falconet D, LeJeune B, Quetier F, Gray MW (1984) Evidence for homologous recombination between repeated sequences containing 18S and 5S ribosomal RNA genes in wheat mitochondria DNA. EMBO J 3:297–302

Farabaugh PJ, Schmeissner U, Hoffer M, Miller JH (1978) Genetic studies of the *lac* repressor. VII. On the molecular nature of spontaneous hotspots in the *lac*I gene of *Escherichia coli.* J Mol Biol 126:847–863

Fox TD, Leaver CJ (1981) The *Zea mays* mitochondrial gene coding cytochrome oxidase subunit II has in intervening sequence and does not contain TGA codons. Cell 26:315–323

Grabau EA (1985) Nucleotide sequence of the soybean mitochondrial 18S rRNA gene: evidence for a slow rate of divergence in the plant mitochondrial genome. Plant Mol Biol 5:119–124

Gray MW, Spencer DF (1983) Wheat mitochondria DNA encodes a eubacteria-like initiator methionine transfer RNA. FEBS 161:323–328

Guzman RM (1978) Una nueva localidad para el teosinte *Zea perennis* y primer reporte de *Zea mexicana* para Jalisco. Bol Inf Inst Bot, Univ Guadalajara 6:9–10

Hiesel R, Brennicke A (1983) Cytochrome oxidase subunit II gene in mitochondria of *Oenothera* has no intron. EMBO J 2:2173–2178

Iltis HH, Doebley JF (1980) Taxonomy of *Zea* (Gramineae). II. Subspecific categories in the *Zea mays* complex and a generic synopsis. Am J Bot 67:994–1004

Iltis HH, Doebley JF, Guzman R, Pazy B (1979) *Zea diploperennis* (Gramineae): a new teosinte from Mexico. Science 203:186–188

Kao T, Moon E, Wu R (1984) Cytochrome oxidase subunit II gene of rice has an insertion sequence within the intron. Nucleic Acids Res 12:7305–7315

Lonsdale DM (1984) A review of the structure and organization of the mitochondrial genome of higher plants. Plant Mol Biol 3:201–206

Maniatis T, Fritsch EF, Sambrook JL (1982) Molecular Cloning. Cold Spring Harbor Laboratory, New York

Mastenbroek I, Cohen CE, deWet JMH (1981) Seed protein and seedling isozyme patterns of *Zea mays* and its closest relatives. Biochem Syst Ecol 9:179–183

Messing J, Crea R, Seeburg PH (1981) A system for shotgun DNA sequencing. Nucleic Acids Res 9:309–321

Moon E, Kao T, Wu R (1985) Pea cytochrome oxidase subunit II gene has no intron and generates two mRNA transcripts with different 5′ termini. Nucleic Acids Res 13:3195–3212

Palmer JD (1983) Chloroplast DNA exists in two orientations. Nature 301:92–93

Palmer JD, Jorgenson RA, Thompson WF (1985) Chloroplast DNA variation and evolution in *Pisum:* patterns of change and phylogenetic analysis. Genetics 109:195–213

Pasupuleti CV, Galinat WC (1982) *Zea diploperennis.* I. Its chromosomes and comparative cytology. J Hered 73:168–170

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

Ripley LS, Glickman BW (1983) DNA secondary structures and mutation. In: Friedberg EC, Bridges BA (eds) Cellular responses to DNA damage. UCLA Symp Mol Cell Biol, Vol II. Alan R Liss, New York, pp 521–540

Saedler H, Nevers P (1985) Transposition in plants: a molecular model. EMBO J 4:585–590

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Schardl CL, Lonsdale DM, Pring DR, Rose KR (1984) Linearization of maize mitochondrial chromosomes by recombination with linear episomes. Nature 310:292–296

Schwarz-Sommer Z, Gierl A, Cuypers H, Peterson PA, Saedler H (1985) Plant transposable elements generate the DNA sequence diversity needed in evolution. EMBO J 4:591–597

Sederoff RR (1984) Structural variation in mitochondrial DNA. In: Scandalios JG (ed) Molecular genetics of plants. Academic Press, New York, pp 1–108

Sederoff RR, Levings CS III, Timothy DH, Hu WWL (1981) Evolution of DNA sequence organization in mitochondrial genomes of *Zea*. Proc Natl Acad Sci USA 78:5953–5957

Shah DM, Levings CS III (1974) Mitochondrial DNA from maize hybrids with normal and Texas cytoplasms. Crop Sci 14:852–853

Smith JSC, Goodman MM, Stuber CW (1984) Variation within teosinte III. Numerical analysis of allozyme data. Econ Bot 36:97–113

Sneath PHA, Sokal RR (1973) Numerical taxonomy. Freeman, San Francisco

Spencer DF, Bonen L, Gray MW (1981) Primary sequence of wheat mitochondrial 5S ribosomal ribonucleic acid: function and evolutionary implications. Biochemistry 20: 4022–4029

Spencer DF, Schnare MN, Gray MW (1984) Pronounced structural similarities between the small subunit ribosomal RNA genes of wheat mitochondria and *Escherichia coli*. Proc Natl Acad Sci USA 81:493–497

Spruill WM Jr, Levings CS III, Sederoff RR (1980) Recombinant DNA analysis indicates that the multiple chromosomes of maize mitochondria contain different sequences. Dev Genet 1:363–378

Stern DB, Lonsdale DM (1982) Mitochondria and chloroplast genomes of maize have a 12-kilobase DNA sequence in common. Nature 299:698

Takaiwa F, Sugiura M (1982) Nucleotide sequence of the 6S-23S spacer region in an rRNA gene cluster from tobacco chloroplast DNA. Nucleic Acids Res 8:2665–2676

Timothy DH, Levings CS III, Pring DR, Conde MF, Kermicle JL (1979) Organelle DNA variation and systematic relationships in the genus *Zea:* teosinte. Proc Natl Acad Sci USA 76:4220–4224

Timothy DH, Levings CS III, Hu WWL, Goodman MM (1983) Plasmid-like mitochondrial DNAs in diploperennial teosinte. Maydica 28:139–149

Upholt WB (1977) Estimation of DNA sequence divergence from comparison of restriction endonuclease digests. Nucleic Acids Res 4:1257–1265

Woese CR, Gutell R, Gupta R, Noller HF (1983) A detailed analysis of the higher order structure of 16S-like ribosomal RNAs. Microbiol Rev 47:621–669

Zurawski G, Clegg MT, Brown AHD (1984) The nature of nucleotide sequence divergence between barley and maize chloroplast DNA. Genetics 106:735–749