MITOCHONDRIAL DNA SEQUENCE DIVERGENCE AMONG LYCOPERSICON AND RELATED SOLANUM SPECIES

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ABSTRACT

Sequence divergence among the mitochondrial (mt) DNA of nine Lycopersi-con and two closely related Solanum species was estimated using the shared fragment method. A portion of each mt genome was highlighted by probing total DNA with a series of plasmid clones containing mt-specific DNA fragments from *Lycopersicon pennellii*. A total of 660 fragments were compared. As calculated by the shared fragment method, sequence divergence among the mtDNAs ranged from 0.4% for the *L. esculentum-L. esculentum* var. cerasiforme pair to 2.7% for the *Solanum rickii-L. pimpinellifolium* and *L. cheesmanii-L. chilense* pairs. The mtDNA divergence is higher than that reported for Lycopersicon chloroplast (cp) DNA, which indicates that the DNAs of the two plant organelles are evolving at different rates. The percentages of shared fragments were used to construct a phenogram that illustrates the present-day relationships of the mtDNAs. The mtDNA-derived phenogram places *L. hirsutum* closer to L. esculentum than taxonomic and cpDNA comparisons. Further, the recent assignment of *L. pennellii* to the genus Lycopersicon is supported by the mtDNA analysis.

VARIABILITY among restriction enzyme digestion patterns of organelle DNA has provided useful information for discerning phylogeny and present-day relationships among various groups of organisms. Comparisons of chloroplast (cp) DNA restriction enzyme fragment patterns of several species of Lycopersicon (PALMER and ZAMIR 1982) and Pennisetum (CLEGG, RAWSON and THOMAS 1984) have produced models of evolutionary relationships among the species. CpDNA restriction enzyme patterns have also aided in discerning the relationships among different polyploid species of Brassica (PALMER *et al.* 1983), Nicotiana (KUNG, ZHU and SHEN 1982) and Triticum and Aegilops (TSUNEWAKI and OGIHARA 1983; BOWMAN, BONNARD and DYER 1983).

Few evolutionary comparisons of plant mtDNA have been performed. Restriction fragment polymorphisms have been used to compare species of coffee

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(BERTHOU, MATHIEU and VEDEL 1983), wheat (VEDEL *et al.* 1978) and maize (TIMOTHY *et al.* 1979; WESSINGER *et al.* 1983; KEMBLE, GUNN and FLAVELL 1983). They have also been used to differentiate normal and male sterile lines of sorghum (CONDE *et al.* 1982) and maize (BORCK and WALBOT 1982). None of these researchers, though, estimated the percent sequence divergence among the species. A few comparisons of mt gene sequences have been made. Wheat and maize 18S rRNA genes are 97% homologous (CHAO, SEDEROFF and LEVINGS 1984; SPENCER, SCHNARE and GRAY 1984). Rice and maize cytochrome oxidase subunit II gene coding regions are 99.5% homologous, whereas the introns are 98.6% homologous, excluding a large insertion in the rice intron (FOX and LEAVER 1981; KAO, MOON and WU 1984).

While actual gene sequence comparisons give an accurate measure of divergence, only a small portion of the genome is sampled. In order to compare a larger portion of the mt genomes of nine Lycopersicon and two related Solanum species, we used the method of restriction fragment comparisons to estimate the amount of divergence among the genomes. We simplified the analysis of the complex plant mt genome by isolating total DNA from each species, transferring the DNA to nitrocellulose (SOUTHERN 1975) and probing with plasmid clones containing fragments of mtDNA from *Lycopersicon pennellii*. These clones were not homologous to either chloroplast or nuclear DNA under our experimental conditions. This procedure allowed us to highlight a portion of the mt genome of each species. The degree of shared fragments (UPHOLT 1977, as modified by GOTOH *et al.* 1979) was then calculated in order to compare the divergence among the species.

We present here the first estimations of plant intrageneric mtDNA sequence divergence, as calculated by the shared fragment method. We show that plant mtDNAs are less divergent than the animal species which have been studied. Using the mtDNA restriction fragment data, we also constructed a maternal phylogenetic tree of the Lycopersicon species and compared it with taxonomic (HOGENBOOM 1979), crossability (RICK 1979) and cpDNA (PALMER and ZAMIR 1982) groupings.

MATERIALS AND METHODS

Plant material: Seeds of all accessions of wild tomato species were obtained from CHARLES RICK, Tomato Genetics Stock Center, University of California, Davis. The species, accession number and site of collection are listed in Table 1. Several sources of *L. esculentum* cultivars were used. Red Cherry seeds were obtained from Herbst Brothers Seedsmen, Inc., Brewster, New York. Fruits of Jumbo were obtained from the Norfolk Food Factory, Norfolk, Virginia. Fruits of Roma were obtained from a local vendor. Fruits of Better Boy, Golden Boy and Walter were obtained from ALLAN STONER, United States Department of Agriculture/Agricultural Research Station, Beltsville, Maryland.

Mitochondrial DNA isolation: Green fruits, suspension cultures and callus were used to isolate mtDNA (HANSON *et al.* 1986). Chopped green fruits were ground in two volumes of grinding buffer [0.3 M mannitol, 50 mM Tris-HCl (pH 8.0), 10 mM EGTA, 5 mM EDTA, 1% polyvinylpyrrolidone (PVP), 0.2% bovine serum albumin (BSA) and 20 mM 2-mercaptoethanol], using two 1-sec bursts on low speed of a Waring Blender and one 6-sec burst on high speed, and were filtered through two layers of cheesecloth

TABLE 1

Accessions of wild species used in evolutionary comparisons

Species	Accesion no.	Site[a]
Solanum rickii	LA 1974	Chuguicamata (Chile)
S. lycopersicoides	LA 1990	Palca
Lycopersicon cheesmanii	LA 1401	Galapagos (Ecuador)
L. chilense	LA 458	Tacna
L. chmielewskii	LA 1306	Tambo
L. esculentum var. cerasiforme[b]	LA 1320	Hda. Carmen
L. esculentum var. cerasiforme	LA 1482	(Malaysia)
L. hirsutum	LA 1392	Huaraz-Casma
L. hirsutum[b]	LA 1777	Rio Casma
L. pennellii	LA 716	Atico
L. peruvianum	LA 111	Rio Supe
L. peruvianum[b]	LA 1955	Matarani
L. peruvianum var. humisifusum	LA 385	San Juan
L. pimpinellifolium	LA 722	Truijillo

[a] All accessions were collected in Peru unless otherwise noted.
[b] These accessions were used in the evolution study. The L. esculentum cultivar used in the evolution study was Red Cherry.

and, then, one layer of Miracloth. Suspension culture cells and callus were ground in one volume of grinding buffer, using a 60-sec burst with a Bead Beater, and were filtered through one layer of Miracloth. The pH of the filtrate was adjusted to 7.5. All filtrates were subsequently treated in the same manner. The filtrate was centrifuged at $1500 \times g$, 4° for 15 min. The supernatant was filtered through one layer of Miracloth and the crude mitochondria were pelleted by centrifugation at $13,000 \times g$, 4° for 15 min. The pellet was resuspended in a small volume of grinding buffer without PVP or 2-mercaptoethanol. The suspension was adjusted to 50 mM $MgCl_2$ and 100 μg/ml DNase and was digested on ice for 30 min. The digestion was halted by adjusting the solution to 50 mM EDTA. The DNase suspension was diluted with 15 volumes of resuspension buffer [0.3 M sucrose, 50 mM Tris-HCl (pH 8.0), 20 mM EDTA and 0.1% BSA] and was centrifuged at $12,000 \times g$, 4° for 15 min. The pellet was resuspended in a small volume of resuspension buffer and was layered on a sucrose step-gradient consisting of 1.6, 1.2 and 0.6 M sucrose. The sucrose solution was made up with 50 mM Tris-HCl (pH 8.0), 20 mM EDTA and 0.1% BSA. The mitochondria were banded by centrifuging at 25,000 rpm in a Sorvall AH 627 at 4° for 1 hr, were removed from the 1.6/1.2 M sucrose interface, were slowly diluted with three volumes of resuspension buffer and were pelleted at $16,000 \times g$, 4° for 20 min. The mitochondrial pellet was resuspended in a minimal volume of 50 mM Tris-HCl (pH 8.0), 20 mM EDTA. After the addition of 200 μl of 20 mg/ml ethidium bromide (EtBr), CsCl was added to a density of 1.6 g/ml. The mtDNA was banded by centrifuging at 38,000 rpm, 20° for 40 hr in a Beckman Ti 75 rotor. The mtDNA band was removed by side puncture and was extracted three times with an equal volume of isopropanol saturated with CsCl to remove the EtBr. The CsCl was removed by dialysis against 10 mM Tris (pH 8.0), 50 mM NaCl and 5 mM EDTA. One-tenth volume 3 M sodium acetate and 2.3 volumes of ethanol were added to the dialysate, and it was stored overnight at −20°. The DNA was pelleted by centrifugation at $15,000 \times g$, 4° for 20 min. The DNA pellet was dried under vacuum and was brought up in a minimal volume of 10 mM Tris (pH 8.0), 1 mM EDTA.

Total DNA isolation: Total DNA was prepared from approximately 400 mg of

lyophilized leaf material, using the procedure of MURRAY and THOMPSON (1980). The first CsCl banding was lengthened to 18 hr, and the second banding was omitted.

Plasmid cloning: *Sal*I digested *L. pennellii* mtDNA was ligated into the *Sal*I site of dephosphorylated pUC9 (VIERA and MESSING 1982). The ligated DNA was used to transform calcium-chloride-treated (COHEN, CHANG and HSU 1972) JM 83 cells.

Electrophoresis, blotting and hybridization: MtDNA (1 μg) and total DNA (4 μg) were separated in 0.8% or 1.0% agarose gels in a buffer containing 40 mM Tris-HCl (pH 7.8), 1 mM EDTA and 5 mM sodium acetate. The total DNA used in the evolution study was digested with *Bam*HI, *Bgl*I, *Hind*III, *Pst*I, *Sal*I and *Sma*I according to the manufacturer's recommendations. The gels were stained with 2 μg/ml EtBr in 400 ml of water and were subsequently photographed. The DNA was then transferred to nitrocellulose (SOUTHERN 1975). The filter was prehybridized at 65° with 6 × SSC (1 × SSC = 0.15 M NaCl, 15 mM sodium citrate) and 0.1% each of Ficoll and PVP for 5 hr to overnight. The filter was then hybridized with 1 × 10^6 to 5 × 10^6 cpm of nick-translated (RIGBY *et al.* 1977) plasmid clone in a solution containing 0.5% SDS, 0.2 mg/ml of calf thymus DNA, 6 × SSC and 0.1% each of Ficoll and PVP. For the evolution study, 1 × 10^6 cpm of each nick-translated clone of the group was used. Hybridization continued overnight. The filter was then washed three times with 0.1 × SSC at 65°, dried and used to expose Kodak SB5 film at −70°.

Estimating sequence divergence; phylogenetic tree construction: Percent sequence divergence was estimated by the method of UPHOLT (1977), utilizing the modifications suggested by GOTOH *et al.* (1979). We used the restriction fragment comparisons as our input data. *F* values were then utilized to produce a phenogram by the unweighted pair-group method (UPGMA; SNEATH and SOKAL 1973).

RESULTS

Restriction analysis of tomato mtDNA variability. MtDNAs isolated from suspension cultures of *Lycopersicon pennellii*, *Solanum rickii* and a sexual hybrid containing *L. peruvianum* cytoplasm (THOMAS and PRATT 1981) and green fruit of the *L. esculentum* cultivar Jumbo were digested with *Sal*I and the fragments were separated on a 0.8% agarose gel (Figure 1). The size distribution of the *Sal*I fragments ranged from approximately 26 kb to 0.5 kb.

Restriction fragment summation of the *Sal*I digest of these tomato species gave an estimate of 300 kb for the genome size of the mtDNA. Several *Sal*I fragments exhibited a higher intensity than neighboring fragments, suggesting that these fragments are represented more than once in the mt genome or that several fragments exhibit similar mobilities. Because each such band was counted only once in the size estimation, the 300-kb figure represents a minimum size estimate.

A survey of 11 cultivars of *L. esculentum*, the common cultivated species, cut with five restriction enzymes, revealed very few differences among the restriction banding patterns (data not shown). The only two detected variant patterns are presented in Figure 2. A *Sal*I digestion of Golden Boy mtDNA produced an extra fragment not found in either Walter or Roma, whereas two unique fragments were found when Roma mtDNA was digested with *Xho*I. The digestion pattern displayed by Walter was identical to that obtained when other cultivars were cut with *Sal*I and *Xho*I.

The interspecific and intraspecific variability is further illustrated in Figure 3. *Sal*I digests of mtDNA of *L. pennellii* and two *L. esculentum* cultivars, Better Boy and Golden Boy, were transferred to nitrocellulose and were probed with
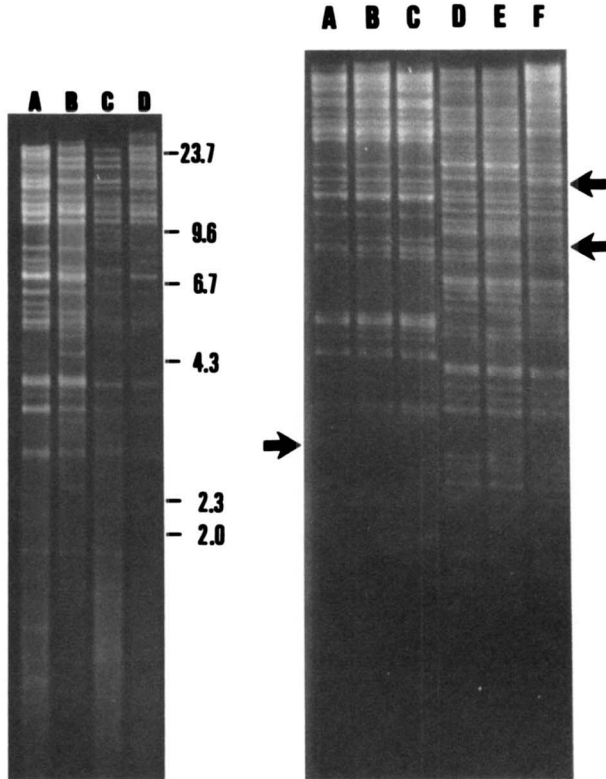
FIGURE 1.—Restriction digestion of tomato mtDNA. One microgram of mtDNA from (A) a sexual hybrid cpontaining *L. peruvianum* cytoplasm (THOMAS and PRATT 1981), (B) *L. pennellii*, (C) *S. rickii* and (D) *L. esculentum* (cultivar Jumbo) was digested with *Sal*I restriction enzyme and was electrophoresed through a 0.8% agarose gel. Marker sizes (expressed as kilobase pairs) are from a *Hind*III digestion of phage λDNA.

FIGURE 2.—Restriction fragment polymorphism of *L. esculentum* cultivars. One microgram of three *L. esculentum* cultivars, Golden Boy (A, D), Walter (B, E) and Roma (C, F) were digested with *Sal*I (A, B, C) and *Xho*I (D, E, F) restriction enzymes and were electrophoresed through a 0.8% agarose gel. Arrows indicate unique bands.

a nick-translated plasmid containing the 2.1-kb *Sal*I fragment of *L. pennellii*. Two fragments of size 9.8 and 3.8 kb were common to all three mtDNAs and were the only Better Boy fragments homologous to the probe. Additionally, *L. pennellii* and Golden Boy shared homology to the cloned 2.1-kb fragment. Golden Boy also contained a 22-kb fragment homologous to the probe, whereas the probe was homologous to *L. pennellii* fragments of 11 and 4.5 kb.

Interspecific comparisons: Unfortunately, many Lycopersicon species produce small fruits and require specific environmental conditions for flowering and fruit set. Suspension and callus cultures of some Lycopersicon species are difficult to initiate and maintain. Thus, we needed an alternative method to examine restriction fragment polymorphisms.

Total DNA from all 11 species was isolated from small amounts of leaf tissue, cut as single digests with six different restriction enzymes and separated
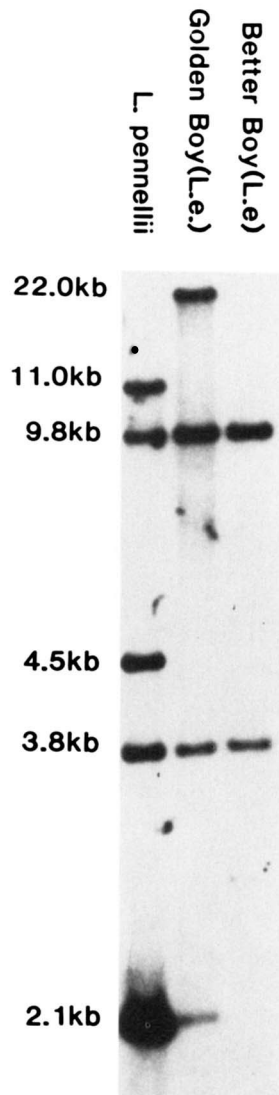
FIGURE 3.—Hybridization of a *L. pennellii* mtDNA clone to several tomato mtDNAs. A nick-translated plasmid clone containing the 2.1-kb *Sal*I fragment of *L. pennellii* was hybridized to a nitrocellulose filter containing *Sal*I mtDNA fragments of *L. pennellii* and *L. esculentum* cultivars Golden Boy and Better Boy. The size of each fragment is given on the left.

by agarose gel electrophoresis. To highlight those mitochondrial-specific fragments, we transferred the DNA to nitrocellulose and probed the blots with one of three groups of plasmids containing *Sal*I mtDNA fragments from *L. pennellii*. The three groups contained fragments of the following sizes: (1) 0.58, 1.88 and 7.10 kb; (2) 1.06, 2.10 and 5.20 kb; and (3) 0.60, 1.14, 2.45, 3.30 and 6.60 kb. These 11 clones do not hybridize detectably either to each other or to chloroplast or nuclear DNA under our experimental conditions (data not
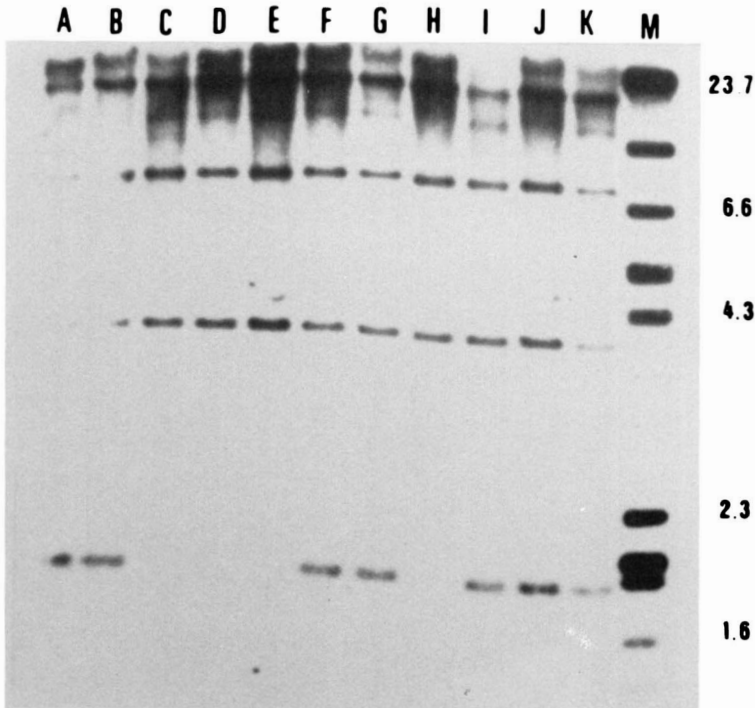
FIGURE 4.—Autoradiogram of total tomato DNA hybridized with *L. pennellii* mtDNA plasmid clones. Four micrograms of total DNA from 11 tomato species were cut with *Sal*I restriction enzyme, transferred to nitrocellulose and simultaneously probed with individually nick-translated plasmids containing the 0.58-, 1.88- and 7.10-kb *Sal*I fragments of *L. pennellii*. A, *S. lycopersicoides*; B, *S. rickii*; C, *L. esculentum,* cultivar Red Cherry; D, *L. esculentum* var. cerasiforme (LA 1320); E, *L. pimpinellifolium*; F, *L. hirsutum* (LA 1777); G, *L. chmielewskii*; H, *L. chilense*; I, *L. peruvianum* (LA 1955); J, *L. pennellii*; and K, *L. cheesmanii*. Lane M contains *Hind*III plus *Hind*III/*Eco*RI digestion products of phage λDNA. Sizes of selected fragments (expressed as kilobase pairs) are given on the right.

shown). An example of a typical autoradiogram is shown in Figure 4. The data obtained from the three groups was then pooled.

The summation distribution of the fragments which hybridize to probes for *Bam*HI and *Sal*I are presented in Figures 5 and 6. The figures reveal that, among the eleven species, between 12 and 15 *Bam*HI fragments and between 10 and 17 *Sal*I fragments were homologous to the probes. A similar number of *Hind*III and *Sma*I fragments were also homologous, whereas 5–8 *Bgl*I and *Pst*I fragments were homologous to the probes.

The *Bam*HI and *Sal*I fragments of *L. pennellii* sum to 84.5 and 73.0 kb, respectively. Using 300 kb as the estimate of the genome size, this suggests that our probes, which represent approximately 10% of the genome, were identifying fragments representing 25% of the mt genome of the Lycopersicon species.

Estimation of divergence by the shared fragment method: The fragment patterns for six restriction enzymes generated from the hybridization proce-
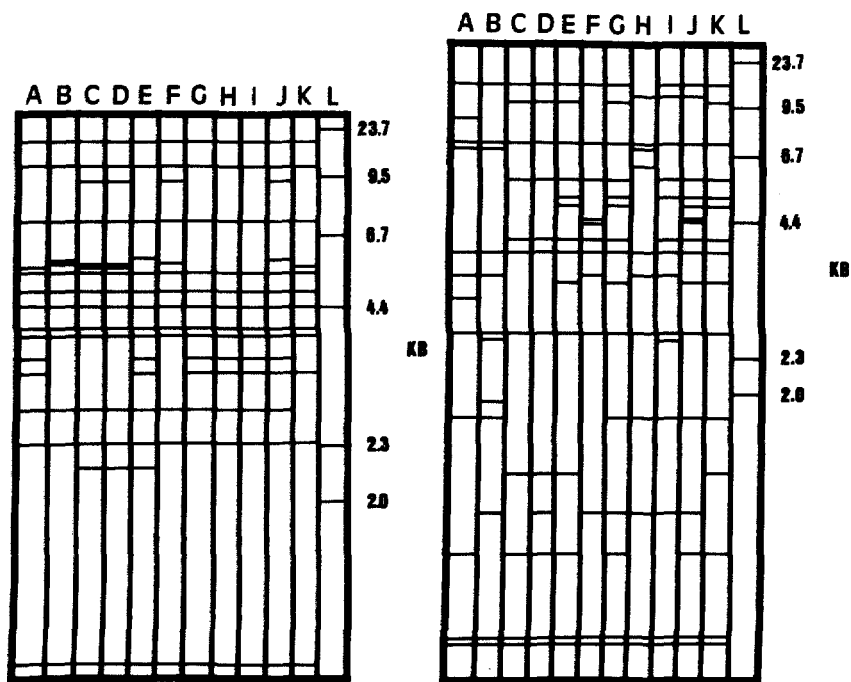
A B C D E F G H I J K L

FIGURE 5.—Diagrammatic representation of *Bam*HI hybridization pattern of 11 *L. pennellii* mtDNA plasmid clones with total tomato DNA. Total DNA from 11 tomato species were digested with *Bam*HI restriction enzyme and were transferred to nitrocellulose. In several experiments, the DNA was probed with different subgroups of 11 unique *L. pennellii* mtDNA clones, and the hybridization patterns were summed over all the experiments. The lane designations for lanes A–K are the same as those used in Figure 4. Lane L depicts fragments from a *Hind*III digestion of phage λDNA. Sizes of these fragments (expressed as kilobase pairs) are given on the right.

FIGURE 6.—Diagrammatic representation of *Sal*I hybridization patterns of 11 *L. pennellii* mtDNA plasmid clones with total tomato DNA. Details of the construction of the diagram, lane designations and marker designations are the same as those used in Figure 5, except that the DNA was cut with *Sal*I.

dure were used to estimate the amount of divergence among the 11 species. The divergence estimates were based on the number of shared fragments developed by UPHOLT (1977), as modified by GOTOH *et al.* (1979) (Table 2). The values entered in the table are the number of base pair differences per kilobase for each species pair. These values ranged from 3.7 for the *L. esculentum-L. esculentum* var. cerasiforme comparison, to 27.3 for the *S. rickii-L. pimpinellifolium* and *L. cheesmanii-L. chilense* comparison. We compared these values with similar estimates generated from restriction digests of mtDNA from *L. esculentum, L. pennellii* and *S. rickii*, using the same enzymes utilized in the hybridization experiments. We obtained divergence values of 11.0 for the *L. esculentum-S. rickii* comparison, 8.3 for the *L. esculentum-L. pennellii* comparison and 11.2 for the *S. rickii-L. pennellii* comparison. The divergence values for the hybridization data for the comparable comparisons were much higher, 22.7, 18.5 and 26.1, respectively. The values generated from the restriction

TABLE 2

Mitochondrial DNA divergence values among tomato species

	S. lycopersicoides	S. rickii	L. esculentum	L. esculentum var. cerasiforme	L. pimpinellifolium	L. hirsutum	L. chmielewskii	L. chilense	L. peruvianum	L. pennellii
Solanum rickii	18.0									
Lycopersicon esculentum	20.1	22.7								
L. esculentum cerasiforme	20.1	22.7	3.9							
L. pimpinellifolium	20.2	27.3	12.9	9.4						
L. hirsutum	23.2	19.8	10.3	9.1	15.3					
L. chmielewskii	19.4	24.7	15.9	13.5	7.4	14.7				
L. chilense	13.7	22.2	22.7	19.8	15.9	15.7	10.6			
L. peruvianum	13.4	17.4	16.7	14.1	13.1	12.9	8.2	14.6		
L. pennellii	20.8	26.1	18.5	18.5	9.7	13.7	7.4	11.4	11.7	
L. cheesmarii	15.8	24.2	15.1	12.6	6.3	12.6	13.6	27.3	12.9	9.4

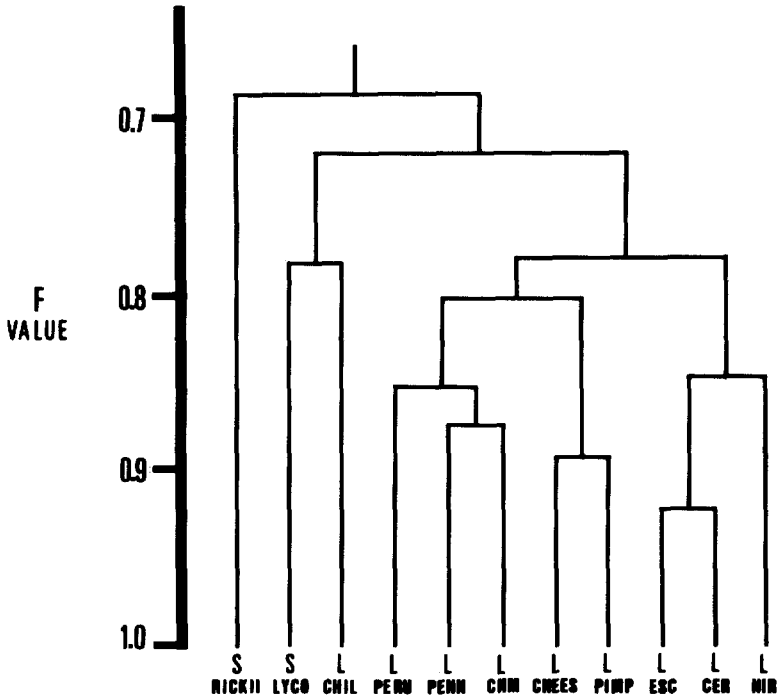Values in the table are expressed as base pair differences per kilobase.

FIGURE 7.—Tomato mtDNA phenogram. F values were used to construct the UPGMA phenogram. The species abbreviations used in the figure are the same as those used in Table 2. The cophenetic correlation is $r = 0.87$. The species abbreviations are S. lyco. = *S. lycopersicoides*; L. esc. = *L. esculentum*; L. cer. = *L. esculentum* var. cerasiforme; L. pimp. = *L. pimpinellifolium*; L. hir. = *L. hirsutum*; L. chm. = *L. chmielewskii*; L. chil. = *L. chilense*; L. peru. = *L. peruvianum*; L. penn. = *L. pennellii*; L. chees. = *L. cheesmanii*.

digestion patterns are probably underestimates. The assumption that comigrating bands contained similar sequence information may not be correct. Thus, a greater degree of error may be associated with estimates made from restriction fragment patterns of complex genomes than with estimates obtained from hybridization data.

Phylogenetic relationships: The F value (GOTOH *et al.* 1979), the ratio of shared fragments to all fragments compared, was used to develop a tree (phenogram) using the UPGMA procedure (SNEATH and SOKAL 1973). No assumptions concerning the origin of restriction fragment differences are made in generating F values. The F values summed over all enzymes were used as measures of similarity to generate the phenogram presented in Figure 7.

Five taxa can be delineated from the phenogram. A taxa is defined here as a pair of species and any additional species or pair of species which join with that group of species at only one higher level. Thus, *L. esculentum* and *L. esculentum* var. cerasiforme form a group, and since *L. hirsutum* is combined with that group by only a single branch, it is considered part of that taxa. Alternatively, *L. peruvianum*, *L. pennellii* and *L. chmielewskii* and *L. pimpinellifolium* and *L. cheesmanii* are taxa, but these taxa are not grouped together,

because another level of branching is needed to include them. The other two taxa are *S. lycopersicoides* and *L. chilense*; and the single species is *S. rickii.*

Intraspecific variability: Two accessions of *L. esculentum* var. cerasiforme (LA 1320 and LA 1428), two accessions of *L. hirsutum* (LA 1392 and LA 1777) and three accessions of *L. peruvianium* (LA 111, LA 1955 and LA 385) were compared with the mtDNA hybridization probes. No differences were detected among the *L. peruvianum* accessions. Differences among *L. esculentum* var. cerasiforme and *L. hirsutum* accessions were detected when the total DNAs were cut with *Hind*III, *Pst*I or *Sal*I and were probed with the group of five clones. The *L. hirsutum* accession LA 1777 contained a 2.0-kb *Hind*III and 3.5-kb *Sal*I fragment, each slightly larger than the corresponding fragment from LA 1392 (data not shown). An additional *Hind*III, *Pst*I and *Sal*I fragment of size 1.1, 6.1 and 2.6 kb, respectively, delineated the two *L. esculentum* var. cerasiforme accessions, LA 1320 and LA 1482 (data not shown).

DISCUSSION

Plants exhibit a wide range of mt genome sizes: The wide variability in mitochondrial genome size was first documented in the family Cucurbitaceae, the species of which range from 320 kb for watermelon to 2400 for muskmelon (WARD, ANDERSON and BENDICH 1981). The most accurate estimate of plant mitochondrial genome size comes from restriction mapping experiments—for example, turnip was calculated to be 218 kb (PALMER and SHIELDS 1984) and maize to be 570 kb (LONSDALE, HODGE and FAURON 1984). A simple summation of restriction fragment sizes, such as our estimate of 300 kb for tomato, usually underestimates the size of the mt genome. SPRUILL, LEVINGS and SEDEROFF (1980) arrived at a 277-kb estimate for the maize mt genome by counting each fragment only once, whereas BORCK and WALBOT (1982) accounted for fragment multiplicity yet estimated the mt genome to be only 475 instead of the 570 kb determined by restriction mapping experiments (LONSDALE, HODGE and FAURON 1984).

Other kingdoms do not exhibit the same wide range of mt genome sizes. Animal mt genomes range in size from 15 to 20 kb (BROWN 1983), whereas the size range for filamentous fungi is 31.5 kb for *Aspergillus nidulans* (MANCINO *et al.* 1980) to 94 kb for *Podospora anserina* (WRIGHT *et al.* 1982). The animal mt genome is considered to be quite conserved and appears to contain little noncoding DNA. If plant and animal mt genomes do indeed perform similar coding roles, then plants possess a large excess of DNA not required for mitochondrial metabolism. One might have expected this large amount of noncoding DNA to allow plant mt genomes a greater flexibility to retain mutations without disrupting function than would exist for animal mt genomes. However, the results we report here show that plant mt genomes are evolving slower than animal mt genomes.

Plant and animal mtDNA comparison: Accurate estimates of divergence among organelle genomes require extensive restriction mapping, a laborious procedure given the large size of plant mt genomes. Animal mt genomes are much smaller and more conserved in size than plant mt genomes and, thus,

are more amenable to restriction enzyme mapping experiments. In some cases, mapping data also allow estimates to be made over a broad phylogenetic range. For example, the common lineage for sea urchin and mammals dates back to 500 million yr ago yet their mt genomes differ in size by only 1 kb (ROBERTS et al. 1983). The contributions of both rearrangement and base pair substitution can thus be estimated from restriction maps of these two genomes. In contrast, only recently have restriction maps of a few mt genomes been constructed. The mapped 218-kb turnip mt genome (PALMER and SHIELDS 1984) is much smaller than the 570-kb genome of corn (LONSDALE, HODGE and FAURON 1984). The vast differences in plant mt genome size minimizes the usefulness of restriction maps to provide an accurate assessment of the mode of mitochondrial evolution of these widely diverged plant species.

We estimated the degree of variability between tomato species by calculating the sequence divergence among the mtDNAs by the shared fragment technique (UPHOLT 1977, as modified by GOTOH et al. 1979). The fragment data was generated by probing total DNA from each species with plasmid clones containing mtDNA from L. pennellii. A potential pitfall with this approach is homologies which exist between mtDNA sequences and sequences in the chloroplast (STERN and PALMER 1984a) and nuclear genomes (JACOBS et al. 1983; WRIGHT and CUMMINGS 1983). We avoided this confounding situation by using clones which did not hybridize, under our experimental protocol, to either chloroplast or nuclear DNA.

The shared fragment method (UPHOLT 1977) assumes that two nucleotide sequences have only diverged by base pair substitutions. Animal mt genomes, though, have been shown to have diverged by length mutations (CANN and WILSON 1983) as well as by rearrangement (ROBERTS et al. 1983). Rearrangements have occurred also between related plant species. SEDEROFF et al. (1981) documented several cases of mtDNA rearrangements among maize and teosinte races. BOESHORE et al. (1983) found that mtDNA of several petunia somatic hybrids were rearranged with respect to their two parents and that not all somatic hybrids exhibited the same pattern of rearrangement. Indeed, Figure 3 shows that rearrangements of some sort have occurred between L. pennellii and L. esculentum, as well as between L. esculentum cultivars. These sorts of rearrangements confound estimates of divergence based on the shared fragment technique. ZIMMER (1980) confronted the same problem of rearrangements when attempting to estimate the divergence among human and ape globin genes and found that rearrangements inflated the nucleotide substitution estimates. It should be noted, then, that our estimates, although expressed as base pair substitutions per kilobases, actually reflect rearrangements as well as substitutions that have occurred between any two mt genomes. In spite of this deficiency, the plant mtDNA divergence estimates we present are still lower than those for animal mtDNA. This implies that the combined mutational effect of point mutations and rearrangements is not generating the degree of mt genome diversity in plants that they do in animals.

The divergence among the tomato species we compared ranged from 3.7 to 27.3 base pairs (bp) per kilobase or, when expressed as percent sequence

divergence, 0.4 to 2.7%. These divergence values are considerably smaller than those reported for related animal species. Comparisons of mtDNAs within rodent genera showed a 13.3% sequence divergence among Rattus species (BROWN and SIMPSON 1981) and 13.2% among Peromyscus species (AVISE LANSMAN and SHADE 1979). Further, intraspecific variability of animal mtDNA was as large as 9.6% for *Rattus rattus* (BROWN and SIMPSON 1981). The results presented here clearly show that the mtDNA of the tomato species have not diverged to the extent that the rodent or primate genera have and that they are less diverged than populations within some individual animal species.

S. *rickii* and *L. esculentum* mtDNAs are diverged by only 2.7%, a value similar to the divergence among mtDNAs of *Mus musculus* subspecies (2.6%) which diverged 1 million yr ago (YONEKAWA *et al.* 1981). An important issue to resolve is whether the tomato species appeared as recently as the mice subspecies and, thus, have similar mtDNA divergence rates or whether they form an older genus and possess a slower rate of mtDNA divergence. The vegetative parts of the tomato plant are fragile and do not lend themselves to fossilization. Thus, the fossil record leaves no clues as to the date of the Solanum-Lycopersicon divergence. Biogeographical evidence, though, can set some limits on the age of the group. RICK (1963) examined the distribution patterns of *L. peruvianum* and suggested that various populations were isolated, perhaps, during the Tertiary period before the Andean uplift. The Andean uplift occurred during the Pliocene epoch (KUMMEL 1970), 3–7 million yr ago, and *L. peruvianum* was distinct from at least the Solanum genera at that time. Therefore, the Solanum-Lycopersicon divergence must also have predated the uplift. This estimate would then support our assertion that the rate of tomato mtDNA divergence must be slower than animal mtDNA divergence.

Two alternatives may account for the slower rate of mtDNA divergence in higher plants. First, separate molecular events could have generated plant and animal and fungi mitochondrial lineages. A comparison of wheat mtrRNA sequences with those of other organisms led SPENCER, SCHNARE and GRAY (1984) to conclude that plant mitochondria were on a separate branch from animal and fungal mitochondria and that the animal-fungal mitochondrial branch was diverged more than the plant mitochondrial branch. KUNTZEL and KOCHEL (1981) also used structural and sequence comparisons of mtrRNA genes to suggest a multiple origin for mitochondrial genomes.

CHAO, SEDEROFF and LEVINGS (1984), though, prefer an alternative hypothesis. They performed a principal coordinate analysis of mtrRNA molecules and found several distinct clusters. The maize 18S mtrRNA molecule clustered with the *E. coli* 16S rRNA molecule. This cluster was unique from a cluster that contained the Aspergillus and yeast 15S rRNA molecules, and both of these clusters were quite distant from a cluster containing human, mouse and bovine 12S rRNA molecules. Although this analysis might also support the independent origins hypothesis, CHAO, SEDEROFF and LEVINGS (1984) suggest that the rates of mt genome evolution vary for each of the individual lineages. Differences in either the mechanism of mutation in mtDNA or the constraints on mtDNA function in different groups of organisms could produce the vari-

able mutation rates among lineages (BROWN, GEORGE and WILSON 1979). However, one might expect the plant genome, with its larger proportion of presumably noncoding DNA, to be the less-constrained molecule.

Tomato mtDNA and cpDNA evolution: PALMER and ZAMIR (1982) performed a detailed restriction enzyme analysis of Lycopersicon cpDNA and found a very low degree of variability. In order to have a common basis for comparison, we used the information in table 2 of PALMER and ZAMIR (1982) to calculate divergence values for cpDNA using the shared fragment technique (UPHOLT 1977, as modified by GOTOH *et al.* 1979). Whereas the tomato mtDNA divergence ranged from 3.9 to 27.3 bp per kilobase, cpDNA pairs were only 0 to 3.6 bp per kilobase diverged. This result suggests that the tomato mitochondrial and chloroplast genomes are evolving at different rates and, possibly, by different mechanisms.

Sequence analysis of mitochondrial 18S rRNA gene of maize and wheat (SPENCER, SCHNARE and GRAY 1984; CHAO, SEDEROFF and LEVINGS 1984) has shown that the two genes have diverged by base pair substitutions and length mutations and differ by 10 bp per kilobase. As with mitochondrial sequences, chloroplast genomes have also diverged by base pair substitutions and length mutations. Restriction fragment analysis of Pisum (PALMER, JORGENSEN and THOMPSON 1985), Triticum and Aegilops (BOWMAN, BONNARD and DYER 1983) and Euoenothera (GORDON *et al.* 1982) cp genomes, for example, revealed that length mutations were the more prevalent form of divergence. But whether one form of divergence is preferentially exhibited by plant mitochondrial genomes can only be determined by estimating the amount of rearrangement that occurs in mitochondrial genomes.

The multipartite structure of plant mt genomes (PALMER and SHIELDS 1984; LONSDALE, HODGE and FAURON 1984), with a master circle containing several sites of intramolecular recombination, suggests one manner in which rearrangements may occur. Maize and turnip mitochondrial genomes contain repeat units that act as regions of recombination to produce smaller circles that are subpopulations of the master mitochondrial circle (PALMER and SHIELDS 1984; LONSDALE, HODGE and FAURON 1984). Other plant mitochondrial genomes contain similar repeats that, presumably, also have the ability to act as regions of recombination (STERN and PALMER 1984b). If the reciprocal recombination event suggested by PALMER and SHIELDS (1984) to generate the subpopulations of mitochondrial circles is inaccurate, rearrangements in the genome would occur. The extent to which this method generates and maintains these rearrangements would depend on the fate of these molecules in subsequent cell generations.

Tomato mtDNA phylogeny: On the basis of morphological and crossability studies, Lycopersicon species have been divided into two complexes. The "esculentum complex" is a broad array of species that includes the red-fruited species *L. esculentum, L. pimpinellifolium* and *L. cheesmanii* and the green-fruited species *L. hirsutum, L. pennellii, L. chmielewskii* and *L. parviflorum* (RICK 1976; HOGENBOOM 1979). The mtDNA phylogeny (Figure 7) groups two red-fruited species, *L. pimpinellifolium* and *L. cheesmanii*. Yet the mtDNA phylogeny most

closely affiliates the green-fruited *L. hirsutum* with the red-fruited species *L. esculentum* and *L. esculentum* var. cerasiforme, a result consistent with the crossability experiments (RICK 1979) that have shown *L. hirsutum* to be compatible to a degree with both *L. esculentum* and *L. esculentum* var. cerasiforme. The mtDNA data, though, group *L. hirsutum* closer to *L. esculentum* and *L. esculentum* var. cerasiforme than do the cpDNA data (PALMER and ZAMIR 1982).

The second Lycopersicon complex is the "peruvianum complex," which consists of the species *L. peruvianum* and *L. chilense* (RICK 1976). The mtDNA phylogeny, though, groups *L. peruvianum* with two "esculentum complex" species, *L. pennellii* and *L. chmielewskii* (Figure 7). Other than this single case, the mtDNA of the "esculentum complex" species forms a single group consisting, as defined here, of three taxa: (1) *L. esculentum*, *L. esculentum* var. cerasiforme and *L. hirsutum*; (2) *L. cheesmanii* and *L. pimpinellifolium*; and (3) *L. pennellii* and *L. chmielewskii*. The last taxa also contains *L. peruvianum*. The mtDNA of the two "esculentum complex" species of the third taxa is more closely related than is their cpDNA (PALMER and ZAMIR 1982).

Crossability experiments (RICK 1979) and the mtDNA data presented here both show *S. rickii*, *S. lycopersicoides* and *L. chilense* to be distant from the other species tested. CpDNA analysis also found *S. lycopersicoides* to be distant from the other species (PALMER and ZAMIR 1982).

Crossability experiments have also shown *L. esculentum* and *L. esculentum* var. cerasiforme to be extremely compatible (RICK 1979). The mtDNA sequence diversity data (Table 2) and the UPGMA phylogeny of the mtDNA (Figure 7) find these two species to be the most closely paired. These results support the contention of JENKINS (1948) that *L. esculentum* var. cerasiforme is the ancestral form of *L. esculentum*.

Until recently, *L. pennellii* was placed in the genus Solanum, a classification based on the anther traits of the species. RICK has questioned this grouping and has presented crossability (RICK 1960; RICK 1979) and isozyme data (RICK and TANKSLEY 1981) suggesting that the species is best placed in the genus Lycopersicon. The mtDNA data presented here support this assignment, a result also consistent with the cpDNA data (PALMER and ZAMIR 1982).

Variability among domesticated tomato: Tomato mt genomes exhibit a small amount of restriction fragment variability. This is evident from the restriction digest (Figures 1 and 2) and hybridization (Figures 3, 4, 5 and 6) data. As with isozymes (RICK 1983), mtDNA variability was more limited among *L. esculentum* cultivars than among different accessions of the wild species sampled. This high degree of similarity of the mtDNAs of the cultivars was not unexpected considering the genetic background used to develop the modern cultivars (RICK 1976). Well-developed cultigens indigenous to Mexico were transferred, starting in the 16th century, to the Old World, where selection identified variants which were adapted to several European growing regions. The reintroduction of selected lines to the New World further narrowed the genetic base used in developing the modern cultivars. Unilateral incongruity between *L. esculentum* and several wild species (HOGENBOOM 1979) requires that *L. esculentum* be used as the female parent when attempting to introduce

wild genes into the cultivated tomato. Although these crosses broaden the nuclear background of *L. esculentum,* maternal inheritance prevents the introduction of mtDNA from the wild species into the maternal parent. Thus, the narrow mitochondrial genetic base of *L. esculentum* cultivars is maintained.

The results we report here emphasize the variable rates of divergence among plant organelle genomes. That mtDNA and cpDNA are diverging at different rates is evidence for several modes of organelle genome evolution in plants. In conclusion, the finding that plant mtDNA may be evolving slower than animal mtDNA suggests that the mtDNAs of each kingdom may be under different restraints, which could be reflective of their ancestral origins.

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