Three copies of a single recombination repeat occur on the 443 kb mastercircle of the *Petunia hybrida* 3704 mitochondrial genome

Otto Folkerts and Maureen R.Hanson

Section of Genetics and Development, Cornell University, Ithaca, NY 14853, USA

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ABSTRACT

At 443 kb, the map of *Petunia hybrida* line 3704 mitochondrial DNA is the largest yet produced from a dicot plant. Regions of similarity to known plant mitochondrial genes and to the chloroplast genome have been placed on a master circle. One long repeated sequence, apparently active in recombination, is present in three copies. Two copies of 6.6 kb occur in a direct orientation and are separated by 199 kb. A third truncated copy of 3.5 kb is inverted relative to the other two and is separated from the others by 99 and 145 kb. The presence of the recombination repeats predicts a multipartite molecular organization, consisting of four master circles and three subgenomic circles. Two other repeated regions were found not to be substrates for, or products of recombination. The absence of recombination at certain reiterated regions indicates that there is specificity of recombination at the recombination repeats.

INTRODUCTION

Higher plant mitochondrial genomes vary greatly in size, from the smallest known in a *Brassica* species (208 kb) to muskmelon, estimated at 2200 kb (1,2). Accurate size estimates depend on genome mapping because of the complexity of the molecular organization of plant mitochondrial genomes. Less than a dozen plant mitochondrial genome maps are presently available. As yet only one mitochondrial genome with a simple circular organization has been identified (*Brassica hirta*; 1). In every other case, reciprocal recombination across recombination repeats converts a master circle into either isomeric molecules or subgenomic molecules.

Recombination repeats were defined by Stern and Palmer (3) as those sequences which are present in at least two copies relative to other sequences in the genome, but which occur in multiple genomic environments. If a sequence is repeated once, the four combinations of sequences flanking the repeat would produce four genomic environments as a result of recombination within the repeat. If a sequence is present in three copies surrounded by different flanking sequences, then nine possible combinations of flanking sequences would be predicted.

The number of direct and inverted recombination repeats determines the complexity of a mitochondrial genome. Maize, wheat and sugarbeet have several repeats; as a result, the predicted molecular organizations of these mt genomes are very complex (4–9). Except for sugarbeet, the previously mapped dicots, spinach, sunflower and several *Brassica* species, have no more than one repeat and therefore a much simpler genome organization (10–12). The sequences which act as recombination repeats differ even between closely related cytoplasms (5,13).

A complete physical map of a mitochondrial genome in the family Solanaceae has not previously been described. Here we present our analysis of the fertile *Petunia hybrida* line 3704, which contains one of the largest and most complex dicot mitochondrial genome yet observed.

MATERIALS AND METHODS

DNA isolation and library construction

DNA was isolated from suspension cultures of *Petunia hybrida* line 3704 as described (14). A cosmid library of mt DNA, which was partially digested with EcoRI and size selected for insert size of 30–45 kb, was prepared in the cosmid vector pJB8 (15) according to standard procedures (16). Packaging extracts (Promega) were used according to the suppliers instructions. Approximately 20,000 colonies per microgram of insert DNA were obtained. A total of 384 colonies were picked into 96-well plates and used in the genome walking.

DNA manipulations and cosmid mapping

All DNA manipulations were carried out according to standard protocols (16), unless otherwise indicated. DNA fragments were purified from gel-slices using the IBI Electrolutor or GeneClean (BIO101) according to suppliers' instructions. DNA fragments were labelled by nick translation or random hexamer labelling (17). Cosmids were labelled by nick-translation. Cosmids were mapped using the *Lambda* terminase procedure of Rackwitz et al. (18). *Lambda* terminase was expressed in and purified from *E.coli* cells as published (19). High molecular weight markers (obtained from BRL) were end-labelled with T4 DNA polymerase. Oligonucleotides complementary to cos-L and cos-R (ON-L and ON-R) were obtained from New England Biolabs, and labelled using T4 kinase and [γ - 32 P] ATP or terminal transferase and [α - 32 P] dCTP (20). All restriction enzymes and DNA modifying enzymes were purchased from BRL. IBI-Pustell software (IBI) was used for sizing of restriction fragments and bands in the terminase mapping autoradiograms on standard and on FIGE gels.

Field Inversion Gel Electrophoresis

FIGE was carried out on a home-made apparatus essentially as described (21). Electrophoresis was carried out for 40 hours, at 150 volts. The switching regime was 0.5 seconds forward, 0.25 seconds reverse polarity. Buffer was recirculated at 200 ml per minute and cooled to 15°C. The cubic spline method of the IBI-Pustell software was used to estimate the molecular weights of fragments run on FIGE.

RESULTS

A physical map of the Petunia mitochondrial genome.

A restriction map of the mt genome of *Petunia hybrida* line 3704 was created by genome walking in a library produced in the cosmid vector pJB8. Starting points for genome walking were produced by isolating cosmids hybridizing to probes for the mitochondrial genes *atp9* and *coxII*. All cosmids of interest were used as hybridization probes against Southern blots of mtDNA digested with several restriction enzymes to verify that the inserted DNA correctly represented the genomic sequence.

The restriction map of the 3704 genome, based on the combined data of 35 overlapping cosmids, is shown in figure 1. The genome is circularly permuted; the map should be read from left to right and from top to bottom. Four enzymes were used in the restriction mapping: PstI, SstI, SalI and BamHI. The total size of the genome (443 kb) was calculated

Figure 1. The restriction map of the *Petunia hybrida* 3704 mitochondrial genome. Restriction maps for PstI (P), SstI (T), SalI (S) and BamHI (B) of overlapping cosmids were used to construct a physical map of the mitochondrial genome. The location and orientation of the recombination repeat copies is indicated by the arrow. The similarity extending upstream of the repeat in two of the three copies is indicated by the dashed line. The numbers of the repeat copies (P8.9, P25.8 and P19.6) correspond to the size of PstI fragments covering the repeat copies indicated. The 2.6 kb SalI fragment and the 1.6 kb BamHI fragment common to all repeat copies are marked with asterisks. Lower case letters designate the different repeat flanks according to figure 3. The locations of all identified mitochondrial genes are indicated by lines pointing toward the area to which specific probes hybridized.

by averaging of the summation of the restriction fragment sizes for each enzyme. The resolution of the map is approximately 0.3 kb; fragments smaller than that may have gone undetected.

One major repeated sequence was identified during analysis of cosmids isolated with the *coxII* probe. This repeat occurs in three copies on the master circle. Two copies (P19.6 and P25.8), separated by 199 kb of single copy DNA, are present in the direct orientation. A third copy (P8.9), separated from the former two by unique DNA regions of 99 and 145 kb, is inverted in orientation. The region common to all three repeats contained within the 2.3 kb SalI and 1.6 kb BamHI fragments is indicated by the horizontal solid arrow. However, the two repeat copies which are in the direct orientation (P19.6 and P25.8) are similar for an additional 2.6 kb upstream (indicated by the dashed line extending the arrow).

Table 1. Heterologous DNA probes used in the localization of mitochondrial genes on the physical map of the *Petunia hybrida* line 3704 mitochondrial genome.

GENE ¹	PROBE ^b	SOURCE
<i>atpA</i>	2.5 kb EcoRI-HindIII (TA22)	maize (CMS-T), Braun and Levings (23)
<i>atp6</i>	1.0 kb EcoRI-HindIII (T25H)	maize (CMS-T), Dewey <i>et al.</i> (24)
<i>atp9</i>	0.28 kb EcoRI-BamHI	<i>Petunia hybrida</i> (3704), Young <i>et al.</i> (22)
<i>cob</i>	0.1 kb Sau3A (M11d)	wheat, Boer <i>et al.</i> (25)
<i>coxI</i>	0.8 kb PstI-HindIII (L4C)	wheat, Bonen <i>et al.</i> (26)
<i>coxII</i>	2.4 kb EcoRI (pZME-1)	maize (N), Fox and Leaver (27)
<i>coxIII</i>	0.9 kb EcoRI-PstI	<i>Oenothera berteriana</i> , Hiesel <i>et al.</i> (28)
<i>nadI</i>	0.97 kb BamHI-HindIII (BH96)	watermelon, Stern <i>et al.</i> (29)
<i>nad4</i>	0.82 kb BglII-HindIII	soybean, Wintz <i>et al.</i> (30)
<i>nad5</i>	1.4 kb HindIII-BamHI (D1D1)	wheat, Bonen (unpublished)
<i>orf25</i>	GCGCCTAAGTGCGAAAAG	Stamper <i>et al.</i> (31)
<i>rps13</i>	0.32 kb AluI	tobacco, Bland <i>et al.</i> (32)
<i>rrn18</i>	3.2 kb PstI (2C70-Pst3.2)	maize, Stern <i>et al.</i> (33)
<i>rrn5</i>	85 bp HpaII-XmnI (206NX)	maize, Maloney (unpublished)
<i>rrn26</i>	4.8 kb SmaI (2C13-Sma4.8)	maize, Stern <i>et al.</i> (33)

The region contained within the 1.6 and 1.0 kb SalI fragments was shown to be identical by restriction mapping and Southern hybridization (data not shown). In addition, similarity also extends further downstream in two repeat copies (P8.9 and P25.8) through the coding sequence of two slightly different *coxII* genes (indicated by the black box and black box with open extension). The repeat flanks are identical up to the point where the two *coxII* genes diverge (22).

Mitochondrial genes in *Petunia*

All of the known mt genes of higher plants listed in Table 1 have been identified in *Petunia* using heterologous probes in hybridizations to Southern blots. The approximate location of genes was determined by hybridization to BamHI- or SalI-digested DNA of 18 cosmids covering the entire mt genome. The exact location of each gene was confirmed by hybridization to Southern blots of individual cosmids digested with the four enzymes used in this study. The results of this analysis are summarized in figure 2, where the genome is represented as a circle, and the three copies of the recombination repeat are indicated by solid boxes on the circle. The locations of the identified mt genes are indicated outside the circle.

Several mt genes are repeated two or more times. The gene for cytochrome oxidase subunit II is present in two copies, *coxII-1* and *coxII-2*, each adjacent to a different copy of the repeat (P8.9 and P25.8). Both genes are transcribed and differ in their extreme 3' end. The divergence occurs four codons before the C-terminus of *coxII-1*; the *coxII-2* reading frame continues for another forty-eight codons (22). The ATP synthase subunit 9 gene occurs in four copies, two of which have been previously isolated and characterized in our laboratory (*atp9-1*, 34; *atp9-2*, 35). Both are transcribed and have identical coding sequences, but diverge in their upstream and downstream flanking sequences. The remaining two copies of *atp9* are currently under investigation. In addition to one full length copy of the ATP synthase subunit alpha gene (*atpA-1*) another truncated copy (*atpA-2*) was identified. All other mitochondrial genes that have been identified are present in only one copy. In figure 2 the location of the following genes is shown: cytochrome oxidase subunits I, II and III (*coxI*, *coxII-1* and *coxII-2*, *coxIII*), ATP synthase subunits 6, 9 and alpha (*atp6*,

Figure 2. Location of mitochondrial genes and regions of chloroplast similarity on the master circle of the mitochondrial genome. Repeat copies are indicated by the black boxes, numbering and flanking sequences as in figure 1. The locations of mitochondrial genes are indicated outside the mastercircle. The approximate location of regions of similarity to the chloroplast genome are indicated by rectangles inside the circle.

atp9-1, *atp9-2*, *atp9-3*, *atp9-4*, *atpA-1* and *atpA-2*), cytochrome b apoprotein (*cob*), NADH dehydrogenase subunits 1, 3, 4 and 5 (*nad1*, *nad3*, *nad4*, *nad5*), ribosomal small subunit proteins 12 and 13 (*rps12*, *rps13*), 26S, 18S and 5S ribosomal RNA's (*rrn26*, *rrn18*, and *rrn5*). The location of the *orf25* gene was determined using an oligonucleotide probe designed from an internal sequence known to be conserved between maize and tobacco *orf25* genes (31; Table 1). Preliminary sequence analysis confirmed the identification of the *Petunia orf25* gene (data not shown). The *orf25* gene is found immediately adjacent to the third copy of the recombination repeat.

Sequences hybridizing to the chloroplast genome

Thirteen different regions of the mitochondrial genome with sequence similarity to the chloroplast (cp) genome were identified. MtDNA was hybridized with purified inserts from a *Petunia* chloroplast library covering the entire chloroplast genome. These regions are indicated by the heavy solid lines inside the circle in figure 2. The size of the heavy lines is not indicative of the amount of similarity present, but rather refers to the smallest area of similarity that could be defined by hybridization of insert probes to Southern blots of cosmids digested with the four enzymes used in the mapping studies. Certain regions hybridized to more than one of the chloroplast probes.

The maximum amount of mt DNA which appears to be derived from the chloroplast genome does not add up to more than five percent of the total genome. Some regions of similarity which were detected may actually represent cross-hybridization between genuine chloroplast genes and mitochondrial genes. There is, for example, considerable

Figure 3. A. Restriction maps of the nine different genomic environments of the three repeat copies resulting from intra-molecular recombination. The area present in all three repeat copies is indicated by the horizontal arrow. The similarity extends upstream in flanks b and c, indicated by the dashed line in front of the arrow. The similarity extending downstream to the point of divergence between the *coxII-1* and *coxII-2* genes in flanks d and f, is indicated by the black rectangle (*coxII-1*) and black rectangle with open extension (*coxII-2*). The open extension on *coxII-2* indicates the extension of the open reading frame relative to the normal gene. The open square in flank e indicates the *orf25* gene. Lower case letters designate the different flanking regions. The numbers of the different repeat copies shown on the right are the predicted sizes of repeat containing PstI fragments, based on restriction maps of overlapping cosmids. **B.** Hybridization to field inversion gels of PstI-digested mitochondrial DNA with a repeat internal 1.6 kb BamHI probe (left panel) and a 2.3 kb HindIII *coxII* probe (right panel). The sizes of the hybridizing restriction fragments are indicated in the center.

sequence conservation between the 18S rRNA gene of mitochondria and the 16S rRNA gene of chloroplasts. Sequence analysis will be necessary to identify exactly which chloroplast sequences are present in the mt genome. By comparison of published sequences, a short region of similarity to the tobacco chloroplast *psbB* gene was found immediately upstream of the *Petunia* mitochondrial *atp9-1* gene (K. Wolfe, personal communication). *Characterization of the recombination repeat*

If the large reiterated *Petunia* sequence functions as a mitochondrial recombination repeat, we would expect to find it present in nine (3^2) genomic environments, resulting from recombination occurring in the area common to all three copies. Cosmids representing the nine genomic contexts of the repeat were isolated and mapped.

The results of the restriction mapping are shown in figure 3A. Each PstI fragment shown represents a unique copy of the repeat with its surrounding sequences. The size of each PstI fragment is shown on the right. Each contains the area common to all of the three copies of the repeat present on the master circle (indicated by the solid arrow, according to the restriction map of figure 1). Each PstI fragment also contains one out of three upstream

Figure 4. A model proposing the origin of the third repeat copy and its downstream abnormal *coxII-2* gene. Duplication of the entire repeat and its flanking sequences in fragment P15.5 (indicated by the area surrounded by the dashed box) in a progenitor genome, followed by integration in the master circle could have generated the third repeat copy and second *coxII* gene. The DNA region into which insertion may have occurred is represented by the unknown PstI fragment, but probably contained a small open reading frame (indicated by the small open rectangle). The insertion may have fused this small ORF to the majority of *coxII-1* coding sequence duplicated in the event, thus creating the extended *coxII-2* copy. The resulting repeat-containing fragment is represented by P25.8. Lower case letters indicate the different repeat flanks, enzymes and *coxII* genes are denoted as in figure 3A.

flanking DNA regions, and one out of three downstream flanking DNA areas. Altogether the nine different PstI fragments represent nine different combinations of flanking sequences. These PstI fragments can be interconverted if recombination occurs in the area in common between PstI fragments. For example, the PstI fragments P28.2 and P8.9 could, through a reciprocal recombination event, give rise to the P21.4 and P15.6 fragments.

In hybridizations with a repeat probe to Southern blots of PstI digested DNA, nine hybridizing bands would be expected. Because several fragments are large and close in size, FIGE was employed to separate them. Figure 3B shows hybridization to a Southern blot of a FIGE gel containing PstI digested mtDNA. The left panel was hybridized with a 1.6kb BamHI fragment internal to the repeat, and visualizes eight out of nine PstI fragments. Only the 13.2 and 13.0 kb fragments comigrate. In the right panel the same blot was hybridized with a *coxII* coding sequence probe, and visualizes only six out of nine PstI fragments as expected. The size of hybridizing restriction fragments determined in FIGE agrees well with the size of PstI fragments predicted by the mapping data.

Recombination probably takes place within the DNA region that is present in all three copies (indicated by the solid arrow in fig.1 and 3A). It is possible that during recent evolution the *Petunia* line 3704 has acquired an additional long copy (represented by P25.8 in figure 3A.) by a duplication event in an unknown progenitor genome. A hypothesis which proposes the creation of this third repeat copy is shown in figure 4. Duplication or excision of the entire repeat and approximately 2.6 kb of DNA upstream and most of the downstream *coxII-1* gene present in the P15.5 repeat area may have occurred. According to the model, this DNA region, indicated by the heavy line, subsequently integrated in either a subgenomic circle or in another mastercircle present in the same

Figure 5. Additional repeated sequences in the mitochondrial genome. **Left panel:** Hybridization of a 138 bp RsaI-HpaII fragment of the tobacco *atp9-rps13* intergenic region to BamHI digested *Petunia* mitochondrial DNA identifies multiple homologous fragments. The size of the BamHI fragments are shown on the right and the mt genes which they are associated with are indicated on the left. The lower case letters a, b and c indicate hybridization of the 138pb probe to the repeat upstream flanks, which are numbered according to figure 3A. **Right panel:** Hybridization to the same Southern blot using a 396 bp AluI fragment from the 5' flank of tobacco *atp9*. Different BamHI fragments are detected with this probe; their sizes are indicated on the right.

mitochondrion. The PstI fragment in which it inserted could have contained an open reading frame (indicated by the small open rectangle) to which the *coxII-1* coding region was fused in frame. This fusion could extend the *coxII-1* reading frame with an additional forty-eight codons, thus creating *coxII-2*. The flanking sequences in the repeat-containing fragment P25.8, which could be the result of such an event, were in this model provided by the DNA regions flanking the insertion point. The unusual *coxII-2* gene is absent from the CMS line 3688 genome (22), which supports the hypothesis that it was created by an integration event occurring in line 3704. Alternatively, its absence in line 3688 could be the result of a deletion.

Recombinationally inactive repeated sequences

During the analysis of the *Petunia coxII* sequence, Pruitt and Hanson found that a 95 bp sequence between -165 and -71 relative to the initiation codon was almost identical to part of the region between *atp9* and *rps13* in tobacco (22). Further searches against the GenBank nucleic acid database revealed that this same sequence could also be found upstream of the *coxI*, *coxIII* and *atp6* genes of *Oenothera berteriana*. In order to determine if this sequence was repeated in *Petunia* we purified and used as a probe a 138 bp RsaI-

Figure 6. Molecular organization of the *Petunia hybrida* line 3704 mitochondrial genome. Four different master circles and three subgenomic circles are predicted based on the restriction mapping data. The different circles are interconvertible by recombination occurring between different repeat units. Recombination repeats are indicated by the black rectangles, numbered according to figure 3A. The repeat flanking sequences are indicated by lower case letters, according to figure 3A as well.

HpaII fragment from the intergenic region of a tobacco *atp9-rps13* clone. Southern blots of mtDNA and cosmids covering the entire genome were probed simultaneously. In figure 5 (left panel), the result of the hybridization to BamHI-digested mtDNA is shown. Eight bands of variable intensity can be observed. All but one band in the digest were also observed in the Southern of BamHI-digested cosmids. Four regions of homology map very closely to the following mt genes: *atp6* (11.1 kb BamHI), *coxIII* (9.0 kb BamHI), *atpA* (7.8 kb BamHI) and *coxII* (1.65 kb BamHI). Sequence analysis of these regions is required to determine the exact extent and location of homology to the 138 bp probe. We observed very faint hybridization to the 5.6 kb BamHI fragment that also harbors the *rps13* gene. The hybridization to the 5' flank of *coxII* maps outside of the recombination repeat, since the area between repeat P19.6 and *ORF25* does not hybridize with this probe. The 138 bp probe also hybridizes to the approximate area of the repeat where the upstream sequences diverge (12.0, 6.5 and 6.1 kb BamHI fragments respectively).

The 16.0 kb BamHI fragment hybridizing with this probe does not have a corresponding BamHI fragment in the restriction digests of BamHI-digested cosmids. This is the only fragment that we cannot account for in the restriction map of the *Petunia* 3704 mt genome. It is possible that this fragment, present at sub-stoichiometric levels (Fig. 4A) is the product of a recombination event between one of the BamHI fragments hybridizing with this probe and another area in this genome. It is also possible that the fragment is carried on a subgenomic molecule not derived from the master circle. Further analysis of this fragment will be needed to clarify its origin and composition.

Another small fragment isolated from the region between 1.3 and 0.5 kb upstream of the tobacco *atp9* ATG was found to be highly reiterated in the *Petunia* mt genome. A 396 bp AluI fragment, and the 0.8 kb PstI-BamHI fragment from which it was isolated, hybridize to several bands in Southern of SalI- and BamHI-digested mt DNA.

Approximately 8 bands of variable intensity were detectable in the BamHI digest (Fig. 5, right panel), all of which are seen in Southern blots of cosmids digested with BamHI. This was also true for any additional bands detected after longer exposures, indicating that none of the fragments containing regions of similarity to this 396 bp AluI fragment are involved in recombination at a detectable rate.

Structure of the mitochondrial genome

The molecular organization of the *Petunia hybrida* 3704 mt genome is complex because all three copies of the repeat recombine. The different products of the genome rearrangement predicted from recombination between the repeats are shown in figure 6. Recombination between the two inverted repeats results in inversion of the unique region between the repeats relative to the other unique regions. Four different mastercircles therefore exist, each with a different arrangement of unique regions. The mastercircle which is shown in figure 2 was arbitrarily chosen.

Recombination between the two direct repeats results in the production of two subgenomic circles of 200 and 243 kb. The larger of the two exists in two forms as a result of recombination between the two inverted repeats. One of these inverted repeats is formed in the recombination event which generated the subgenomic circle, and the other was located between the direct repeats.

In the Southern hybridization of the repeat internal probe to a PstI digest of mt DNA (figure 3B, left panel) not all bands appear to have the same intensity. This suggests that not all recombination products are present at stoichiometric levels. However, the reduced intensity of the higher molecular weight bands may actually be the result of the effect of breakage of mt DNA during isolation and sample preparation.

DISCUSSION

At 443 kb the *Petunia hybrida* 3704 mitochondrial genome is the largest dicot mt genome mapped to date. The 3704 mt genome is also the only dicot found to contain 3 copies, two in direct and one in inverted orientation, of a sequence apparently active in recombination. Presently the prevailing view is that genomic isomers and subgenomic circles are the products of frequent recombination at repeats, rather than independent separately replicating molecules.

All genes present in other plant mitochondrial genomes have also been found in *Petunia hybrida* 3704. It appears that the gene content of plant mitochondrial genomes is quite conserved (for a review of all cloned plant mitochondrial genes see 36,37). At a gross genomic level there is little conservation of gene order between the *Petunia* mt genome and other mt genomes characterized previously. This is not unexpected, since it has been shown that the major force in evolution of mt genomes in *Brassica* is genome rearrangement (12). Some regional similarities between the *Petunia hybrida* 3704 mt genome and other characterized gene regions or genomes do occur. For example, the gene for the 5S rRNA (*rrn5*) is situated immediately downstream of the 18S rRNA gene (*rrn18*) as in most mt genomes. The *Petunia nad3* and *rps12* genes are separated by only 46 bp; this situation is similar in wheat, where these genes are separated by 47 bp (38,39).

A striking example of the plasticity of higher plant mitochondrial genomes is provided by comparison of the *Petunia atp9-2-rps13* region with the tobacco *atp9-rps13* DNA region. The *Petunia* 3704 mt genome contains four copies of the *atp9* gene, in contrast to the single *atp9* gene in tobacco. Comparison of the 5' and 3' flanking sequences of *Petunia atp9-1* and *atp9-2* (35) with the tobacco *atp9* flanking sequences (32) shows that the *Petunia*

atp9-1 region most resembles the tobacco *atp9* region. However, the *rps13* gene, in tobacco situated immediately downstream of *atp9*, in *Petunia* is present in the *atp9-2* 3' flank. In addition, the region between tobacco *atp9* and *rps13* is present in an entirely different location since no significant hybridization to the *atp9* and *rps13* regions in *Petunia* could be detected using a 138 bp *RsaI*-*HpaII* probe from the intergenic region in tobacco. In *Petunia* this sequence was also found to be reiterated approximately eight times. This observation suggests that extensive rearrangement, through recombination and duplication events, of a particular region of the common progenitor genome has occurred during the relatively short period of divergence of the *Petunia* and tobacco mitochondrial genomes.

The 138bp probe derived from the tobacco *rps13* 5' flank hybridized to five mt genes in *Petunia* (*coxII-1*, *coxII-2*, *coxIII*, *atp6* and *atpA-1*). This result corroborates and extends similar observations in tobacco mitochondria, where the same sequence similarity was detected upstream of *atp6* (40). In *Oenothera*, linkage to three mt genes was observed (*coxI*, *coxIII* and *atp6*). The regions homologous to the 138 bp fragment are all included in the transcripts of the *Oenothera* genes, the *Petunia* *coxII* genes and the tobacco *rps13* and *atp6* genes. The area of sequence identity 5' to the *coxI* and *coxIII* genes in *Oenothera* is part of a 657 bp conserved sequence block upstream of both genes, which has been implied to be involved in rearrangement of the *Oenothera* mt genome (28). In *Petunia* the recombination repeat is flanked at the right end in two of its three copies (to the right of the point of divergence between flanks d and f and flank e) by the 91 bp sequence present in this conserved sequence block. Similarity to the 138 bp probe, covering this 91 bp sequence is also found in the left end of all three repeat copies (just to the right of the point where flanks b and c diverge from flank a). While the function of this conserved and reiterated sequence is unknown, its location in the 5' flank of genes from several genera may point to a regulatory function, or to a role in creation of genomic organizational diversity.

A 396 bp *AluI* fragment isolated from a tobacco mt DNA clone also detected highly reiterated sequences in this *Petunia* mt genome. In contrast with the preceding observations, this sequence appears not to have any special features. It does not show linkage to any other known sequence, and is not involved in frequent or detectable recombination events. Recombination between different copies of this sequence must be very rare, if it occurs at all, since novel fragments hybridizing with the 396 bp probe could not be detected.

Short repeated sequences such as the two described above have been found in other plant mitochondrial genomes. They may be substrates for rare recombination events causing inversions or duplications of sections of the genome. Duplication of the tRNA-Pro gene in wheat may have involved recombination between short repeated sequences found in the proximity of the progenitor tRNA-Pro gene (41). Recombination at short repeats evidently occurred to produce male fertile revertants following genome rearrangements in the maize CMS-T mitochondrial genome (42). Recombinant mitochondrial genomes are also created during the process of somatic hybridization (43,44). It was previously shown that recombination occurred between two copies of *atp9*, 88-*atp9-1* from the sterile parent 3688 and 04-*atp9-2* from the fertile parent 3704, thus creating a novel gene, 133-*atp9-R*, in the somatic hybrid line 13-133 (45,56). Although recombination occurred between two *atp9* genes in the parental mitochondrial genomes following protoplast fusion, none of the four different *atp9* copies present in the 3704 genome described in this paper recombine at a detectable level (data not shown, 45).

In *Petunia* and other plant mitochondrial genomes, repeated sequences can be divided into two classes—one in which recombination occurs to maintain genomic isomers and

subgenomic circles, and another in which detectable recombination does not occur under normal conditions. This implies that general homologous recombination is not the mechanism through which multiple circles are produced. The recombination process must entail some sequence specificity, which is not yet understood. Under certain rare conditions, general homologous recombination may occur between short repeats, producing duplications or major genome rearrangements which become fixed. The recombination repeats remain one of the most intriguing mysteries regarding plant mitochondrial genomes. Only speculations can be made concerning their origin and effect on mitochondrial function.

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