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## Inheritance of mitochondrial DNA in Oenothera berteriana and Oenothera odorata Hybrids

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Restriction patterns of mitochondrial DNAs (mtDNA) from different *Oenothera berteriana* and *Oenothera odorata* bastards are compared and the identity of mtDNA in hybrid forms with different combinations of nuclear, plastid, and mitochondrial genomes has been determined. Inheritance of mtDNA in these hybrids follows the pattern predicted by the genetic analysis. One hybrid is shown to have a *Oenothera berteriana* nuclear and plastidal genome with a mitochondrial genome of the *Oenothera odorata* type.

Extrachromosomal inheritance has been studied in *Oenothera* subsection *Munzia* species with genetic analyses for a number of years [1, 2]. The molecular basis for traits inherited in a nonmendelian fashion will have to be looked for in the extrachromosomal genomes in the first place. Analysis of plastidal DNAs in Oenothera subsection Euoenothera has revealed those distinct sequence arrangements for the five plastomes which have been postulated from the genetic crosses [3]. Likewise the plastid DNA from Oenothera berteriana and Oenothera odorata of the subsection Munzia can be distinguished through their different fragment patterns generated by restriction enzymes [4, 5]. The identity of the chloroplast genomes could be verified in hybrids with different nuclear genomes.

We have now analysed restriction fragments of mitochondrial DNA (mtDNA) in a number of hybrids in order to determine inheritance origins of the mitochondrial genomes. Mitochondrial DNA was isolated from tissue cultures as previously described [6] and digested with restriction enzymes as indicated by the supplier (Boehringer, Mannheim). Gel electrophoresis was performed in horizontal or vertical agarose gels.

Two of these comparisons shall be discussed here: Fig. 1 shows a comparison of restriction fragments

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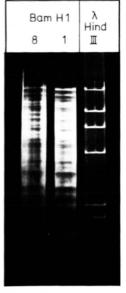


Fig. 1. Comparison of restriction fragments of mitochondrial DNA from *Oenothera berteriana* (track 1) and the hybrid  $B \cdot I$  (track 8). Both forms contain identical plastidal and mitochondrial genomes, but different nuclear chromosome complexes.

generated from one of the parent forms Oenothera berteriana (track "1" in the figure) with the restricted mitochondrial DNA from a hybrid form with a different nuclear genome. Oenothera berteriana contains the heterozygous nuclear genome of the chromosome complexes  $B \cdot I$ , whereas the hybrid analysed here (track "8" in Fig. 1) is heterozygous with the complexes  $B \cdot I$ . Both mitochondrial genomes appear to be identical as judged from restriction patterns of various enzymes (not shown).

The most interesting hybrid analysed, however, is the one named "2" by J. Schwemmle in 1938 [2]. This bastard was postulated in reciprocal crossings to have the *Oenothera berteriana* nuclear and plastid genome, but the mitochondrial genome of *Oenothera odorata*. The identity of the chloroplast genome was shown by Alt *et al.* (1982) [5] to be of *Oenothera berteriana* origin. We have now analysed the mitochondrial DNA in this hybrid and found that the mitochondrial genome is distinctly different from the *Oenothera berteriana* mitochondrial DNA (Figure 2) and identical to the *Oenothera odorata* mtDNA.



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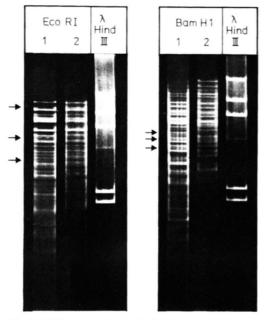


Fig. 2. Differences in restriction fragments generated with the enzymes Eco RI and Bam HI between the mitochondrial DNAs of *Oenothera berteriana* (lanes 1) and the hybrid "2" (lanes 2) are indicated by arrows. Hybrid "2" contains the same plastidal and nuclear genome, but *Oenothera odorata* mitochondrial DNA. Length standards in Fig. 1 and 2 are Hind III restriction fragments with the sizes (top to bottom): 23.6 kb; 9.46 kb; 6.78 kb; 4.34 kb; 2.26 kb; 1.98 kb. (The 0.56 kb fragment is not visible in these reproductions.)

In these experiments we could thus verify the postulated arrangement of the three genomes in these particular hybrids of *Oenothera*. It is now possible to compare in more detail DNA sequence arrangements of two genetically and physiologically

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distinguishable mitochondrial genomes [7, 8] in connection with a common nuclear and chloroplast genome background. With the hybrids available we can furthermore study the behaviour of identical mitochondrial genetic information combined with different nuclear genomes. We have begun to identify and sequence coding regions of Oenothera berteriana mtDNA [9] and want to identify functional information in the DNA sequences rearranged between the Oenothera berteriana and Oenothera odorata mtDNA. The transmission pattern of information necessary for the concerted cooperation of the different cellular genomes should have a molecular basis. In its expression the differences between the Oenothera mitochondrial genomes must play a role as they can be distinguished by their genetic behaviour.

Mitochondrial DNA of tobacco has been found to rearrange sequences rapidly in tissue cultures [10]. *Oenothera berteriana* mtDNA however, appears to be fairly stable, as restriction digests of the independently derived tissue cultures are identical for the parent form *Oenothera berteriana* and hybrid forms ("8") with the same mtDNA. These forms were separated more than 50 years ago and have been selfpollinated since then. Over this period of time and in spite of the transition into tissue culture cells, so far no change in the restriction patterns of mtDNA could be observed.

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