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Dwarfism and male sterility in interspecific hybrids of *Epilobium*

2. Expression of mitochondrial genes and structure of the mitochondrial DNA

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Summary. Mitochondrial DNA (mtDNA) and transcriptional patterns of mitochondrial genes have been examined in dwarf, normal, fertile and male sterile *Epilobium* hybrids. No alterations or rearrangements of mitochondrial DNA could be detected in the developmentally disturbed hybrids. They exhibit restriction patterns of mtDNA that correspond exactly to those of their female parents. However, the transcription of at least one mitochondrial gene is significantly altered in the male sterile hybrid *E. hirsutum* × *montanum*. In normal plants, one mRNA of 1.6 kb hybridizes to the cytochrome c oxidase subunit II gene, while in male sterile plants a transcript of this size is lacking and instead a major transcript of 2.0 kb and two smaller ones occur. The transcript pattern of the F_1 ATPase alpha subunit (*atpA*) gene exhibits slight alterations in sterile plants also. Since these hybrids have the same cytoplasm as normal plants, an incompatibility between the nuclear and the mitochondrial genotype may be responsible for the altered mitochondrial gene expression. No alteration of the transcripts of the mitochondrial genes tested could be detected in dwarf hybrids. The coincidence of male sterility with an altered transcription pattern of mitochondrial genes suggests that the mitochondria are involved in the occurrence of this phenotype.

Key words: *Epilobium* – Male sterility – Dwarfism – Mitochondrial DNA – Gene expression

Introduction

Differences in reciprocal crosses between various *Epilobium* species have been investigated intensively by classical crossing experiments (Renner and Kupper 1921; Geith 1924; Michaelis 1940, 1951; Michaelis and von Dellinghausen 1942; Michaelis and Wertz 1935). When the possibilities of preferential segregation, sex linkage or virus infections can be excluded, such differences may be considered to be determined by the cytoplasm (Jinks 1964). They arise because of unequal contribution of cytoplasmic determinats from the male and female gametes to the zygote and their inheritance is normally through the female parent. In *Epilobium* a number of approaches have been used to localize the genetic factors responsible for the occurrence of reciprocal differences (reviewed by Michaelis 1961, 1966). However, a clear determination of the genetic compartment responsible for the expression of such traits is often not very accessible by classical genetics.

Recently we have started to characterize the genetic material of plastids and mitochondria in different *Epilobium* species with molecular genetic methods (Schmitz and Kowallik 1986a, b; Schmitz 1988b). The plastome has been analysed in interspecific combinations where differences between reciprocal hybrids occur, and we have concluded that the plastids are most probably not responsible for the expression of the male sterile or the dwarfed phenotype (Schmitz 1988a). Because of these findings, we were interested in investigating whether the mitochondrial genetic compartment in these hybrids might be involved.

Materials and methods

Plant material and isolation of nucleic acids from mitochondria

The origin and source of the plant material has been described elsewhere (Schmitz and Kowallik 1986a). Growth conditions and crossing experiments have been reported in part 1 of this

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paper (Schmitz 1988 a). Nucleic acids from mitochondria of *Epilobium* leaves and stems were isolated as previously described (Schmitz 1988 b). Mitochondrial RNA (mtRNA) was purified immediately after lysis of mitochondria by several phenol/chloroform extractions and precipitation in 2 *M* LiCl at 4 °C for 10 h. It was stored in sterile TE-buffer (10 m*M* Tris/HCl, ph 7.5; 1 m*M* EDTA) at −70 °C.

Elektrophoresis, transfer and labeling of nucleic acids

MtDNA was digested with Bam HI and Eco RI according to the manufacturer's (Boehringer Mannheim) instructions. Restricted DNA was subjected to gel electrophoresis on 0.7% horizontal agarose gels. MtRNA was heat-denatured in the presence of formamide and formaldehyde according to Carmichael (1980) and separated on formaldehyde gels (1% agarose). The size of individual RNA species was determined using the BRL RNA ladder as a length standard. Nucleic acids were transferred to nitrocellulose filters (Schleicher and Schüll) according to Thomas (1980).

Mitochondrial gene probes from *Oenothera* were a kind gift of Dr. Brennicke, Tübingen. DNA fragments harbouring the genes for subunits I, II and III of cytochrome c oxidase and the alpha subunit of ATPase were isolated from bacterial plasmids and labelled with alpha-^{32}P dATP as described previously (Schmitz 1988 b). Hybridization was carried out for 24 h in the presence of 65% formamide, 5× SSC and 0.1% SDS at 42 °C. Using a Quanta II screen (Du Pont), exposure time was 4–7 days.

Results

Two types of reciprocal differences affecting plant development have been described in interspecific *Epilobium* crosses (Schmitz 1988 a). The cross between *E. lanceolatum* and *E. watsonii* produces heterogenous progeny, consisting of dwarf and normal plants. In two other crosses (*E. hirsutum* × *E. montanum* and *E. parviflorum* × *E. montanum*), a homogenous male sterile F_1 was found. Mitochondrial DNA from plants representing all of the F_1 types has been analysed with the restriction enzymes Bam HI and Eco RI. All *Epilobium* species tested so far have a species-specific restriction fragment pattern (Schmitz 1988 b). As shown in Fig. 1, mtDNA from dwarf plants exhibits the same restriction pattern as that from normal plants in the progeny of the cross *E. lanceolatum* × *E. watsonii*. Furthermore, the fragment pattern corresponds exactly to that of *E. lanceolatum*, indicating a maternal inheritance of mtDNA to dwarf and normal plants.

Figure 2 illustrates the analysis of mtDNA of the male sterile hybrids, the reciprocal fertile hybrids and the parental plants. The sterile plants show restriction patterns that differ from those of the reciprocal hybrids but are identical to those of their female parents. No rearrangements of the mtDNA or deviations from the maternal mode of mitochondrial inheritance are encountered in the male sterile hybrids.

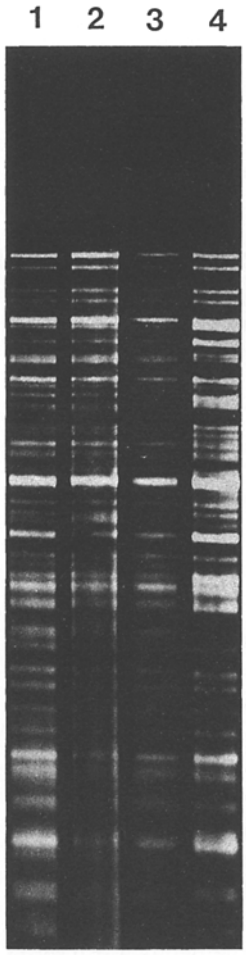

Fig. 1. Bam HI restriction fragment patterns of mtDNA from dwarf (*lane 3*) and normal hybrids (*lane 2*) of the cross *E. lanceolatum* × *E. watsonii*. The corresponding fragment patterns of the parental plants are shown in *lane 1* (*E. lanceolatum*) and *lane 4* (*E. watsonii*)

Since no alterations were found on the structural level of mtDNA in dwarf and male sterile hybrids, we wondered whether the expression of mitochondrial genes might be affected in these plants. Thus, we analysed the transcription of four mitochondrial genes using radioactively labelled gene probes from *Oenothera*. Hybridizations of mitochondrial genes coding for cytochrome c oxidase subunits I, II, III and the alpha subunit of ATPase against total mtRNA from *Epilobium* are illustrated in Figs. 3 and 4. In normal plants of different species (*E. lanceolatum*, Fig. 3 and *E. hirsutum*, Fig. 4), these genes hybridize to transcripts of the same size. The *cox* I gene shows a more complex transcription pattern with a large transcript of 6.0 kb and two smaller ones of 2.9 and 2.0 kb. In contrast, the *cox* II and *cox* III genes hybridize to single transcripts of 1.7 kb and 3.0 kb, respectively. Also, the *atpA* gene probe gives only one signal with a transcript of 2.1 kb in normal plants.

In dwarf hybrids of *E. lanceolatum* × *E. watsonii* the same transcript pattern of mitochondrial genes is found as in normal plants of *E. lanceolatum* (Fig. 3). In contrast, some mRNAs in the mitochondria of male sterile

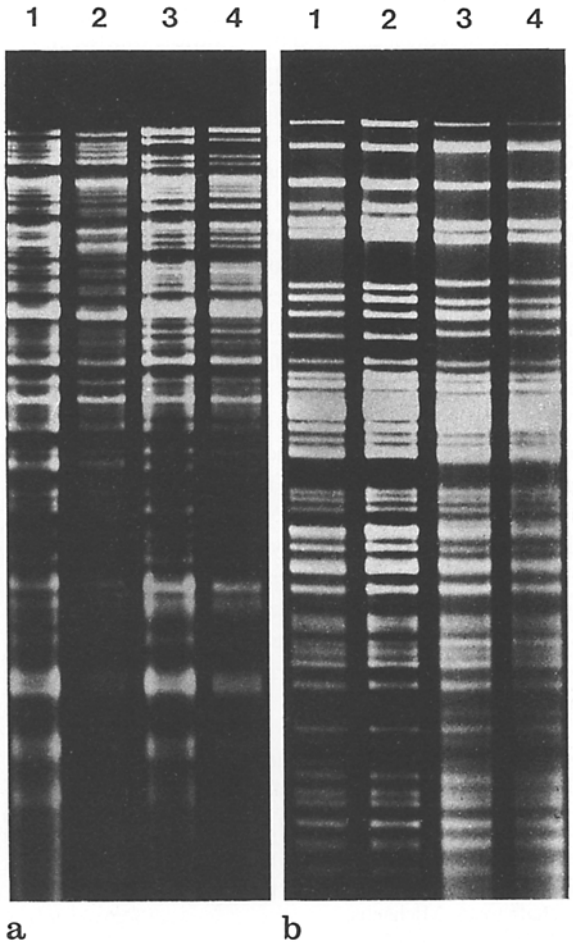

Fig. 2a and b. Gel electrophoresis of mtDNA from male sterile (*lane 2*) and fertile hybrids (*lane 3*) after restriction with EcoRI (**a**) and Bam HI (**b**). *Lanes 1* and *4* contain the restriction digests of mtDNA of the parental plants. **a**: *E. hirsutum* (*lane 1*), *E. hirsutum* × *E. montanum* (*lane 2*), *E. montanum* × *E. hirsutum* (*lane 3*), *E. montanum* (*lane 4*). **b**: *E. parviflorum* (*lane 1*), *E. parviflorum* × *E. montanum* (*lane 2*), *E. montanum* × *E. parviflorum* (*lane 3*), *E. montanum* (*lane 4*)

E. hirsutum × *E. montanum* differ from those found in normal *E. hirsutum*. Both plants have the same mitochondria as deduced from their mtDNA fragment patterns (Fig. 2), but their transcript patterns differ. As shown in Fig. 4, the 2.1 kb transcript for the *atpA* gene is faint in these hybrids and two mRNAs of 1.6 and 1.1 kb are found. While this alteration may not be significant, as the 2.1 kb mRNA is present in both fertile and sterile plants, the transcript pattern of the *cox* II gene of male sterile plants shows distinct differences in comparison to the normal plants. The 1.7 kb transcript found in normal *E. hirsutum* and *E. lanceolatum* is absent, even after long exposure time. Instead, three transcripts of 2.0, 1.2 and 0.8 kb hybridize to the *cox*II gene probe. Possibly, the two smaller transcripts occur due to degradation

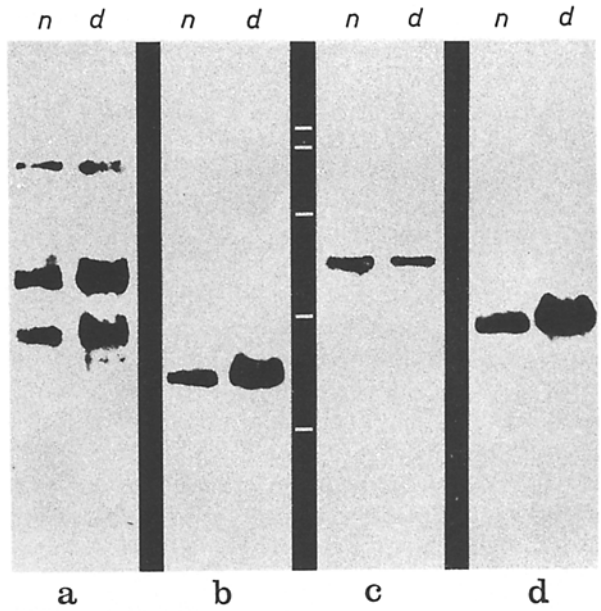

Fig. 3a–d. Northern hybridization of mitochondrial RNA from dwarf hybrids of the cross *E. lanceolatum* × *E. watsonii* (*d*) and the normal female parent *E. lanceolatum* (*n*). RNA was separated on formaldehyde gels (1% agarose), transferred to nitrocellulose and probed with radioactively labelled DNA, representing *Oenothera* mitochondrial genes coding for COXI (**a**), COXII (**b**), COXIII (**c**) and ATPA (**d**). The positions of RNA bands used as length standards (9.49 kb, 7.46 kb, 4.4 kb, 2.37 kb, 1.35 kb) are indicated as *white bars* between "**b**" and "**c**"

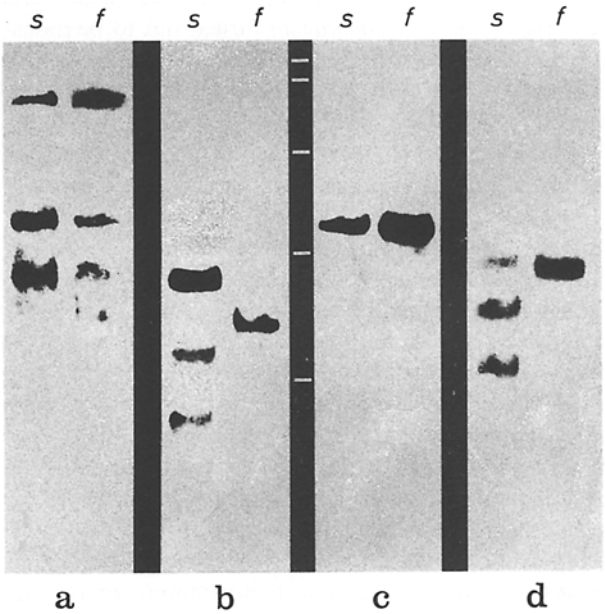

Fig. 4a–d. Northern hybridization of mtRNA from the male sterile hybrid *E. hirsutum* × *E. montanum* (*s*) and the fertile female parent *E. hirsutum* (*f*). Electrophoresis, hybridization conditions and probes used were identical to Fig. 3

of the 2.0 kb RNA at a specific site. The transcripts for the other two mitochondrially encoded subunits of the cytochrome c oxidase complex, COX I and COX III, have identical sizes in fertile and male sterile plants (Fig. 4a and c).

Discussion

As reported recently, distinctive differences in reciprocal crosses between certain *Epilobium* species occur in terms of dwarfism and male sterility (Schmitz 1988a). These morphological abnormalities do not seem to be correlated with the genetic compartment of the plastids, because no differences in plastid DNA organization or plastid gene expression could be detected between normal and developmentally impaired plants. In this report, the mitochondria have been analysed in dwarf and male sterile hybrids.

Mitochondrial DNA of *Epilobium* exhibits a complex restriction fragment pattern and is inherited maternally in normal hybrids (Schmitz 1988b). We show here that in dwarf and male sterile hybrids mitochondria are contributed to the progeny only via the gamete of the female parent. In contrast to *Sorghum*, where the occurrence of mtDNA alteration in different nuclear backgrounds has been reported (Bailey-Serres et al. 1986), in *Epilobium* no nuclear effect on the mitochondrial genome organization could be detected. The restriction fragment patterns of mitochondrial DNA in dwarf hybrids from the cross *E. lanceolatum* × *E. watsonii* and male sterile hybrids from the crosses *E. hirsutum* × *E. montanum* and *E. parviflorum* × *E. montanum* turned out to be indentical to those of the female parents. However, the nuclear background obviously has a distinct effect on the transcription of mitochondrial genes. In the male sterile hybrid *E. hirsutum* × *E. montanum* the transcript pattern of the *cox* II gene is significantly altered in comparison to the *E. hirsutum* maternal parent. These differences may reflect an incompatibility between the mitochondria of *E. hirsutum* and nuclear genes of *E. montanum*. This hypothesis is confirmed by the results of backcrosses. As reported earlier (Schmitz 1988a), the backcross of the male sterile hybrid with *E. montanum* once again produces a totally male sterile progeny. However, the backcross with *E. hirsutum* yields some fertile plants. The restoration of male fertility is probably due to the addition of certain genes from *E. hirsutum* that are required in the homozygous condition to allow proper pollen development.

As shown by Southern hybridization against restricted mtDNA, the fragments containing the *cox* II gene differ in size when *E. hirsutum* and *E. montanum* are compared (Schmitz 1988b). In contrast, the fragments carrying the *cox* I, *cox* III and *atpA* genes do not differ in an obvious way. Assuming that the coding region of the *cox* II gene is conserved, it will be interesting to determine whether species-specific differences in the adjacent regulatory regions of this gene are involved in the occurrence of variable transcripts in reciprocal hybrids.

While the influence of nuclear genes on the transcription of mitochondrial genes is very well established in yeast (Lisowski et al. 1987; Masters et al. 1987; Schmidt et al. 1987), for plant mitochondria our knowledge is very limited. In maize, recent reports have indicated that nuclear restorer genes alter the transcript pattern of a 13 kd protein within T-cytoplasm mitochondria (Dewey et al. 1986, 1987; Walker et al. 1987).

While the male sterile phenotype in *E. hirsutum* × *E. montanum* correlates with the occurrence of altered transcripts of mitochondrial genes, no such differences could be found between dwarf and normal plants. Further, analysis of these hybrids may allow us to determine whether the dwarf phenotype can be linked to a molecular alteration of a genetic factor of the cytoplasm. It will also be interesting to investigate in more detail how alterations of mitochondrial transcripts and male sterility are associated in *Epilobium*.

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