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CMS sources in sunflower: different origin but same mechanism?

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Abstract The presence of *orfH522*, *orfH708* and *orfH873* in the mtDNA, as well as the expression of mitochondrially encoded proteins, were investigated for 28 sources of cytoplasmic male sterility (CMS) and HA89, a fertile line of *Helianthus annuus*. The whole 5-kb insertion, found in PET1, is also present in all PET1-like CMS sources. However, with regard to the 11-kb inversion ANO1 demonstrated a different organization at the *cob* locus from the other PET1-like CMS sources. Only *orfH873* gave hybridization patterns in all investigated cytoplasms. For the fertile cytoplasm, as well as ANN4, ANN5, ANL1, ANL2, ARG2 and MAX1, hybridizations obtained with *orfH708* were highly polymorphic. Hybridization signals with *orfH522* were only detectable in the PET1-like CMS sources and MAX1. Comparing the mitochondrially encoded proteins of the CMS sources characteristic patterns could be detected for seven cytoplasms in addition to the PET1-like CMS sources expressing the 16-kDa protein. For ANN1 and ANN3 three CMS-associated proteins of 16.3 kDa, 16.9 kDa and 34.0 kDa could be identified among the *in organello* translation products. Also ANT1 expressed three additional proteins of 13.4 kDa, 17.8 kDa and 19.7 kDa, respectively. In ARG3 and RIG1 one protein of 17.5 kDa was missing and instead a new protein of 16.9 kDa appeared. In addition, in GIG1 and PET2 a unique protein of 12.4 kDa could be identified. These results indicate that certain types of cytoplasmic male sterility are preferentially present in sunflower.

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Introduction

Hybrid breeding leading to high-yielding F_1-plants requires an efficient and complete control of pollination. Up to now hybrid production in sunflower has relied on one system of cytoplasmic male sterility (CMS), the so called PET1 cytoplasm, which has been obtained by an interspecific cross between *Helianthus petiolaris* and *Helianthus annuus* (Leclercq 1969). Altogether, more than 50 CMS sources have been described in the genus *Helianthus* (Serieys 1996). However, no molecular characterization exists for these new CMS sources, and the corresponding maintainer and restorer lines are missing. Therefore, these male-sterile sources have not been used in commercial hybrid breeding although they might offer the chance to broaden the genetic basis in order to reduce the potential risk of vulnerability by pathogens. Apart from this, sunflower offers the possibility to obtain more general information about the CMS phenotype by the availability of a large number of CMS sources within one genus.

Due to its importance for hybrid breeding the molecular basis of PET1 has been carefully studied. Comparing the mitochondrial DNA organization of fertile and male-sterile lines (PET1), a region of approximately 17 kb, including a 5-kb insertion and an 11-kb inversion, proved to be rearranged (Siculella and Palmer 1988; Köhler et al. 1991). A new open reading frame *orfH522* in the 3′-flanking region of the *atpA* gene was found to be associated with the CMS phenotype (Köhler et al. 1991; Laver et al. 1991). Using specific antibodies against the gene product of *orfH522* it was demonstrated that *orfH522* encodes the 16-kDa protein (Monéger et al. 1994; Horn et al. 1996) which represents the unique difference between the *in organello*

translation products of fertile and male-sterile lines (Horn et al. 1991). Comparing the mitochondrially encoded proteins of 28 CMS sources in sunflower, nine additional male-sterile cytoplasms could be identified which also have the same CMS mechanism as PET1 (Horn et al. 1996). This was surprising as these CMS sources are reported to have a different origin. These cytoplasmic male-sterile germplasms have been produced either by different interspecific crosses involving *Helianthus argophyllus* (ARG1), *Helianthus neglectus* (NEG1), *Helianthus exilis* (EXI2), *Helianthus anomalus* (ANO1) and two subspecies of *Helianthus praecox* (PRR1, PRH1), or by mutagenesis of two maintainer lines for the PET1 cytoplasm (MUT1 and MUT2). In addition, one of the CMS types that appeared spontaneously (ANN10) expresses the 16-kDa protein. All these PET1-like cytoplasms have the same organization at the *atpA* locus (Hornet et al. 1996). In order to explain how, or whether, this CMS mechanism evolved several times independently it is necessary to know if the entire rearranged area is present in these cytoplasms. Southern hybridizations elucidated the organization of the mitochondrial DNA of these CMS cytoplasms with regard to the open reading frames *orfH522*, *orfH708* and *orfH873* and allowed a comparison with other CMS sources. In addition, new CMS-specific mitochondrially encoded proteins could be identified for seven CMS sources.

Materials and methods

CMS sources

The origin of the 28 investigated CMS sources, which were kindly provided by Dr. H. Serieys (INRA, Montpellier), is given in Table 1. Our study included CMS sources which occurred spontaneously within *H. annuus* as well as cytoplasms that originated from inter- or intra-specific crosses. Two of the CMS sources were induced by mutagenesis.

Isolation of the mitochondrial DNA

Mitochondria were isolated from etiolated sunflower seedlings by differential centrifugation as described by Horn et al. (1991). However, the mitochondria were not further purified by Percoll density gradient centrifugation but immediately treated with DNase and

Table 1 Overview of the investigated CMS sources (references are cited in Serieys 1996)

Name	FAO-code	Origin	Reference
Spontaneously occurring CMS sources			
H. annuus 367	ANN1	*H. annuus*	Serieys and Vincourt 1987
H. annuus 517	ANN2	*H. annuus*	Serieys and Vincourt 1987
H. annuus 519	ANN3	*H. annuus*	Serieys and Vincourt 1987
H. annuus 521	ANN4	*H. annuus*	Serieys and Vincourt 1987
NS-ANN-81	ANN5	*H. annuus*	Skoric 1988
AN-67	ANN10	*H. annuus*	Christov 1992
Intraspecific crosses			
Kouban	ANL1	*H. annuus* ssp. *lenticularis*	Anaschenko et al. 1974
Indiana1	ANL2	*H. annuus* ssp. *lenticularis*	Heiser 1982
Fundulea 1	ANT1	*H. annuus* ssp. *texanus*	Vranceanu et al.1986
Interspecific crosses			
Anomalus	ANO1	*H. anomalus*	Serieys and Vincourt 1987
Argophyllus	ARG1	*H. argophyllus*	Christov 1990
Argophyllus	ARG2	*H. argophyllus*	Christov 1990
Argophyllus	ARG3	*H. argophyllus*	Christov 1992
Bolanderi	BOL1	*H. bolanderi*	Serieys and Vincourt 1987
Exilis	EXI1	*H. exilis*	Serieys and Vincourt 1987
Exilis	EXI2	*H. exilis*	Serieys 1991
CMG2	GIG1	*H. giganteus*	Whelan 1981
CMG3	MAX1	*H. maximiliani*	Whelan and Dedio 1980
Neglectus	NEG1	*H. neglectus*	Serieys and Vincourt 1987
Fallax	PEF1	*H. petiolaris* ssp. *fallax*	Serieys and Vincourt 1987
PET/PET	PEP1	*H. petiolaris* ssp. *petiolaris*	Serieys and Vincourt 1987
Petiolaris	PET1	*H. petiolaris*	Leclercq 1969
CMG1	PET2	*H. petiolaris*	Whelan and Dedio 1980
PHIR 27	PRH1	*H. praecox* ssp. *hirsutus*	Christov 1993
PRUN 29	PRR1	*H. praecox* ssp. *runyonii*	Christov 1993
Vulpe	RIG1	*H. rigidus*	Vulpe 1972
Induced by mutagenesis			
HEMUS	MUT1	Irradiation of 'Hemus'	Christov 1993
PEREDOVIK	MUT2	Sonification of 'Peredovik'	Christov 1993

then centrifuged for 10 min at 10 000 rpm, 8°C. The sediments were dissolved in 1 ml of lysis buffer (50 mM Tris-HCl pH7.2, 5 mM EDTA) and frozen at −80°C. Isolation of the mitochondrial DNA was performed according to Rogers and Bendich (1985) using the CTAB procedure. The mtDNA was digested with *Sal*I or *Bgl*II and separated on 0.8% agarose gels. For Southern hybridizations specific probes for *orfH522* (*TaqI* fragment of 406 bp), *orfH708* (*Sma*I/*Hin*dII fragment of 339 bp), *orfH873* (*Sma*I/*Eco*RI fragment of 404 bp), and *cob* (*Eco*RI 1.5 kb fragment) were used. As all open reading frames share homology in the 5′-coding region with *orfB* (Köhler et al. 1991) it was important to exclude these regions from the probes to avoid cross reaction. The probes were labelled non-radioactively by the ECL system according the Amersham Life Science protocol.

In organello translation

Mitochondria of etiolated sunflower seedlings of the male-sterile and fertile lines were isolated by differential centrifugation and Percoll density gradient centrifugation. The mitochondrially encoded proteins were labelled by *in organello* translation with ^{35}S-methionine as described by Horn et al. (1991). After 90 min protein synthesis was stopped and aliquots were taken to estimate the incorporation of ^{35}S-methionine into the proteins. The mitochondria were washed and sedimented. Twenty seven CMS sources were investigated (Table 1). Due to a lack of seed material ANN2 could not be included in the study of the*in organello* translation products. For SDS-polyacrylamide gel-electrophoresis the labelled mitochondria were solubilized in Laemmli sample buffer and heated for 10 min at 80°C. Equal amounts of TCA-precipitable radioactivity were loaded into each lane. The gels were submitted to fluorography.

Results

Organization of the rearranged area in the PET1-like CMS sources

Hybridization with *orfH522* as a probe demonstrated that this CMS-specific open reading frame is localized in the 3′-flanking region of the *atpA* gene in nine other cytoplasms in addition to PET1 (Horn et al. 1996). However, it was unknown whether the whole 5-kb insertion also exists in these PET1-like cytoplasms (Fig. 1). Therefore, hybridizations were performed with *orfH522*, *orfH708*, and *cob* against mitochondrial DNA digested with *Sal*I (Fig. 2). The 9.2-kb fragment detected in all PET1-like CMS sources by *orfH522* covers the whole 5-kb insertion. As the last 228 bp of *orfH708* are present distal of the insertion in PET1 (Horn et al. 1995) this sequence can be used as a probe to verify the result; *orfH708* hybridized to the same mtDNA fragment as *orfH522*. The fertile line HA89 gave a characteristic band of 1.8 kb as *orfH708* is localized in the 3′-flanking region of the *cob* gene in the fertile mtDNA. Hybridizing the *cob* gene against the mitochondrial DNA revealed that ANO1 differs from the other PET1-like CMS sources which gave an 11.8-kb signal. ANO1 showed the same 9.9-kb fragment which is typical for the fertile line. This indicates that in ANO1, as in the fertile line, the *Sal*I restriction site in *orfH*708 (position 262) is still present in the 3′-flanking region of the *cob* gene.

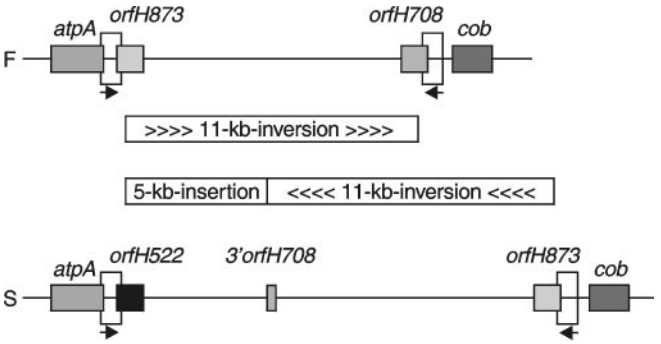

Fig. 1 Organization of the mitochondrial DNA flanked by the *atpA*- and the *cob*-gene of fertile und male-sterile (PET1) lines (Horn et al. 1995, modified according to Köhler et al. 1991). *Boxes with arrows* give the orientation of the inverted repeat of 261 bp

Molecular structure of other CMS sources

Three open reading frames, *orfH522*, *orfH708* and *orfH873*, have been identified in the rearranged area of PET1 (Fig. 1). Southern hybridizations were performed against *Bgl*II digested mtDNA using these orfs as probes. Apart from the PET1-like CMS sources (Horn et al. 1996) MAX1 is the only other CMS type which hybridized to *orfH522*, although it only has a homology of 93% (Hahn and Friedt 1994). Instead, the *orfH873* with an unknown function showed hybridization signals with all 28 investigated CMS sources and the fertile cytoplasm (Fig. 3). However, polymorphisms in the size of the restriction fragments could be observed. A 9.1-kb fragment was detected in ANO1, BOL1, EXI1, PEF1, PEP1 and MAX1. The other PET1-like CMS sources and ANN1, ANN2 and ANN3 showed a 7.9-kb fragment. The remaining ten CMS sources and the fertile cytoplasm shared a 7.4-kb fragment in the hybridization experiment with *orfH873*.

For *orfH708* the situation is different (Fig. 3); all CMS sources that showed a hybridization signal with *orfH522* (PET1-like and MAX1) also hybridized to *orfH708*. However, all PET1-like CMS sources showed a 1.1-kb signal whereas MAX1 had a unique band of 2.2 kb. Apart from these CMS sources only ANN4, ANN5, ANL1, ANL2, ARG2 and the fertile cytoplasm contain regions in the mitochondrial DNA that show homology to *orfH708*. The patterns obtained by *orfH708* for these CMS sources were highly polymorphic. In ANL1, ANL2 and the fertile cytoplasm *orfH708* hybridized to two fragments of 6.0 kb and 3.0 kb. ANN4 and ANN5 showed a signal of 1.2 kb. ARG2 had a single fragment of 3.0 kb, and more than one-third of the investigated CMS sources did not hybridize to *orfH708* at all.

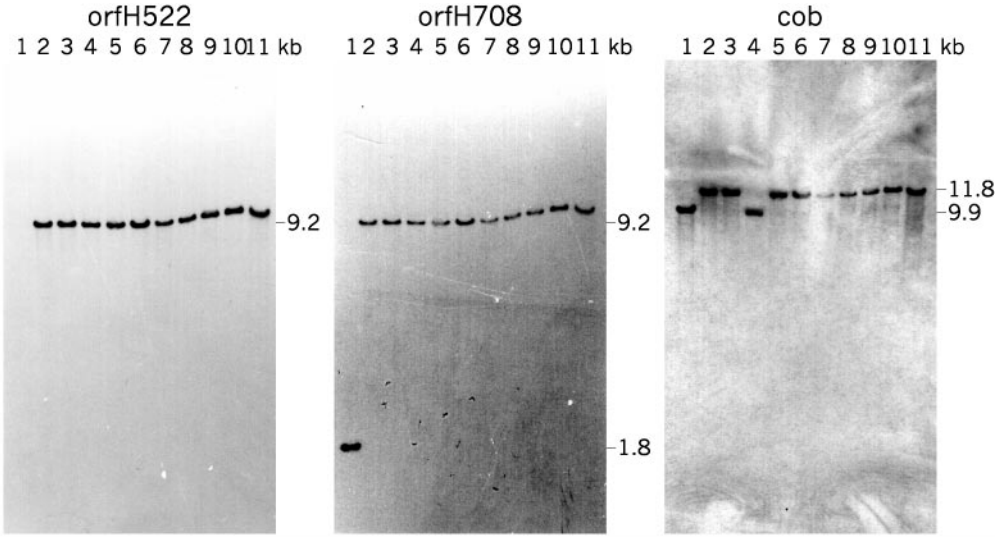

Fig. 2 Investigation of the presence of the 5-kb insertion in the PET1-like CMS sources. MtDNA digested with *SalI* was hybridized against *orfH522* (*Taq*I digested), *orfH708* (*Sma*I/*Hin*dII digested) and *cob* (*Eco*RI digested) as probes. *Lanes*: *1*, HA89; *2*, PET1; *3*, ANN10; *4*, ANO1; *5*, EXI2; *6*, ARG1; *7*, NEG1; *8*, PRH1; *9*, PRR1; *10*, MUT1; *11*, MUT2

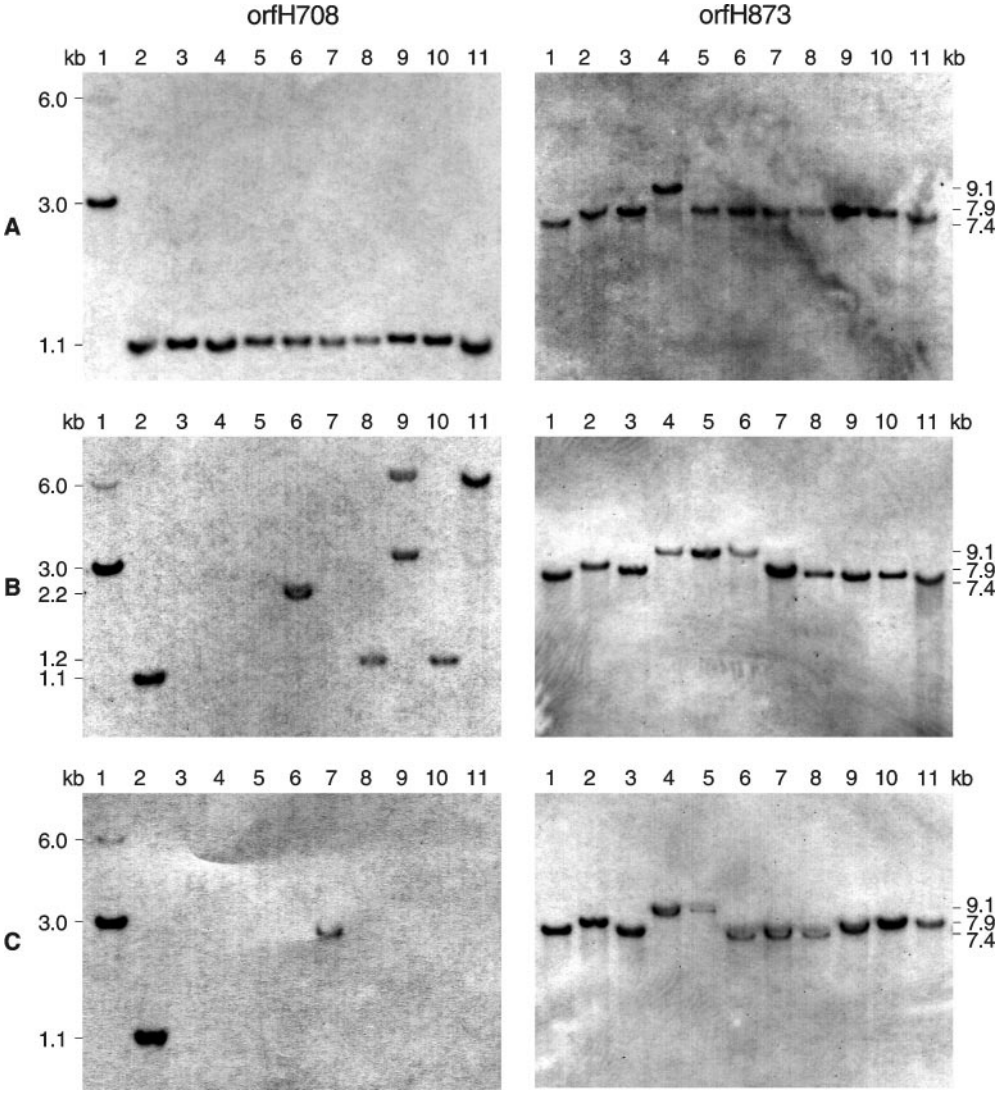

Fig. 3A–C Hybridization of the mitochondrial DNA of new CMS sources against *orfH708* and *orfH873*. The mtDNA was digested with *Bgl*II. **A** *Lanes: 1*, HA89; *2*, PET1; *3*, ANN10; *4*, ANO1; *5*, EXI2; *6*, ARG1; *7*, NEG1; *8*, PRH1; *9*, PRR1; *10*, MUT1; *11*, MUT2. **B** *Lanes: 1*, HA89; *2*, PET1; *3*, PET2; *4*, PEF1; *5*, PEP1; *6*, MAX1; *7*, ANT1; *8*, ANN5; *9*, ANL2; *10*, ANN4; *11*, ANL1. **C** *Lanes: 1*, HA89; *2*, PET1; *3*, GIG1; *4*, BOL1; *5*, EXI1; *6*, RIG1; *7*, ARG2; *8*, ARG3; *9*, ANN1; *10*, ANN2; *11*, ANN3

Identification of new CMS-specific proteins in sunflower

Comparing the mitochondrially encoded proteins of different CMS sources, 17 of the investigated 28 cytoplasms did not express the 16-kDa protein typical for the PET1-like cytoplasms. In addition these cytoplasm do not show any homology to *orfH522* at the mitochondrial DNA level (data not shown). The patterns of the mitochondrially encoded proteins obtained for the investigated cytoplasms showed a high similarity although they originate from different wild species of the genus *Helianthus*. Twenty *in organello* translation products ranging in molecular mass between 9 and 54 kDa were present in all CMS-inducing cytoplasms as well as in the fertile cytoplasm of *H. annuus*. For seven of the new CMS sources modifications in the pattern of the mitochondrially encoded proteins could be identified. ANN1 and ANN3, which occurred spontaneously in *H. annuus*, expressed three additional proteins with a molecular mass of 16.3, 16.9 and 34.0 kDa, respectively (Fig. 4 A). In GIG1 and PET2, originating from two different interspecific crosses, an additional protein of 12.4 kDa could be identified (Fig. 4 B). In ARG3 and RIG1 which had been developed by interspecific hybridizations with *H. argophyllus* and *Helianthus rigidus* as female, respectively, one protein of 17.5 kDa present in all other cytoplasms was missing and, instead, a new protein of 16.9 kDa appeared (Fig. 5 A). However, it is unknown whether the additional 16.9-kDa protein in ANN1 and ANN3 is identical with the protein in ARG3 and RIG1.

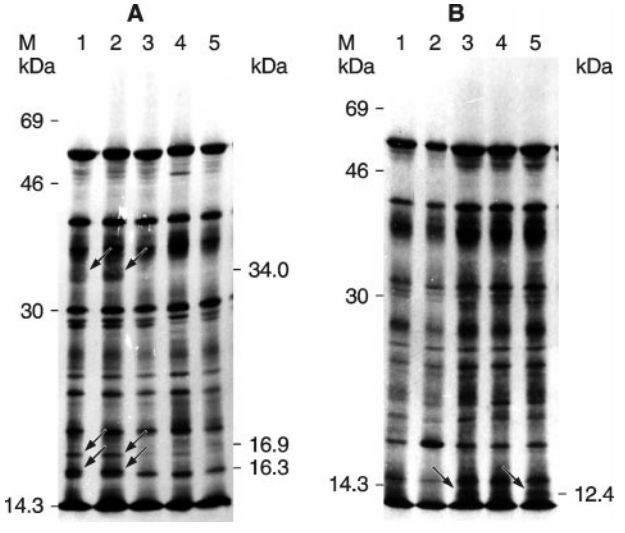

Fig. 4A, B Comparison of the *in organello* translation products of the new CMS sources. Mitochondrially encoded proteins were labelled with ^{35}S-methionine and separated on 12.5% SDS-polyacrylamide gels. Differences in the protein pattern are marked by *arrows*. **A** *Lanes: 1*, ANN1; *2*, ANN3; *3*, ANN4; *4*, ANL1; *5*, ANL2. **B** *Lanes: 1*, HA89; *2*, PET1, *3*, PET2; *4*, MAX1; *5*, GIG1

Also ANT1 coming from an intraspecific cross derived from *H. annuus* ssp. *texanus* was characterized by three additional proteins of 13.4, 17.8 and 19.7 kDa (Fig. 5 B). None of the ANT1-specific proteins were observed in the other cytoplasms. In the other male-sterility sources no differences in the protein patterns were observed. However, it cannot be excluded that CMS-specific proteins which were not detected by our analyses might also be present in these cytoplasms.

Discussion

The PET1-like CMS sources described here have a different origin. However, these CMS sources possess the same CMS mechanism characterized by the presence of *orfH522* and its gene product, i.e. the 16-kDa protein (Horn et al. 1996). Test crosses using nine restorer and two maintainer lines (data not shown), in addition to the molecular analyses, support this hypothesis. Southern hybridizations using *orfH522* and *orfH708* as probes revealed that in all PET1-like CMS sources the whole 5-kb insertion is also present. However, the occurrence of an *orfH522*-homologous region in MAX1 (Hahn and Friedt 1994) in a different environment demonstrates that other organizations of *orfH522* which do not seem to have any function may exist. In MAX1, this organization of the mitochondrial DNA was only preserved due to the simultaneous presence of another yet-unknown CMS mechanism.

In addition, ANO1 contains the whole 5-kb insertion and hybridization using *cob* as a probe revealed that the *Sal*I restriction site of *orfH708*, which is lost in the other PET1-like cytoplasms due to the inversion, is still present in ANO1. The evidence for different recombination events in MAX1 and ANO1 indicates that the rearranged areas of the PET1-like CMS sources may exist due to independent multiple-recombination events. However, the composition of the 5-kb insertion from sequences showing homology to the nuclear DNA, mitochondrial DNA and unknown sequences (Horn et al. 1995) makes it difficult to explain the different origin of cytoplasms if there is not a very strong selection for this configuration.

However, it is a fact that CMS sources from different interspecific crosses (Serieys 1996), e.g. *H. exilis* × *H. annuus* or *H. argophyllus* × *H. annuus*, lead both to CMS lines which express the 16-kDa protein (EXI2, ARG1) and to others having a different CMS mechanism (EXI1, ARG2 and ARG3).

Comparing the mitochondrially encoded proteins of additional CMS sources, new proteins were detected for groups of cytoplasms such as, for extample, ARG3/RIG1, ANN1/ANN3 and GIG1/PET2. For these CMS types we found the same situation as for the PET1-like cytoplasms (Horn et al. 1996), a different origin but the same CMS-associated proteins. Therefore,

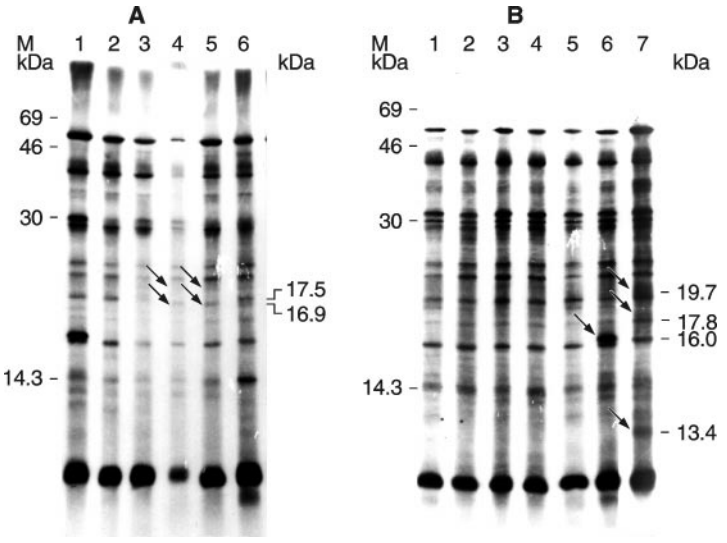

Fig. 5A, B Mitochondrially encoded proteins of the new CMS sources. Proteins were labelled with ^{35}S-methionine and separated on 16% SDS-polyacrylamide gels. Differences in the protein pattern are marked by *arrows*. **A** *Lanes: 1*, PET1; *2*, ARG2; *3*, ANN5; *4*, RIG1; *5*, ARG3; *6*, ANN3. **B** *Lanes: 1*, PEF1; *2*, PEP1; *3*, BOL1; *4*, EXI1; *5*, ANN4; *6*, ANO1; *7*, ANT1

it seems as if certain types of male sterility are preferentially present in sunflower. However, it is unknown whether the CMS-specific mtDNA configurations leading to the expression of these proteins were already been present in the wild *Helianthus* species or have been produced by interspecific hybridization.

For the CMS-associated *pvs*-region in common bean (Johns et al. 1992) it has been demonstrated that this sequence is widespread in *Phaseolus coccineus* and *Phaseolus vulgaris* (Hervieu et al. 1993). For rapeseed, PCR experiments identified the presence of normal and Ogura-type cytoplasms in Japanese wild radishes and in Asian radish cultivars (Yamagishi and Terachi 1996). However, Rieseberg et al. (1994) could not amplify PET1-specific fragments by PCR in the 55 accessions of *H. annuus* and 26 of *H. petiolaris* which they investigated.

According to another hypothesis the organization of the *atpA* locus typical for PET1 already exists in a very low concentration as a sublimon in fertile *H. annuus* (Laver et al. 1991; Monéger et al. 1994). Due to the interspecific hybridization this sublimon would only be amplified instead of being newly created.

One reason for the frequent occurrence of the PET1-like CMS sources is probably the selection of stable male-sterile types by the breeder, since recombination events which did not lead to male sterility were not maintained. Therefore, these configurations of the mtDNA are not currently available for molecular investigation. The configurations of the studied CMS sources do not represent the normal distribution of cytotypes in the wild species but rather a subclass selected for CMS.

The three investigated open reading frames, *orfH522*, *orfH708* and *orfH873*, which have been identified in the rearranged area in PET1 share homology with *orfB* in the 5′-coding region (Köhler et al. 1991). *OrfB* was first reported in *Oenothera* (Hiesel et al. 1987), later in wheat (Gualberto et al. 1991), and in normal as well as Ogura cytoplasm in rapeseed (Bonhomme et al. 1992). In wheat, the expression of *orfB* was immunologically verified (Gualberto et al. 1991). However, the role of *orfB* in higher plants has still to be elucidated. In sunflower it is localized in the flanking region of *coxIII* (Quagliariello et al. 1990). Although *orfB* in sunflower and wheat share a 90% homology in amino-acid sequence the antibody reacting with the *orfB* translation product in wheat did not detect a protein in the fertile or PET1 cytoplasm (data not shown). In sunflower, the first 57 bp of *orfH*522 are identical to *orfB* (Köhler et al. 1991) and 30 bp of these are also part of the 261-bp inverted repeat present at the *atpA*, *cob* and *coxIII* loci (Köhler et al. 1991). In rapeseed (*Brassica napus*), the 5′-coding region of the open reading frame *orf224* associated with 'Polima' CMS also shows 174-bp homology to *orfB* (Handa et al. 1995). In 'Ogura' rapeseed the CMS-specific *orf138* is flanked by a 209-bp repeat which includes the first 13 bp of *orf158*, the *orfB* equivalent in radish (Bonhomme et al. 1992). However, this coincidence might only exist because the presence of these sequences in several regions of the mitochondrial DNA, as e.g. described in sunflower (Quagliariello et al. 1990; Köhler et al. 1991), might up open the possibility for recombination leading to the creation of CMS-specific regions.

Whereas all investigated mtDNAs in sunflower show homology to *orfH873* only some of the CMS sources and the fertile cytoplasm react in Southern hybridizations with *orfH708*. In PET1, only the last 228 bp of *orfH708* are present (Horn et al. 1995). In addition, all PET1-like cytoplasms show the same *Bgl*II fragment in Southern hybridizations. Signals obtained with *orfH708* for the other CMS sources are highly polymorphic. Therefore, it is unclear whether the whole *orfH708*, or only parts of it, are present in these mtDNAs. No transcripts of *orfH708* and *orfH873* have been detected in etiolated seedlings of PET1 and the fertile cytoplasm (Köhler et al. 1991). This does not

exclude the possibility that these orfs might be transcribed in other tissues or other developmental stages.

However, the 3′-coding and 3′-flanking regions of the *atpA* gene represent a recombination hot spot due to the 261-bp inverted repeat (Köhler et al. 1991). Comparison of the *atpA* locus by hybridization revealed a considerable variation between different CMS sources which was not observed with other probes (Crouzillat et al. 1991, 1994).

The results of the mtDNA analyses and the patterns of the mitochondrially encoded proteins of the different CMS sources indicate either that certain configurations of the mitochondrial DNA are preferentially present in the wild species of the genus *Helianthus* or are newly created by interspecific hybridization. However, the occurrence of these configurations seems to be independent of the species.

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References

Bonhomme S, Budar F, Lancelin D, Small I, Defrance MC, Pelltier G (1992) Sequence and transcript analysis of the Nco2.5 Ogura-specific fragment correlated with cytoplasmic male-sterility in *Brassica* cybrids. Mol Gen Genet 235:340–348

Crouzillat D, de la Canal L, Perrault A, Ledoigt G, Vear F, Serieys H (1991) Cytoplasmic male sterility in sunflower: comparison of molecular biology and genetic studies. Plant Mol Biol 16:415–426

Crouzillat D, de la Canal L, Vear F, Serieys H, Ledoigt G (1994) Mitochondrial DNA RFLP and genetical studies of cytoplasmic male sterility in the sunflower (*Helianthus annuus*). Curr Genet 26:146–152

Gualberto JM, Bonnard G, Lamattina L, Grienenberger JM (1991) Expression of the wheat mitochonrial *nad3-rps12* transcription unit: correlation between editing and mRNA maturation. Plant Cell 3:1109–1120

Hahn V, Friedt W (1994) Molecular analysis of the cms-inducing MAX1 cytoplasm in sunflower. Theor Appl Genet 89:379–385

Handa H, Gualberto JM, Grienenberger JM (1995) Characterization of the mitochondrial *orfB* and its derivate, *orf224*, a chimeric open reading frame specific to one mitochondrial genome of the 'Polima' male-sterile cytoplasm in rapeseed (*Brassica napus* L.). Curr Genet 28:546–552

Hervieu F, Charbonnier L, Bannerot H, Pelletier G (1993) The cytoplasmic male-sterility (CMS) determinant of common bean is widespread in *Phaseolus coccineus* L. and *Phaseolus vulgaris* L. Curr Genet 24:149–155

Hiesel R, Schobel, Schuster W, Brennicke A (1987) The cytochrome subunit I and subunit III genes in *Oenothera* mitochondria are transcribed from identical promotor sequences. EMBO J 6:29–34

Horn R, Köhler RH, Zetsche K (1991) A mitochondrial 16-kDa protein is associated with cytoplasmic male sterility in sunflower. Plant Mol Biol 7:29–36

Horn R, Köhler RH, Lössl A, Kräuter R, Gerlach J, Hustedt JEG, Hahn V, Hain T, Zetsche K, Friedt W (1995) Development and molecular characterization of alloplasmatic CMS in *Helianthus annuus*. In: Kück U, Wricke G (eds) Advances in plant breeding, No. 18: pp. 89–110. Blackwell Wissenschaftsverlag, Berlin

Horn R, Hustedt JEG, Horstmeyer A, Hahnen J, Zetsche K, Friedt W (1996) The CMS-associated 16-kDa protein encoded by *orfH522* is also present in other male-sterile cytoplasms of sunflower. Plant Mol Biol 30:523–538

Johns C, Lu MQ, Lyznik A, Mackenzie S (1992) A mitochondrial DNA sequence is associated with abnormal pollen development in cytoplasmic male sterile bean plants. Plant Cell 4:435–449

Köhler RH, Horn R, Lössl A, Zetsche K (1991) Cytoplasmic male sterility in sunflower is correlated with the co-transcription of a new open reading frame with the *atpA* gene. Mol Gen Genet 227:369–376

Laver HK, Reynolds SJ, Moneger F Leaver CJ (1991) Mitochondrial genome organization and expression associated with cytoplasmic male sterility in sunflower (*Helianthus annuus*). Plant J 1:185–193

Leclercq P (1969) Une stérilité mâle chez le tournesol. Ann Amélior Plant 19:99–106

Monéger F, Smart CJ, Leaver CJ (1994) Nuclear restoration of cytoplasmic male sterility in sunflower is associated with tissue-specific regulation of a novel mitochondrial gene. EMBO J 13:8–17

Quagliariello C, Sariardi A, Gallerani R (1990) The cytochrome oxidase subunit III gene in sunflower mitochondria is co-transcribed with an open reading frame conserved in higher plants. Curr Genet 18:355–363

Rieseberg LH, Van Fossen C, Adrias D, Carter RL (1994) Cytoplasmic male sterility in sunflower: origin, inheritance, and frequency in natural populations. J Hered 85:233–238

Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol Biol 5:69–76

Serieys H (1996) Identification, study and utilisation in breeding programs of new CMS sources. FAO progress report. Helia 19 (special issue):144–158

Siculella L, Palmer JD (1988) Physical and gene organization of mitochondrial DNA in fertile and male-sterile sunflower. Nucleic Acids Res 16:3787–3799

Yamagishi, H, Terachi, T (1996) Molecular and biological studies on male-sterile cytoplasm in *Cruciferae*. III. Distribution of Ogura-type cytoplasm among Japanese wild radishes and Asian radish cultivars. Theor Appl Genet 93:325–332