Theor Appl Genet (1988) 76:587–592

Structural variations in *Vicia faba* mitochondrial genome

V. I. Negruk and N. K. Kaushik
Institute of Plant Physiology, USSR Academy of Sciences, Botanicheskaya 35, I27276, Moscow, USSR

Received February 10, 1988; Accepted February 28, 1988
Communicated by Y. Gleba

Summary. A comparative analysis of the presence of minicircular DNA CCCIB in 16 different lines and cultivars of fertile *Vicia faba* L. plants was conducted. It was found that copy number of CCCIB ranged from several copies per mitochondrial genome to – probably – zero, depending on cultivar or line. Fertility of plants in these cases was not altered. We chose 10 cultivars and lines among 16 analysed. Mitochondria of five cultivars and lines contained about two CCCIB molecules per one CCCIA. The sixth cultivar contained CCCIB at copy number several times lower. In the last four cultivars CCCIB could not be identified. Copy number analysis of CCC2 in ten chosen cultivars and lines revealed that in eight cases the quantitiy of CCC2 was equal to CCCIA. However, two other cultivars contained about two times lower quantity of CCC2. Parallel to that we observed an increase in quantity of one sequence homologous to CCC2, which in the first eight cultivars and lines could be found only in minor quantities. Comparative restriction analysis revealed notable rearrangement events in mitochondrial DNAs of ten cultivars and lines being investigated. We did not find any correlations between patterns of restriction fragments and copy number of CCCIB. In some cases, rearrangements in *Vicia faba* mitochondrial genomes caused a duplication of sequences homologous to the *Zea mays* coxII gene.

Key words: Mitochondrial DNA of plants – Minicircular DNA copy number – Molecular rearrangements – *Vicia faba*

Introduction

Comparative restriction analysis of mitochondrial DNAs isolated from fertile and cms plants and from callus tissues obtained from corresponding plants showed that mitochondrial genomes of this group of eukaryotic organisms undergo molecular rearrangements (Pring et al. 1977; Pring and Levings 1978; Kemble and Bedbrook 1980; Boutry and Briquet 1982; Leaver and Gray 1982; McNay et al. 1984; Kemble and Shepard 1984; Negruk et al. 1986). Such rearrangements could be identified by notable changes in ratios of different restriction fragments. Electron microscopic analysis of circular DNA molecules from mitochondria of whole plants and suspension cultures of *Vicia faba* demonstrated that these changes could, at least partly, be caused by alterations in the population of subgenomic circular DNA (Negruk et al. 1986). Based upon analysis of the molecular rearrangements of the mitochondrial genome after in vitro cultivation of higher plant cells, we suggest that this feature is generally specific for the mitochondrial genome and in vitro cultivation only increases its frequency. To verify this suggestion, comparative restriction analysis of mitrochondrial DNA isolated from different cultivars and lines of *Vicia faba* was done. A number of notable differences between mitochondrial genomes of different cultivars and lines were observed. These concern both restriction fragment patterns and sets of minicircular DNA molecules, as well as copy number of some regions of the mitochondrial genome.

Materials and methods

Plant material

Twenty eight different lines and cultivars of *Vicia faba* L. were used in this research. Fifteen lines were previously constructed in the laboratory of Prof. R. Rieger (A, B, C, D, E, F, G, H, I, K, N, EFK, BKH, DK-14 and BDK-14; Schubert et al. 1982). These lines and 11 cultivars of *Vicia faba* were kindly provided by Prof. R. Rieger and Dr. I. Schubert from the Zentralinstitut

Table 1. The content of minicircular DNA sequences CCCIA and CCCIB in 16 different lines and cultivars of *Vicia faba*

Subspecies and varieties	Lines and cultivars	CCCIA	CCCIB[a]
Subspecies *minor*	EFK	+	++
variety *minor*	BKH	+	++
	DK-14	+	++
	Fribo	+	+/−
	251	+	−
	199	+	++
	31	+	++
	91	+	++
	Aushra	+	++
Subspecies *faba*	Black Russians	+	++
variety *equina*	122	+	−
	39	+	++
	28	+	++
	163	+	−
Subspecies *faba*	148	+	++
variety *faba*	353	+	−

[a] ++ = quantity of CCCIB is an average twice that of CCCIA; +/− = quantity of CCCIB is several times less than CCCIA; − = CCCIB is completely absent

für Genetik und Kulturpflanzenforschung der Akademie der Wissenschaften der DDR in Gatersleben. Among the 11 cultivars are cv "Fribo"; the other 10 cultivars from different geographical regions of Europe and Asia are designated by catalog numbers: 251, 199, 31, 91, 122, 39, 28, 163, 148 and 353. Seeds of cv "Black Russians" were obtained from the All-Union Institute of Selection and Seed Production of Vegetable Crops, Moscow Distr., USSR. Cultivar "Aushra" was obtained from the Lithuanian Institute of Agriculture, Kedanski Distr., LiSSR. More detailed characteristics of lines and cultivars investigated are presented in Table 1.

Mitochondrial DNA isolation and analysis

Preparations of mitochondria were made according to Synenki et al. (1978). Mitochondrial DNA was isolated by a phenol deproteinization procedure. Mitochondrial DNA preparations digested by restriction endonucleases were analysed by electrophoresis in 0.8% agarose gels. The gels were stained with ethidium bromide and photographed under short-wavelength ultraviolet light. Southern blotting, nick translation and molecular hybridization were carried out according to Maniatis et al. (1982). Plasmid-like DNA sequences of *Vicia faba* mitochondria (Negruk et al. 1985) and cloned *Zea mays* COXII gene sequence, kindly supplied by C. S. Levings III via T. Börner, were used as probes.

Results

It was necessary to evaluate the stability of the minicircular DNA family in mitochondria of different lines and cultivars of *Vicia faba*. During earlier work with cv "Black Russians", we found a family of minicircular DNAs with sizes from 1–2 kb, in which three types of molecules called CCCIA, CCCIB and CCC2 were predominant (Negruk et al. 1982, 1985, 1986). In 1%–1.5% agarose gels these minicircular DNAs migrated as two bands. The slow migrating band contained similarly sized CCCIA and CCCIB sequences (of about 1.7 kb) showing different physical maps. For example, HindIII cut CCCIA into two large fragments 1.13 and 0.5 kb in length, respectively. CCCIB was cut by HindIII into one fragment 1.47 kb in length. Minicircular DNA CCC2 was located in the band with higher electrophoretical mobility. It was resistant to HindIII, but BamHI cut CCC2 at one site and yielded a 1.45 kb fragment. CCCIA and CCCIB were resistant to BamHI. Apparently, the same molecules were described by Goblet et al. (1985) and Wahleithner and Wolstenholme (1987). Comparing physical maps of minicircular DNA molecules isolated in our laboratory and in two other laboratories, we came to the conclusion that their primary structures are rather conservative.

CCCIA and CCC2 are regulary present in mitochondria of *Vicia faba*, but CCCIB was not found in all cases. Goblet et al. (1985) suggested the association of CCCIB with cytoplasmic male sterility (cms). In our case and in the case described from the laboratory of Wolstenholme (Wahleithner and Wolstenholme 1987) CCCIB was also found in mitochondria of fertile plants. To clarify the correlation between fertility and CCCIB disappearance we conducted a comparative hybridization analysis of the ^{32}P-labeled cloned CCCIB sequence with HindIII-fragments from 16 different lines and cultivars, which belong to all three varieties of *Vicia faba*: faba, equina and minor (Fig. 1 and Table 1). In many cases CCCIB was found in mitochondria of fertile plants and in all of them fertility was not decreased. The relative quantity of CCCIB could vary significantly: an average of two CCCIB molecules were found per one CCCIA molecule in some cases, CCCIB was completely lost in others, and as an intermediate case, the number of CCCIB molecules was several times less than CCCIA (Fig. 1 and Table 1).

Considering genetical reasons for intermediate case (Fig. 1 f) we could suggest two possibilities: (1) Existence of two types of mitochondrial genomes – with CCCIB (where the average copy number of CCCIB was two times that of CCCIA) and without CCCIB. In this case an intermediate variant probably contains the mixture of two types of mitochondrial genomes; and (2) Existence of at least three types of mitochondrial genomes – with CCCIB, without CCCIB and with low copy number of CCCIB. To select one of two possibilities we hybridized ^{32}P-labeled cloned CCCIB sequence to total DNA isolated from 11 individual seedlings of cv "Fribo" and digested with HindIII. These experiments (not shown) demonstrated that the copy number of CCCIB in each individual seedling was the same as in mtDNA preparation isolated simultaneously from several dozen cv

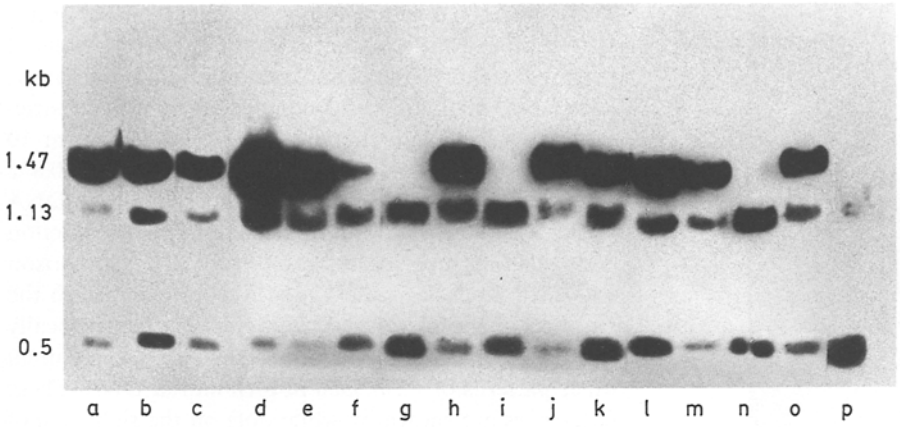

Fig. 1. 0.8% agarose gel electrophoresis of total mtDNA preparations from 6-day-old etiolated seedlings digested with HindIII-restriction endonuclease. The autoradiograph of the hybridization of ^{32}P-labeled cloned CCCIB to mtDNA from lines EFK (*a*), BKH (*b*), DK-14 (*c*), and cvs "Black Russians" (*d*), "Aushra" (*e*), "Fribo" (*f*), 251 (*g*), 199 (*h*), 122 (*i*), 31 (*j*), 39 (*k*), 28 (*l*), 91 (*m*), 163 (*n*), 148 (*o*) and 353 (*p*). The *numbers* at the left of the electropherogram (*a*) show the sizes of the CCCIA and CCCIB HindIII-fragments in kb

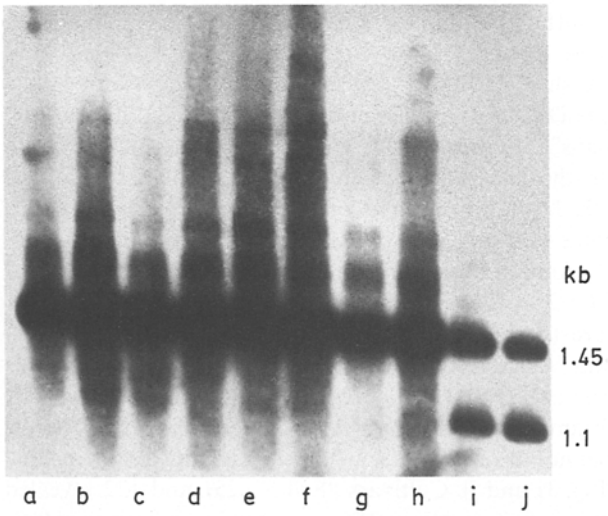

Fig. 2. 0.8% agarose gel electrophoresis of total mtDNA preparations from 6-day-old etiolated seedlings digested with BamHI-restriction endonuclease. The autoradiograph of the hybridization of ^{32}P-labeled cloned CCC2 sequence to mtDNA from lines EFK (*a*), BKH (*b*), DK-14 (*c*), and cvs "Black Russians" (*d*), "Aushra" (*a*), "Fribo" (*f*), 251 (*g*), 122 (*h*), 163 (*i*) and 353 (*j*). The *numbers* at the right of the electropherogram (*j*) show the sizes of CCC2 BamHI-fragment and smaller fragment homologous to CCC2 in kb

Table 2. The content of minicircular DNA sequences CCCIA, CCCIB and CCC2 in 10 selected lines and cultivars of *Vicia faba*

Subspecies and varieties	Lines and cultivars	CCCIA	CCCIB[a]	CCC2[b]
Subspecies *minor* variety *minor*	EFK	+	++	++
	BKH	+	++	++
	DK-14	+	++	++
	Aushra	+	++	++
	Fribo	+	+/−	++
	251	+	−	++
Subspecies *faba* variety *equina*	Black Russians	+	++	++
	122	+	−	++
	163	+	−	+
Subspecies *faba* variety *faba*	353	+	−	+

[a] Designations for quantity of CCCIB are analogous to Table 1
[b] Designations for quantity of CCC2: ++ = normal content of CCC2; + = content of CCC2 is on average decreased by one half

"Fribo" seedlings. Based on these data we came to a conclusion regarding the existence of more than two types of mitochondrial genomes with different CCCIB copy number among different *Vicia faba* cultivars and lines. Mitochondria of cv "Fribo" contain CCCIB at low copy number. It is interesting that in prolonged suspension culture of *Vicia faba* originated from cv "Black Russians" we also observed a decrease of the CCCIB copy number compared with the original plant (Negruk et al. 1986).

For further analysis we chose ten different lines and cultivars of *Vicia faba*. Five of them contained three types of minicircular DNAs, where CCCIB was in high copy number (three lines from Prof. Rieger's collection – EFK, BKH and DK-14, plus cvs "Black Russians" and "Aushra"). The sixth cultivar ("Fribo") contained CCCIB at low copy number and the last four cultivars (122, 163, 251 and 353) did not contain any notable quantity of CCCIB. Hybridization analysis of the ^{32}P-labeled cloned CCC2 sequence with BamHI-fragments from these ten lines and cultivars of *Vicia faba* mitochondrial DNA showed, in all cases, the existence of linearized CCC2 (Fig. 2 and Table 2). In addition to that, mitochondrial DNA of each line or cultivar contained sequences different in size but homologous to CCC2. In eight lines and cultivars these sequences were present in minor quantities, however, the situation was completely different in mitochondria of cultivars 163 and 353. Here the content of CCC2 decreased, on average, by one half. Conversely, the content of one of the homologous sequences significantly increased (Fig. 2).

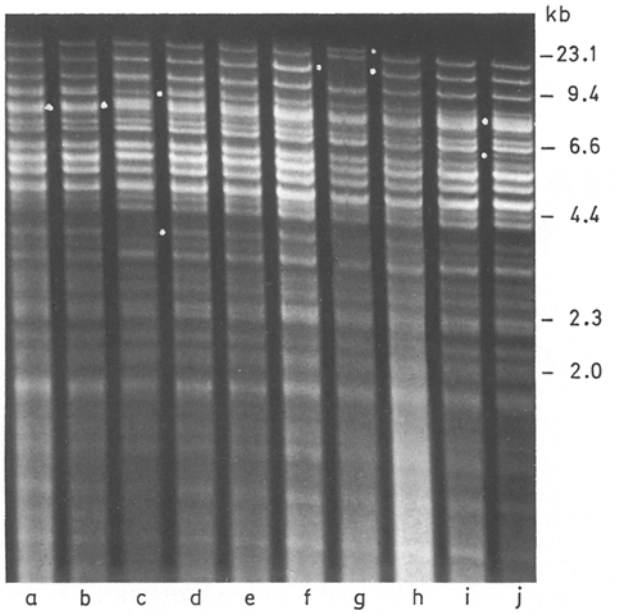

Fig. 3. 0.8% agarose gel electrophoresis of total mtDNA preparations from 6-day-old etiolated seedlings digested with EcoRI restriction endonuclease. Designations are the same as in Fig. 2. The *numbers* on the right side of the electropherogram (*j*) show the sizes of HindIII-fragments of λ DNA in kb

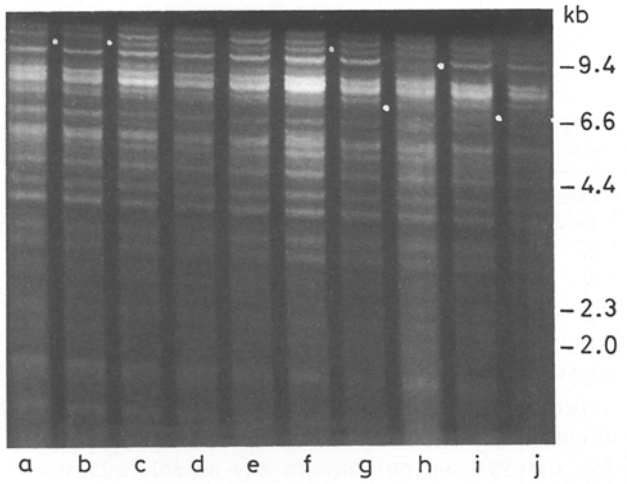

Fig. 4. 0.8% agarose gel electrophoresis of total mtDNA preparations from 6-day-old etiolated seedlings digested with BamHI restriction endonuclease. Designations are the same as in Fig. 2. The *numbers* on the right side of the electropherogram (*j*) show the sizes of HindIII-fragments of λ DNA in kb

Thus, the copy number of minicircular DNAs in mitochondria of *Vicia faba* could vary significantly depending on the line or cultivar studied. This is true for CCCIB. However, in two cultivars of *Vicia faba* (163 and 353) we also found notable variations with respect to the quantity of CCC2.

Based upon data previously obtained in the laboratory of Briquet (Boutry and Briquet 1982), the possibility of rearrangements in the main mitochondrial DNA can be inferred. To verify this we conducted comparative restriction analysis of mitochondrial DNA isolated from 10 lines and cultivars chosen earlier. In Figs. 3 and 4, 0.8% agarose gel electropherograms of mitochondrial DNA preparations digested with EcoRI and BamHI restriction endonucleases are shown, respectively. In comparison with cvs "Black Russians" and "Aushra" (for which the patterns of visual restriction fragments are practically identical), we found and analysed some differences in all other patterns of *Vicia faba* mitochondrial DNA. These differences are shown by white dots on the right side of the lanes. Among five lines and cultivars containing CCCIA, CCIB and CCC2, restriction fragment patterns of mtDNA from lines EFK and BKH were similar. But these patterns differed in cvs "Black Russians" and "Aushra". MtDNA restriction fragment pattern in the line DK-14 was different both from the pair "Black Russians", "Aushra" and from the pair EFK, BKH, in spite on the fact that nuclear genomes of EFK, BKH and DK-14 are of the same origin but differ with respect to some rearrangements in the nuclear chromosomes.

Among four cultivars containing only CCCIA and CCC2, cv 122 had a mitochondrial genome close in structure to the mitochondrial geomes of "Black Russians" and "Aushra", though some differences were found in this case also (Fig. 4h). Cultivars 163 and 353 showed similar total mitochondrial DNA restriction fragment patterns, but notably differed from "Black Russians" and "Aushra" (Fig. 3i and j). Cultivars "Fribo", 251 and 122 revealed unique features (Figs. 3f, g and 4h). Comparative analysis of EcoRI and BamHI-restriction fragments among ten chosen lines and cultivars allowed to differentiate three pairs of lines and cultivars showing similar restriction fragment patterns (EFK–BKH, "Black Russians"–"Aushra" and 163–353) and four unique types of mitochondrial genome (in line DK-14 and in cvs "Fribo", 251 and 122). In our case, we could not find any correlations between the number of minicircular DNA types, patterns of restriction fragments and fertility of plants.

It is clear that restriction analysis by itself allows only general evaluations of intensity and specificity of rearrangements in mitochondrial genomes from different lines and cultivars. For a more detailed characterization of rearrangements we conducted the hybridization of ^{32}P-labeled cloned *Zea mays* coxII gene sequences to mitochondrial DNAs from ten chosen lines and cultivars digested with BamHI-restriction endonuclease (Fig. 5). All lines and cultivars contained a 6.5 kb BamHI-fragment homologous to the *Zea mays* coxII gene. Line DK-14 and cvs "Black Russians" and "Aushra" contained an additional 2.6 kb BamHI-fragment, which also hybridized to the coxII gene (Fig. 5c–e). In the case of

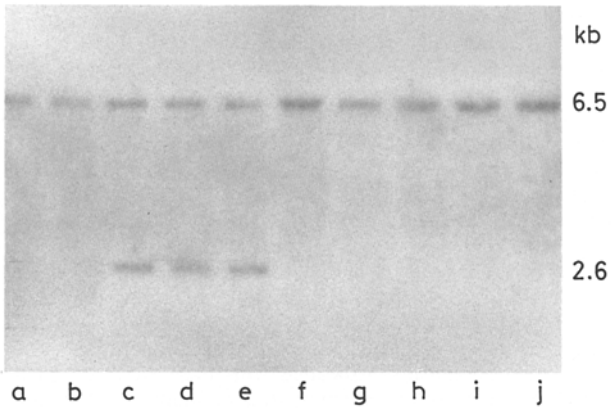

Fig. 5. 0.8% agarose gel electrophoresis of total mtDNA preparations from 6-day-old etiolated seedlings digested with BamHI-restriction endonuclease. The autoradiograph of the hybridization of ^{32}P-labeled cloned *Zea mays* coxII gene sequences to mtDNA of the same lines and cultivars shown in Fig. 2. The *numbers* on the right side of the electropherogram (*j*) show the sizes of BamHI-fragments homologous to the coxII gene of *Zea mays* in kb

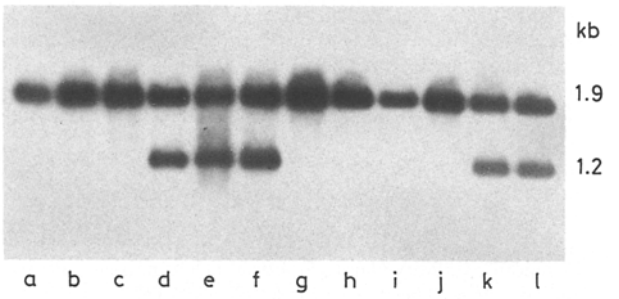

Fig. 6. 0.8% agarose gel electrophoresis of total mtDNA preparations from 6-day-old etiolated seedlings digested with EcoRI-restriction endonuclease. The autoradiograph of the hybridization of ^{32}P-labeled cloned *Zea mays* coxII gene sequences to mtDNA from lines A (*a*), B (*b*), C (*c*), D (*d*), E (*e*), F (*f*), G (*g*), H (*h*), I (*i*), K (*j*), N (*k*), and BDK-14 (*l*). The *numbers* on the right side of the electropherogram (*l*) show the sizes of EcoRI-fragments homologous to the coxII gene of *Zea mays* in kb

the 2.6 kb fragment, the intensity of hybridization was almost the same as in the case of the 6.5 kb fragment. Hybridization analysis of the ^{32}P-labeled cloned coxII gene sequences to EcoRI digests of the same lines and cultivars also revealed one large homologous fragment (1.9 kb) in all preparations and an additional smaller EcoRI-fragment (1.2 kb) in mtDNA preparations of cvs "Black Russians" and "Aushra" and line DK-14 (not shown). Based on these data we suggest that in mitochondrial genomes of line DK-14 and cvs "Black Russians" and "Aushra" we are dealing with a duplication of the coxII gene sequence.

Thus, mtDNA of the line DK-14 was notably different from lines EFK and BKH, both by restriction fragment patterns and by sequences homologous to the coxII gene. But, as we know, lines DK-14, EFK and BKH were obtained by sexual hybridization between primary lines with different chromosomal abberations (Schubert et al. 1982). Therefore, it was necessary to analyse mitochondrial genomes in original primary lines. In this case coxII probe hybridization (Fig. 6), as well as comparative restriction analysis (not shown), revealed the existence of two types of mitochondrial genomes. Mitochondrial genomes from lines A, B, C, G, H, I and K belonged to type I as well as from lines EFK and BKH (Figs. 5a, b and 6a–c, g–j). Mitochondrial genomes from lines D, E, F and N, as well as from lines DK-14 and BDK-14, belonged to type II (Figs. 5c, d, e and 6d–f, k, l). Mitochondrial genomes from cvs "Black Russians" and "Aushra" contained two BamHI- and EcoRI-restriction fragments homologous to the coxII gene sequence and therefore could be attributed to type II. However, some differences in EcoRI-restriction fragment patterns force us to suggest two possibilities: (1) Mitochondrial genomes from cvs "Black Russians" and "Aushra" have some differences compared with mitochondrial genomes of type II; and (2) Mitochondrial genomes from cvs "Black Russians" and "Aushra" contain a mixture of the mitochondrial genome of type II and of some other(s).

Discussion

We reported previously that minicircular DNAs migrating in 1%–1.5% agarose gels as two bands were an essential component of the *Vicia faba* mitochondrial genome (Negruk et al. 1982, 1985). These data have been confirmed by other authors (Boutry and Briquet 1982; Goblet et al. 1985; Wahleithner and Wolstenholme 1987). Two bands of supercoiled minicircular DNA molecules could be found in every line or cultivar of *Vicia faba*. However, the copy number of some types of these plasmid-like molecules could be different. Concerning the CCCIB sequence we found three situations: (1) When the copy number of CCCIB was twice that of CCCIA; (2) When the copy number of CCCIB was several times less than CCCIA; and (3) When CCCIB was completely absent. All lines and cultivars investigated in our case were fertile. Therefore, based on our data and data obtained by Goblet et al. (1985) and by Boutry and Briquet (1982), we suggest that cytoplasmic male sterility in *Vicia faba* is probably determined not only by the presence of CCCIB but by the combination of the mitochondrial genome containing CCCIB with a special type of the nuclear genome. Such combination could cause a functional imbalance between nuclear and mitochondrial genomes. It is possible that a functional imbalance is caused not only by the presence of CCCIB, but by the special rearrangement events in main mtDNA. We cannot exclude the existence of several types of rearrangement events, or the

existence of several different combinations of nuclear and mitochondrial genomes eventually leading to the functional imbalance. The replacement of one nuclear genome by another, which can coexist with a given type of mitochondrial genome with CCCIB and some rearrangements in the main mtDNA, may possibly cause the reconstitution of functional balance. Thus, it is possible that in the case of *Vicia faba* we are dealing with a situation close to that described for *Zea mays* (Laughnan and Gabay-Laughnan, 1983).

Comparative restriction analysis revealed notable rearrangement activity of the *Vicia faba* mitochondrial genome (Figs. 3 and 4). In the ten different lines and cultivars investigated, seven different variants of restriction fragment patterns were found; three lines, EFK, BKH and DK-14, were the most interesting. These lines have the same origin and differ from each other only by several rearrangements of the nuclear chromosomes (Schubert et al 1982). Concerning the mitochondrial genome, two of them (EFK and BKH) showed the same mitochondrial DNA restriction fragment patterns, but in the third line (DK-14) the restriction fragment pattern was different. Comparative restriction and hybridization analysis of 11 primary lines with chromosomal abberations revealed that the original cultivar used as a source of these lines probably contained a mixture of two types of mitochondrial genomes. Lines EFK and BKH inherited mitochondrial genome of type I and lines DK-14 and BDK-14 inherited mitochondrial genome of type II. Minicircular DNA copy number in all lines and in cvs "Black Russians" and "Aushra" did not changed significantly. From this observation we suggest that the main mitochondrial DNA rearrangements and alterations in CCCIB copy number are not linked processes.

A more detailed analysis of *Vicia faba* mitochondrial DNA rearrangements, which was conducted with ^{32}P-labeled cloned *Zea mays* coxII gene sequences, revealed that in some cases mitochondrial DNA rearrangements resulted in duplication of some regions. Thus, the duplication of some regions of the mitochondrial genome can take place not only in one species compared with another (as mentioned by Dawson et al. 1986), but among different fertile lines and cultivars of one plant species. Sequences homologous to the coxII gene are being cloned and are now under investigation. In this regard we can not exclude that the coxII gene duplicate is a pseudogene, as was shown for the ATPase α-subunit gene in mitochondrial genome of Oenothera (Schuster and Brennicke 1986) and for the coxII gene of wheat (Bonen et al. 1984).

Acknowledgements. We thank Prof. C. S. Levings III and Prof. T. Börner for the *Zea mays* coxII gene probe. We also thank Prof. R. Rieger and Dr. I. Schubert for kindly providing seeds of *Vicia faba*, helpful discussion, critical reading of the manuscript and for improvement of English.

References

Bonen L, Boer PH, Gray MW (1984) The wheat cytochrome oxidase subunit II gene has an intron insert and three radical amino acid changes relative to maize. EMBO J 3:2531–2536

Boutry M, Briquet M (1982) Mitochondrial modification associated with the cytoplasmic male sterility in *Vicia faba*. Eur J Biochem 127:129–135

Dawson AJ, Hodge TP, Isaac PG, Leaver CJ, Lonsdale DM (1986) Location of the genes for cytochrome oxidase subunits I and II, apocytochrome b, α-subunit of the F, ATPase and ribosomal RNA genes on the mitochondrial genome of maize (*Zea mays* L.). Curr Genet 10:561–564

Goblet JP, Flamand MC, Briquet M (1985) A mitochondrial plasmid specifically associated with male sterility and its relation with other mitochondrial plasmids in *Vicia faba*. Curr Genet 9:423–426

Kemble RJ, Bedbrook JR (1980) Low molecular weight circular and linear DNA in mitochondria from normal and male-sterile *Zea mays* cytoplasm. Nature 284:565–566

Kemble RJ, Shepard JF (1984) Cytoplasmic DNA variation in a potato protoclonal population. Theor Appl Genet 69: 211–216

Kemble RJ, Gunn RE, Flavell RB (1980) Classification of normal and male-sterile cytoplasms in maize. Electrophoretical analysis of DNA species in mitochondria. Genetics 95:451–458

Laughnan JR, Gabay-Laughnan SJ (1983) Cytoplasmic male sterility in maize. Ann Rev Genet 17:27–48

Leaver CJ, Gray MW (1982) Mitochondrial genome organization and expression in higher plants. Annu Rev Plant Physiol 33:373–402

Maniatis T, Fritsch EF, Saulbrook J (1982) A laboratory manual molecular cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor

McNay JW, Chourey DS, Pring DR (1984) Molecular analysis of genomic stability of mitochondrial DNA in tissue cultured cells of maize. Theor Appl Genet 67:433–437

Negruk VI, Cherny DI, Nikiforova ID, Alexandrov AA, Butenko RG (1982) Isolation and characterization of minicircular DNAs found in mitochondrial fraction of *Vicia faba*. FEBS Lett 142:115–117

Negruk VI, Goncharova NP, Gelnin LG, Mardamshin AG (1985) Molecular hybridization analysis of cloned *Vicia faba* minicircular DNA sequences. Mol Gen Genet 198:486–490

Negruk VI, Eisner GI, Redichkina TD, Dumanskaya NN, Cherny DI, Alexandrov AA, Shemyakin MF, Butenko RG (1986) Diversity of *Vicia faba* circular mtDNA in whole plants and suspension cultures. Theor Appl Genet 72: 541–547

Pring DR, Levings CS III (1978) Heterogeneity of maize cytoplasmic genomes among male sterile cytoplasms. Genetics 89:121–136

Pring DR, Levings CS III, Hu WWL, Timothy DH (1977) Unique DNA associated with mitochondria in the S-type cytoplasm of male sterile maize. Proc Natl Acad Sci USA 74:2904–2908

Schubert I, Michaelis A, Rieger R (1982) Karyotype variability and evolution in *Vicia faba* L. Biol Zbl 101:793

Schuster W, Brennicke A (1986) Pseudocopies of the ATPase α-subunit gene in Oenothera mitochondria are present on different circular molecules. Mol Gen Genet 204:29–35

Synenki RM, Levings CS III, Shah DM (1978) Physico-chemical characterization of mitochondrial DNA from soybean. Plant Physiol 61:460–464

Wahleithner JA, Wolstenholme DR (1987) Mitochondrial plasmid DNAs of broad beans: nucleotide sequences, complex secondary structures and transcription. Curr Genet 12: 55–57