Plant Molecular Biology **56:** 811–820, 2004.

A fragment of chloroplast DNA was transferred horizontally, probably from non-eudicots, to mitochondrial genome of *Phaseolus*

Magdalena Woloszynska[1,*], Tomasz Bocer[1], Pawel Mackiewicz[2], Hanna Janska[1]
[1]*Institute of Biochemistry and Molecular Biology, University of Wroclaw, Department of Cell Molecular Biology, Wroclaw, Poland (*author for correspondence; e-mail Magdalena.Woloszynska@ibmb.uni.wroc.pl);*
[2]*Institute of Genetics and Microbiology, University of Wroclaw, Department of Genomics, Wroclaw, Poland*

Received 10 August 2004; accepted in revised form 21 October 2004

Key words: chloroplast *trnA* gene, horizontal gene transfer, intracellular gene transfer, plant mitochondrial DNA

Abstract

The mitochondrial genomes of some *Phaseolus* species contain a fragment of chloroplast *trnA* gene intron, named *pvs-trnA* for its location within the *Phaseolus vulgaris* sterility sequence (*pvs*). The purpose of this study was to determine the type of transfer (intracellular or horizontal) that gave rise to *pvs-trnA*. Using a PCR approach we could not find the respective portion of the *trnA* gene as a part of *pvs* outside the *Phaseolus* genus. However, a BLAST search revealed longer fragments of *trnA* present in the mitochondrial genomes of some *Citrus* species, *Helianthus annuus* and *Zea mays*. Basing on the identity or near-identity between these mitochondrial sequences and their chloroplast counterparts we concluded that they had relocated from chloroplasts to mitochondria via recent, independent, intracellular DNA transfers. In contrast, *pvs-trnA* displayed a relatively higher sequence divergence when compared with its chloroplast counterpart from *Phaseolus vulgaris*. Alignment of *pvs-trnA* with corresponding *trnA* fragments from 35 plant species as well as phylogenetic analysis revealed that *pvs-trnA* grouped with non-eudicot sequences and was well separated from all *Fabales* sequences. In conclusion, we propose that *pvs-trnA* arose via horizontal transfer of a *trnA* intron fragment from chloroplast of a non-eudicot plant to *Phaseolus* mitochondria. This is the first example of horizontal transfer of a chloroplast sequence to the mitochondrial genome in higher plants.

Abbreviations: bp, base pair; *cox1*, gene encoding subunit 1 of cytochrome oxidase; HGT, horizontal gene transfer; kb, kilo base; LRT, likelihood ratio test; ML, maximum-likelihood; *nad1*, gene encoding subunit 1 of respiratory chain NADH dehydrogenase; *pvs*, *Phaseolus vulgaris* sterility sequence; *trnA*, gene encoding alanine tRNA; *trnI*, gene encoding isoleucine tRNA; WNJ, weighted neighbour-joining

Introduction

Genetic information is viewed primarily as being transmitted by vertical transfer, that is inherited from generation to generation. This paradigm was changed by the discoveries of lateral gene flow between cellular genomes and, even more so, of interspecies horizontal gene transfer (HGT). The intracellular transfer of genetic information in plant cells is a well known fact; DNA can be transmitted between the nucleus and mitochondria in both directions as well as from chloroplasts to the nucleus and mitochondria. Interestingly, neither nuclear nor

mitochondrial sequences have been found in plastid genomes. Although the transfer of organellar genes to the nucleus occurred mainly at an early stage of eukaryotic evolution (Gray, 1992), in plants this is still a frequent and ongoing phenomenon (Adams *et al*., 2002; Stegemann *et al*., 2003; Martin, 2003). The number and variety of plastid-derived sequences found in plant mitochondria indicate that DNA flow from chloroplasts to mitochondria is also common (Unseld *et al*., 1997; Notsu *et al*., 2002).

Interspecies HGT in prokaryotes is a generally accepted fact, especially in the case of genes responsible for antibiotic resistance (de la Cruz and Davies, 2000). However, the sequencing of almost 200 microbial genomes demonstrated that HGT among bacterial species was much more frequent than previously thought (reviewed by Lawrence and Hendrickson, 2003). This way of transmission has been experienced even by genes involved in the most fundamental cellular processes like replication, transcription and translation (reviewed by Lawrence and Hendrickson, 2003). The 'genomic era' has resulted in a burst of HGT examples and fired up the discussion on the exact role and impact of HGT on the evolution of prokaryotes. Opinions vary from those viewing HGT as a massive phenomenon and the essence of the phylogenetic process (Doolittle, 1999) to statements arguing that its impact on evolution is marginal (Kurland *et al*., 2003).

Little is known, however, about HGT between prokaryotes and eukaryotes or between eukaryotic organisms, with the latter event being still largely enigmatic. However, recent years have seen a rapid accumulation of data indicating HGT in the eukaryotic domain of life. A significant proportion of the genes of alga *Bigelowiella natans* encoding plastid proteins has been acquired by HGT from other algae (Archibald *et al*., 2003). Cho *et al*. (1998) established that group I intron from a fungal donor has invaded mitochondrial *cox1* genes of angiosperms over 1000 times during evolution. The scenario of those events is not clear yet. There are also reports demonstrating DNA flow between unrelated higher plants. Intron 2 and adjacent exons of the mitochondrial *nad1* gene were transferred horizontally from an asterid to *Gnetum* (Won and Renner, 2003). Bergthorsson *et al*. (2003) reported five cases of 'intra-eukaryotic' horizontal transfers for three standard mitochondrial genes encoding ribosomal and respiratory proteins. Most recently, Davis and Wurdack (2004) argued that a parasitic relationship may facilitate HGT between flowering plants. They showed that part of the *Rafflesiaceae* mitochondrial genome was acquired horizontally from their host *Tetrastigma*.

The mitochondrial genomes of higher plants are distinguished from their animal and fungal counterparts by a unique set of features (Janska and Woloszynska, 1997). The plant mitochondrial DNA is extraordinarily large (up to 2500 kb) and displays very low nucleotide sequence variability and high recombination activity. Frequent and reversible recombinations across large repeats (minimal size 1 kb) determine the organization of the main mitochondrial genome viewed as a pool of DNA molecules of various sizes. The main genome is often accompanied by sublimons – DNA molecules present at very low numbers (Janska *et al*., 1998; Arrieta-Montiel *et al*., 2001) and generated by rare, irreversible recombinations via short repeats (below 1 kb). Consequently, plant mitochondrial genomes often exhibit heteroplasmy – the presence of a mixed population of highly abundant main genome molecules and sublimons (Small *et al*., 1989; Janska *et al*., 1998).

In the genus *Phaseolus* heteroplasmy is associated with cytoplasmic male sterility - a maternally inherited inability to produce or shed viable pollen. In *Phaseolus* sp. this trait is determined by the mitochondrial sequence *pvs* (Chase and Ortega, 1992; Johns *et al*., 1992). Some portions of *pvs* demonstrate similarity to chloroplast or nuclear sequences (Chase and Ortega, 1992; Johns *et al*., 1992; Arrieta-Montiel *et al*., 2001). It has been suggested that *pvs* is a chimeric sequence that arose by multiple recombinations (Arrieta-Montiel *et al*., 2001). One of the *pvs* regions, named *pvs-trnA*, is 190 bp in size, and is homologous to a fragment of the unique intron of the chloroplast gene *trnA* (Johns *et al*., 1992). In our study we present evidence that this chloroplast-derived portion of mitochondrial DNA was acquired by horizontal rather than intracellular DNA transfer. This is the first, to our knowledge, example of chloroplast sequence transmission via HGT in higher plants.

Materials and methods

Plant materials

To assess the occurrence of *pvs-trnA* 41 plant species were examined. The two *Phaseolus* species (*P. lunatus* and *P. glabellus*) were as described by Woloszynska *et al.* (2001) and Arrieta-Montiel *et al.* (2001). The remaining samples were collected in the Botanical Garden of the University of Wroclaw: *Aesculus hippocastanum*, *Arabidopsis thaliana*, *Calycanthus floridus*, *Chimonanthus praecox*, *Citrus meyeri*, *C. reticulate, Cocos nucifera*, *Cornus florida*, *Cucumis sativus*, *Echinodorus parviflorus*, *Elaeagnus multiflora*, *Euonymus fortunei*, *Geranium pratense, Glycine max, Helianthus annuus*, *Helleborus foetidus*, *Hibiscus rosa sinensis*, *Hypericum perforatum*, *Jasminum nudiflorum*, *Lens culinaris*, *Lupinus luteus, Lycopersicon esculentum*, *Magnolia grandiflora*, *Medicago sativa*, *Oenothera biennis*, *Oxalis deppei*, *Panicum vigantum*, *Philodendron scandens*, *Phoenix dactylifera*, *Pisum sativum, Rhododendron impeditum*, *Rosa jacutica*, *Spinacia vulgaris, Staphylea trifolia*, *Tellima grandiflora*, *Trollius europaeus*, *Vicia faba*, *V. villosa*, *Zygophyllum fabago*.

Gene isolation and characterization

Genomic DNA extracted from leaf samples using NucleoSpin Plant kit, Marchery–Nagel was used as a template in PCR. The PCR reaction mixture components and the thermocycler used were as described by Woloszynska *et al.* (2001). Typical PCR consisted of initial denaturation at 94 °C, 3 min, followed by 30 cycles of 94 °C for 15 s, 54 °C for 30 s, and 72 °C for 30 s (1 min in the case of the *Citrus* species and *Helianthus annuus* in PCR assays performed to obtain sequences homologous to *pvs-trnA*). The last step was the final elongation, 72 °C for 5 min. For sequencing the PCR products were purified using Concert Rapid PCR Purification System, (Gibco BRL). Two independent PCRs for each template were performed and both products were sequenced using tRNA1 and tRNA2 primers. The conditions of Southern hybridization were exactly as described by Janska *et al.* (1998) and Woloszynska *et al.* (2001).

Primers

To detect *pvs-trnA* the following primers were used for PCR: trn1 (5′-GGG ATC CCA GAT GAG GGA AC-3′) and trn2 (5′-AAT AAT GGG GAA GAG GAC CG-3′) homologous to the 5′ and 3′ termini of *pvs-trnA*, respectively, PVS9 (5′-TGA CAA CTT CCT CAC CCC-3′) and PVS13 (5′-ACG CTT ACG CCC CTC GAC-3′) homologous to sequences, respectively, upstream and downstream of *pvs-trnA*. All these primers were derived from non-coding sequences. The expected sizes of PCR products were 577 bp (trn1, PVS13) and 529 bp (PVS9, trn2). All the primers used to obtain sequences homologous to *pvs-trnA* derived from the intron of chloroplast *trnA* genes were designed basing on well conserved fragments of the higher plant *trnA* intron sequences available in the BLAST database. In the case of *Phaseolus vulgaris*, *Cocos nucifera*, *Philodendron scandens*, *Phoenix dactylifera, Magnolia grandiflora* and *Chimonanthus praecox*, tRNA1 (5′-GCT CAA AGG GTT GAA GGG-3′) and tRNA2 (5′-CGT TGC GAT TAC GGG TTG-3′) primers were used. Both oligonucleotides were derived from portions of the *trnA* intron flanking the fragment homologous to *pvs-trnA*. The obtained products were about 320 bp long, the exact size depending on the plant species. For the *Citrus* species and *Helianthus annuus* we used an alternative pair of primers: tRNA3 (5′-CAG CAG CAG TTC GAA AGG T-3′) and tRNA4 (5′- GGG CTA TTA GCT CAG CGG -3′). The tRNA3 oligonucleotide was located upstream of the *trnA* gene while tRNA4 hybridized to the 3′ exon of the *trnI* gene downstream of *trnA*. This pair of primers yielded a product of 2260 bp. Neither tRNA3 nor tRNA4 could anneal to the *Citrus* sp. or *Helianthus annuus* mitochondrial DNA. A 152 bp fragment of the mitochondrially encoded *cob* gene was amplified as a positive PCR control using the cob1 (5′-CGGTTCGTTAGCTGGTATTTG–3′) and cob2 (5′–CATGCATATAACGGAGCA ACC–3′) primers. The primers were derived from the *cob* gene sequence of *Vicia faba*.

Phylogenetic analyses

The sequences were aligned using the CLUSTAL W 1.83 program (Thompson *et al.*, 1994). The alignment was edited manually and ambiguously

aligned regions and gaps were excluded from further analyses. Phylogenetic trees were constructed using three approaches. A distance matrix was calculated with TREE-PUZZLE 5.1 (Schmidt *et al*., 2002) assuming HKY model of nucleotide substitution and among-site rate variation modelled on a Γ distribution with an invariable sites parameter and 10 variable rate categories. A distance tree was constructed using the weighted neighbour-joining (WNJ) method using WEIGHBOR (Bruno *et al*., 2000). In the second approach a maximum-likelihood tree was inferred using DNAML from the PHYLIP 3.6 package (Felsenstein, 2004). The tree was searched with 10 random taxon additions followed by global rearrangements. The rate variation among sites was modelled using the 'R' option with nine categories of substitution rates including invariable sites. The α shape parameter (the coefficient of variation), fraction of invariable sites and nucleotide frequencies were assumed as estimated by TREE-PUZZLE for the HKY + Γ + Inv model. A second maximum-likelihood tree was constructed using PHYML (Guindon and Gascuel, 2003) for the F84 model of base substitution including rate variation among sites (ten categories) and invariable sites for parameters estimated from the input data. The F84 + Γ + Inv model was chosen as a result of comparing different models using the likelihood ratio (LRT) and because this model minimized the AIC index (Akaike, 1974). Besides, the trees constructed using other models gave the same tree topology as the chosen model did. Bootstrap data sets were created with SEQBOOT from the PHYLIP 3.6 package (1000 for WNJ distance tree; 1000 for ML tree constructed by PHYML; 100 for ML tree constructed by DNAML). Bootstrap trees were constructed as described above but with one input order jumble in the case of the ML trees created by DNAML.

In order to compare competing tree topologies (supporting horizontal or vertical transfer of *pvs-trnA*) we applied the following tests: SH (Shimodaira and Hasegawa, 1999), two-sided KH (Kishino and Hasegawa, 1989), one-sided KH (Goldman *et al*., 2000) and ELW (expected likelihood weights; Strimmer and Rambaut, 2002) as implemented in TREE-PUZZLE.

Results

The presence of the chloroplast-derived pvs-trnA *sequence in the mitochondrial genome is limited to some particular species of the* Phaseolus *genus*

Complete *pvs* has been reported only within mitochondrial genomes of specific lines of *P. vulgaris*, *P. coccineus* and *P. polyanthus* (Mackenzie, 1991; Hervieu *et al*., 1993; Arrieta-Montiel *et al*., 2001). It seems to be unique to the *Phaseolus* genus since it has not been found in the investigated plants representing grasses, cucurbits, legumes, solanaceous, and brassica species (Johns *et al*., 1992). *P. vulgaris*, *P. coccineus* and *P. polyanthus* belong to the same phylogenetic complex and are believed to originate from a common wild ancestor (Schmit *et al*., 1993). To search for *pvs-trnA,* the chloroplast-derived part of *pvs,* outside this phylogenetic complex, we amplified *pvs-trnA* with a portion of its upstream or downstream flanking sequences. We started the search with two species of the *Phaseolus* genus: *P. lunatus* and *P. glabellus*, distantly related to each other and to the phylogenetic complex (Schmit *et al*., 1993). Regardless of the pair of primers used, we did not obtain any PCR product. Similarly, we were not able to detect *pvs-trnA* in any of the remaining 39 plant species examined.

To check whether *pvs-trnA* is present as a sublimon in the examined plant species, we transferred electrophoresed products of PCRs to a nylon membrane and hybridized it with a radioactively labelled *pvs-trnA* probe. Usually, sublimons cannot be detected by conventional PCR, but they can be visualized by PCR associated with product detection by Southern hybridization (Janska *et al*., 1998; Woloszynska *et al*., 2001). Employing such an approach we did not obtain any hybridization signal, which indicated that *pvs-trnA* was not present at a substoichiometric level in the mitochondrial genomes of the analyzed plants.

These results suggested that the putative transfer of the chloroplast *trnA* intron fragment to the mitochondrial genome occurred recently, e.g. within the *Phaseolus* genus, or that the fragment in question was present in different genomic environments in the mitochondrial genomes of plants other than the three *Phaseolus* species. We could not test the second possibility directly, since

primers homologous exclusively to the intron sequence would result in the amplification of the respective fragment from chloroplast DNA.

Chloroplast genome fragments containing the trnA gene were frequently transferred

A BLASTN search using *trnA* as a query allowed us to identify homologous sequences in the mitochondrial genomes of some plant species. A 2.3 kb portion of chloroplast DNA including the whole *trnA* gene and a fragment of *trnI* was identified in mitochondria of *Citrus* species (GenBank accession no. AB061306). The mitochondrial genome of *Helianthus annuus* contains an about 0.8 kb fragment of chloroplast-derived sequence encompassing exon 1 of *trnA* and 541 bp of the intron including the fragment homologous to *pvs-trnA* (GenBank accession no. X95260). Several segments of chloroplast sequence reside in the *Zea mays* mitochondrial genome, one of which, of about 12 kb, starts within the *trnA* gene and contains 576 bp of intron encompassing the *pvs-trnA* homology (GenBank accession no. AY506529). In summary, apart from *Phaseolus* sp., *trnA* or its fragments were found in the mitochondrial genomes of *Citrus* sp., *H. annuus* and *Z. mays*. Since those fragments were longer than *pvs-trnA* and located in different genomic environments, they could not have been detected using our PCR approach.

Sequence alignment and the phylogenetic position of pvs-trnA

We have compared the nucleotide sequences of *pvs-trnA* and its homologs found in the mitochondrial genomes of *Citrus* sp., *H. annuus* and *Z. mays* with their chloroplast counterparts. In Figure 1 we present only this fragment of the alignment which covers *pvs-trnA*. Within this region the pairs of nucleotide sequences from both organellar genomes of the same plant are identical in *H. annuus* and *Z. mays* or nearly identical (one substitution) in the *Citrus* sp. Moreover, the high level of homology between the mitochondrial and chloroplast sequences is maintained along the whole alignment (99.52 % identity for *Citrus* species, 97.17 % for *H. annuus* and 97.67 % for *Z. mays*). In contrast, the *trnA* sequence from the mitochondrial genome of *Phaseolus vulgaris* differs in 10 positions from its chloroplast counterpart. The significant similarity of chloroplast and mitochondrial sequences in the *Citrus* species, *H. annuus* and *Z. mays* suggests that the transfers of *trnA* or its fragments from the chloroplast to the mitochondrium were intracellular and occurred recently. When compared to those transfers, the event involved in *pvs-trnA* evolution appears to be a clearly different phenomenon.

To further clarify the evolutionary history of the fragment of *trnA* gene transferred to the mitochondrium in *Phaseolus*, we aligned *pvs-trnA* with sequences of the appropriate fragments of chloroplast *trnA* introns from 35 plant species (Figure 1). In this comparison three nuclear homologs of *pvs-trnA* found in *Solanum tuberosum* were also included (GenBank accession nos. AF265664 and AJ011801). For most plant species the sequences examined were retrieved from the GenBank database, except for the following plant species for which the DNA sequences were obtained by PCR amplification and DNA sequencing: *Chimonanthus praecox* (GenBank accession no. AY687351), *C. meyeri* (AY690322), *C. reticulate* (AY690321), *Cocos nucifera* (AY690325), *Helianthus annuus* (AY690323), *Magnolia grandiflora* (AY687352), *Phaseolus vulgaris* (AY690327), *Philodendron scandens* (AY690326), *Phoenix dactylifera* (AY690324). The sequence divergences between *pvs-trnA* and its chloroplast counterparts of all eudicots analyzed, including *Phaseolus* and other *Fabales* species, were relatively uniform. It has to be noted that the *pvs-trnA* sequence does not possess any features characteristic of the sequences of *Fabales*. Surprisingly, *pvs-trnA* was found to be much more similar to the respective fragment of *trnA* intron of monocots and magnoliids than to the chloroplast *trnA* gene of *Phaseolus*. The most similar to *pvs-trnA* were sequences of *Magnolia grandiflora* and *Philodendron scandens*. These two sequences are identical to each other and differ from *pvs-trnA* in only three sites. The presented alignment suggests that the DNA transfer which gave rise to *pvs-trnA* was not of the intracellular type and did not take place within *Phaseolus* or any other *Fabales* plant. It rather implies that the relocation of *trnA* occurred by horizontal DNA transfer from the chloroplast genome of a non-eudicot plant to *Phaseolus* mitochondria.

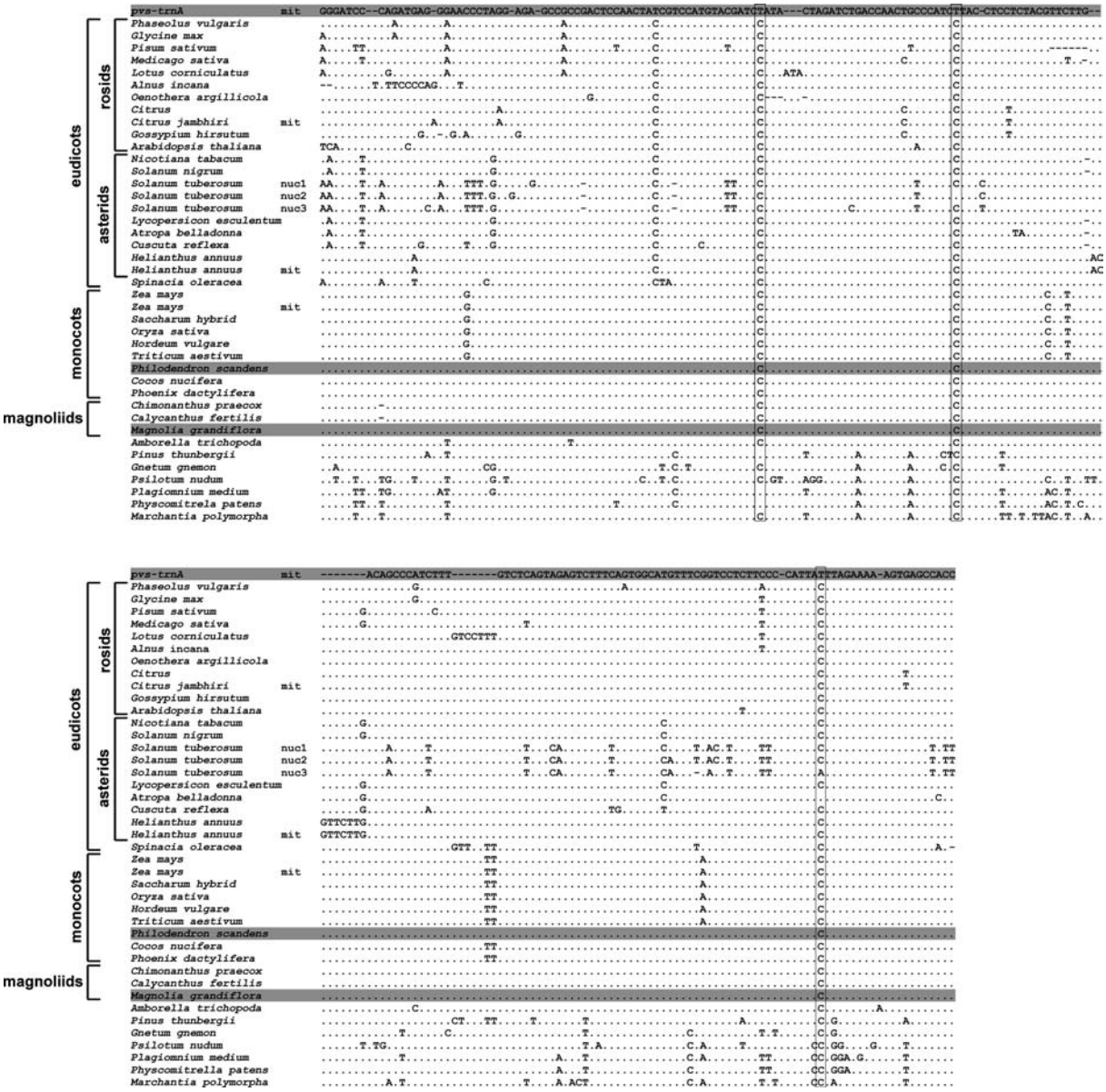

Figure 1. Alignment of *pvs-trnA* and corresponding fragments of chloroplast *trnA* introns, and mitochondrial (mit) and nuclear (nuc) homologs from variety of plant species. The *pvs-trnA* sequence and its closest homologs from *Magnolia grandiflora* and *Philodendron scandens* are shaded. Dots indicate identity to *pvs-trnA*, dashes represent gaps. The C to T substitutions distinguishing *pvs-trnA* from almost all remaining sequences are framed. The sequence of the chloroplast *trnA* intron fragment was identical in all three *Citrus* species analyzed and is described as "*Citrus*".

This conclusion was further supported by the phylogenetic analysis. All trees constructed using the three methods described earlier gave the same topology. The ML tree constructed for the F84 + Γ + Inv model by PHYML including bootstrap values for all three approaches is presented in Figure 2. The position of *pvs-trnA* is not completely resolved because of the weak phylogenetic signal in the analyzed sequences. However, *pvs-trnA* is placed among monocots and magnoliids but does not group with *Fabaceae* (*Phaseolus vulgaris*, *Glycine max*, *Pisum sativum*, *Medicago sativa* and *Lotus corniculatus*) which form the clade with a relatively strong bootstrap support. The

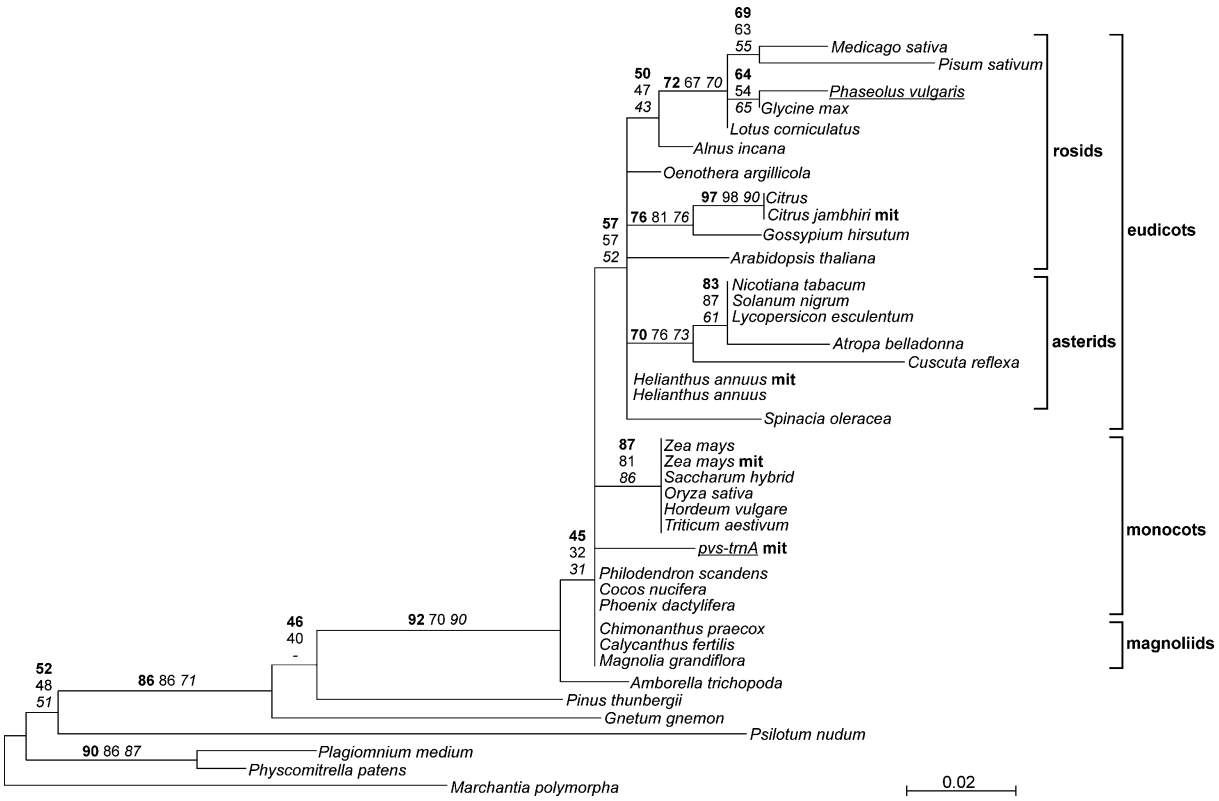

Figure 2. Maximum-likelihood tree of *pvs-trnA* and its chloroplast and mitochondrial (mit) homologs, constructed on the assumption of the F84 + Γ + Inv model of nucleotide substitutions. Bootstrap values based on three following phylogenetic methods are shown at nodes: maximum-likelihood by PHYML (in bold), maximum-likelihood by PHYLIP (in regular font) and weighted neighbour-joining (in *italics*). The scale bar represents the number of nucleotide substitutions per site.

phylogenetic relationships of the analyzed species are generally congruent with those inferred from the analysis of plastid and nuclear genes (Soltis *et al.*, 1999; Wikström *et al.*, 2001). The topology of the obtained tree was compared with five competing tree topologies assuming potential vertical transfer of *pvs-trnA* (i.e., grouping this sequence with other representatives of *Fabaceae*, including the *Phaseolus vulgaris* chloroplast sequence). However, all the tests applied (SH, KH and ELW) rejected the topologies of the vertical transfer in favour of the horizontal transfer with 5% significance level.

Sequence alignment (Figure 1) and phylogenetic analysis (Figure 2) indicate also that the four mitochondrial *trnA* homologs are less closely related to each other than to their chloroplast counterparts. This result together with the lack of sequences homologous to *trnA* in the completely sequenced mitochondrial genomes of *A. thaliana*, sugar beat and rice imply that these mitochondrial sequences originated from separate transfer events. The three nuclear *trnA* homologs included in our analysis (Figure 1) are probably also the result of another independent transfer, but this time from the chloroplast to the nucleus. Despite little divergence, all three copies exhibit specific substitutions discriminating them from all the analyzed sequences. On the other hand, they are most closely related to the *Solanaceae* chloroplast *trnA* sequence containing almost all features characteristic of the *trnA* sequences of this plant family. Therefore, we think that part of *trnA* intron was relocated to the nucleus within *Solanaceae* followed by its spread and divergence in the nuclear genome.

Discussion

From the results presented here we conclude that the chloroplast *trnA* gene or its portions

underwent four independent transfers from chloroplasts to mitochondria during higher plant evolution. Three of these transfers were intracellular and occurred within individual plant lineages, while the fourth transfer, the one that gave rise to *pvs-trnA,* most likely represents transmission between unrelated plants. To our knowledge, this is the first study documenting horizontal transfer of a chloroplast sequence to the mitochondrial genome in higher plants. A portion of chloroplast DNA crossed not only the interorganismal barriers but was also transmitted to another organelle. It has to be emphasized that our conclusion cannot be attributable to contamination of DNA preparations since *pvs-trnA* is a truly mitochondrial sequence identified in the mitochondrial genomes of *Phaseolus vulgaris* (Johns *et al*., 1992; Chase and Ortega, 1992;) as well as *P. polyanthus* and *P. coccineus* (Hervieu *et al*., 1993) by independent scientific groups.

The recent, independent and intracellular nature of the transfers in the *Citrus* species, *H. annuus* and *Z. mays* was obvious based on the high sequence homology between the chloroplast and the corresponding mitochondrial sequences from the same species. In contrast, the chloroplast-derived portion of the *trnA* intron found in *Phaseolus* mitochondrial genome, *pvs-trnA*, showed a relatively high sequence divergence compared to the respective fragment of the *Phaseolus* chloroplast DNA. Moreover, *pvs-trnA* exhibited surprising similarity to the chloroplast *trnA* intron of monocots and magnoliids. This fact may be explained in two ways: (i) either the fragment of the *trnA* intron was intracellularily transferred from chloroplasts to mitochondria in the common ancestor of eudicots and non-eudicots (monocots, magnoliids) and subsequently lost in the majority of descendant lineages, or (ii) it was acquired by horizontal transfer probably from monocots or magnoliids to the *Phaseolus* genus. According to Wikström *et al*. (2001) eudicots diverged from magnoliids and monocots about 160 million years ago. Thus, in order to maintain such a high homology, the *pvs-trnA* sequence would have to present extraordinary conservativeness over this long evolutionary time, both in the chloroplast genomes of non-eudicots and in mitochondria of *Phaseolus*. This is rather doubtful regarding that chloroplast-derived DNA sequences, commonly found in higher plant mitochondrial genomes following intracellular transfer, have been shown to be unstable due to rearrangements and base substitutions (Watanabe *et al*., 1994; Kanno *et al*., 1997). Moreover, since we did not detect mitochondrial sequences closely related to *pvs-trnA* outside the *Phaseolus* genus, the putative ancient transfer ought to be followed by massive and independent losses from mitochondria of all other eudicot plants. In the light of the above arguments, the ancient intracellular transfer is highly unlikely and in our view horizontal DNA transfer is the only plausible explanation. The most probable donor was a non-eudicot plant, possibly a monocot or magnoliid. However, the evidence for this event is relatively weak since the bootstrap value supporting eudicot monophyly to the exclusion of *pvs-trnA*, is only 52–57%. As regards the recipient plant, our speculations base mostly on the fact that we did not identify *pvs-trnA* outside of the phylogenetic complex *P. vulgaris, P. cossineus* and *P. polyanthus*. This finding suggests that the *trnA* transposition took place in a *Phaseolus* ancestor common to these three species. However, a precise estimation of the identity of the donor and the recipient as well as the timing of the horizontal transfer is not possible based on our analyses.

Numerous studies document the presence of chloroplast sequences in plant mitochondrial genomes. The best insight into this issue was given by Cummings *et al*. (2003), who documented five independent transfers of the chloroplast gene *rbcL* to the mitochondrial genome in angiosperms. It is generally expected that the acquisition of chloroplast-derived sequences by mitochondria occurred by intracellular gene transfer in a single plant species, which we also observed in the cases of dicots (*Citrus* sp. and *H. annuus*) and monocots (*Z. mays*). However, our results concerning the evolutionary history of the part of chloroplastic *trnA* intron found in the mitochondrial genome of *Phaseolus* imply that horizontal acquisition is also possible. It is intriguing that so many examples of HGT occurring within eukaryotic organisms concern transfers to plant mitochondria (Cho *et al*., 1998; Bergthorsson *et al*., 2003; Won and Renner, 2003). Since the data available in this field is still scarce it is unclear whether this bias in reports reflects the real situation. It is, however, possible that plant mitochondrial genomes are particularly prone to the incorporation of the foreign DNA supplied by intracellular or horizontal gene

transfer. This could be a consequence of the plant mitochondrial DNA organization comprising mostly noncoding sequences, and of its unusual recombinational activity.

Acknowledgements

This work was supported by the State Committee for Scientific Research (Grant No. 3 P06A 007 25).

References

Adams, K.L., Qiu, Y.L., Stoutemyer, M. and Palmer, J.D. 2002. Punctuated evolution of mitochondrial gene content: high and variable rates of mitochondrial gene loss and transfer during angiosperm evolution. Proc. Natl. Acad. Sci. USA 99: 9905–9912.

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19: 716–723.

Archibald, J.M., Rogers, M.B., Toop, M., Ishida, K.I. and Keeling, P.J. 2003. Lateral gene transfer and the evolution of plastid-targeted proteins in the secondary plastid-containing alga, *Bigelowiella natans*. Proc. Natl. Acad. Sci. USA 100: 7678–7683.

Arrieta-Montiel, M., Lyznik, A., Woloszynska, M., Janska, H., Tohme, J. and Mackenzie, S. 2001. Tracing evolutionary and developmental implications of mitochondrial stoichiometric shifting in the common bean. Genetics 158: 851–864.

Bergthorsson, U., Adams, K.L., Thomason, B. and Palmer, J.D. 2003. Widespread horizontal transfer of mitochondrial genes in flowering plants. Nature 424: 197–201.

Bruno, W.J., Socci, N.D. and Halpern, A.L. 2000. Weighted neighbor joining: a likelihood-based approach to distance-based phylogeny reconstruction. Mol. Biol. Evol. 17: 189–197.

Chase, C.D., Ortega, V.M. 1992. Organization of ATPA coding and 3′ flanking sequences associated with cytoplasmic male sterility in *Phaseolus vulgaris* L. Curr. Genet. 22: 147–153.

Cho, Y., Qiu, Y.L., Kuhlman, P. and Palmer, J.D. 1998. Explosive invasion of plant mitochondria by a group I intron. Proc. Natl. Acad. Sci. USA 95: 14244–14249.

Cummings, M.P., Nugent, J.M., Olmstead, R.G. and Palmer, J.D. 2003. Phylogenetic analysis reveals five independent transfers of the chloroplast gene *rbcL* to the mitochondrial genome in angiosperms. Curr Genet 43: 131–138.

Davis, C.C. and Wurdack, K.J. 2004. Host-to-parasite gene transfer in flowering plants: phylogenetic evidence from *Malpighiales*. Science 305: 676–678.

de la Cruz, F. and Davies, J. 2000. Horizontal gene transfer and the origin of species: lessons from bacteria. Trends Microbiol. 8: 128–133.

Doolittle, W.F. 1999. Phylogenetic classification and the universal tree. Science 284: 2124–2128.

Felsenstein, J. 2004. PHYLIP (Phylogeny Inference Package) version 3.6 b. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Gray, M.W. 1992. The endosymbiont hypothesis revisited. Int. Rev. Cytol. 141: 233–357.

Goldman, N., Anderson, J.P. and Rodrigo, A.G. (2000) Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49: 652–670.

Guindon, S. and Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52: 696–704.

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

Janska, H., Sarria, R., Woloszynska, M., Arrieta-Montiel, M. and Mackenzie, S. 1998. Stoichiometric shifts in the common bean mitochondrial genome leading to male sterility and spontaneous reversion to fertility. Plant Cell 10: 1163–1180.

Janska, H. and Woloszynska, M. 1997. The dynamic nature of plant mitochondrial genome organization. Acta Biochim. Polon. 44: 239–250.

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

Kanno, A., Nakazono, M., Hirai, A. and Kameya, T. 1997. Maintenance of chloroplast-derived sequences in the mitochondrial DNA of *Gramineae*. Curr. Genet. 32: 413–419.

Kishino, H. and Hasegawa, M. (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J. Mol. Evol. 29: 170–179.

Kurland, C.G., Canback, B. and Berg, O.G. 2003. Horizontal gene transfer: A critical view. Proc. Natl. Acad. Sci. U. S. A. 100: 9658 - 9662.

Lawrence, J.G. and Hendrickson, H. 2003. Lateral gene transfer: when will adoloscence end? Mol. Microbiol. 50: 739–749.

Mackenzie, S.A. 1991. Identification of a sterility-inducing cytoplasm in a fertile accession line of *Phaseolus vulgaris* L. Genetics 127: 411–416.

Martin, W. 2003. Gene transfer from organelles to the nucleus: Frequent and in big chunks. Proc. Natl. Acad. Sci. USA 100**:** 8612–8614.

Notsu, Y., Masood, S., Nishikawa, T., Kubo, N., Akiduki, G., Nakazono, M., Hirai, A. and Kadowaki, K. 2002. The complete sequence of the rice (*Oryza sativa* L.) mitochondrial genome: frequent DNA sequence acquisition and loss during the evolution of flowering plants. Mol. Genet. Genomics 268: 434–445.

Schmidt, H.A., Strimmer, K., Vingron, M. and von Haeseler, A. 2002. TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. Bioinformatics 18: 502–504.

Schmit, V., du Jardin, P., Baudoin, J.P. and Debouck, D.G. 1993. Use of chloroplast DNA polymorphisms for the phylogenetic study of seven *Phaseolus* taxa including *P. vulgaris* and *P. coccineus*. Theor. Appl. Genet. 87: 506–516.

Shimodaira, H. and Hasegawa, M. (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16: 1114–1116.

Small, I.D., Suffolk, R. and Leaver, C.J. 1989. Evolution of plant mitochondrial genomes via substoichiometric intermediates. Cell 58: 69–76.

Soltis, P.S., Soltis, D.E. and Chase, M.W. 1999. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. Nature 402: 402–404.

Stegemann, S., Hartmann, S., Ruf, S. and Bock, R. 2003. High-frequency gene transfer from the chloroplast genome to the nucleus. Proc. Natl. Acad. Sci. USA 100: 8828–8833.

Strimmer, K. and Rambaut, A. (2002) Inferring confidence sets of possibly misspecified gene trees. Proc. R. Soc. Lond. B, 269, 137–142.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673–4680.

Unseld, M., Marienfeld, J.R., Brandt, P. and Brennicke, A. 1997. The mitochondrial genome of *Arabidopsis thaliana* contains 57 genes in 366,924 nucleotides. Nat Genet. 15: 57–61.

Watanabe, N., Nakazono, M., Kanno, A., Tsutsumi, N. and Hirai, A. 1994. Evolutionary variations in DNA sequences transferred from chloroplast genomes to mitochondrial genomes in the *Gramineae*. Curr. Genet. 26: 512–518.

Wikström, N., Savolainen, V. and Chase, M.W. 2001. Evolution of the angiosperms: calibrating the family tree. Proc R Soc Lond B Biol Sci. 268: 2211–2220.

Woloszynska, M., Kieleczawa, J., Ornatowska, M., Wozniak, M. and Janska, H. 2001. The origin and maintenance of the small repeat in the bean mitochondrial genome. Mol. Genet. Genomics 265: 865–872.

Won, H. and Renner, S.S. 2003. Horizontal gene transfer from flowering plants to *Gnetum*. Proc. Natl. Acad. Sci. USA 100: 10824–10829.