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Divergent evolution of two plastid genes, *rbcL* and *atpB*, in a non-photosynthetic parasitic plant

Philippe Delavault [1], Vehary Sakanyan [2] and Patrick Thalouarn [1,*]
[1] *Laboratoire de Cytopathologie Végétale, Faculté des Sciences et des Techniques, Université de Nantes, 44072 Nantes Cedex 03, France (*author for correspondence);* [2] *Laboratoire de Biotechnologie, Faculté des Sciences et des Techniques, Université de Nantes, 44072 Nantes Cedex 03, France*

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Abstract

Plastid DNA (ptDNA) regions for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubiso) (*rbcL*) and the β-subunit of ATP synthase (*atpB*) genes of the holoparasite *Lathraea clandestina* L. were sequenced. These regions were obtained by cloning either a *Bam* HI endonuclease generated fragment from the *Lathraea* ptDNA or polymerase chain reaction (PCR) amplified products. The *Lathraea* ptDNA contains the entire sequence for the *rbcL* gene which shares 94.5% homology with the *Nicotiana tabacum* gene, whereas *atpB* is maintained as a pseudogene. The intergenic region between divergently transcribed *rbcL* and *atpB* genes is shorter (758 bp) in *L. clandestina* plastid genome in comparison with *N. tabacum* (823 bp), however they have a noticeable similarity, mainly in the *rbcL* 5′-upstream region. A low level of the *rbcL* gene transcription was detected whereas no *atpB* transcripts were found in *Lathraea*. The plasmid *rbcL* gene of the hemiparasite *Melampyrum pratense* and the autotroph *Digitalis purpurea* both from the Scrophulariaceae were cloned by PCR amplification and then sequenced. The *L. clandestina rbcL* gene is highly homologous to the *M. pratense* and *D. purpurea* genes. The data indicate that the evolution of the plastid *atpB-rbcL* region was different in parasites from the Scrophulariaceae and Orobanchaceae families.

Introduction

Holoparasitic plants receive assimilates essential for their growth and development from the host plants. Most of them lack typical plastids and contain only amyloplastids devoid of chlorophyll and lamellae system [6]. This raises the question as to whether holoparasites have retained a plastid genome comparable in size, gene arrangement and functional capacity to that of land plants. It was demonstrated that the parasitic flowering plant *Epifagus virginiana* from Orobanchaceae contains a chloroplast genome of 71 kb in which all photosynthesis-related genes are absent or are pseudogenes [5, 27] whereas the plastid translational machinery is more or less conserved [26,

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers X83719, X83720 and X83721.

29]. The greatest extent of deletions and other mutations was observed in the single-copy regions (LSC and SSC) of *Epifagus* [27, 28] and of other holoparasites, *Conopholis americana* [25] and *Orobranche hederae* [24]. Thus the *rbcL* gene for the large subunit of Rubisco which is located in the LSC of the plastid DNA (ptDNA) of higher plants, has lost several segments (about half of the intact gene) in *Epifagus*. The *rbcL* gene is not deleted in *O. hederae*. Nevertheless, the corresponding transcript was not detected [24]. In the nearly holoparasitic plant *Cuscuta reflexa*, which still contains small chloroplasts with few thylakoid membranes [12], the *rbcL* gene encodes for a polypeptide with 18–23 amino acids more than in other higher plants [9]. Therefore, the *rbcL* gene might be a convenient indicator for an evolutional relaxation of a selection pressure towards ptDNA in parasitic plants.

The *rbcL* gene was previously amplified from the ptDNA of some photosynthetic hemiparasites (e.g. *Melampyrum pratense*) and a non-photosynthetic holoparasite (*Lathraea clandestina*) by polymerase chain reaction (PCR) [22]. However, PCR products themselves are not sufficient to reach a conclusion on an intact nature of the amplified genes. In this work we have sequenced a ptDNA region that contains a supposed *rbcL* gene of *L. clandestina*. The established *rbcL* gene sequence of *Lathrea* has been compared with those from the autotroph *Digitalis purpurea* and the hemiparasite *M. pratense* of the Scrophulariaceae family as well as with *Nicotiana tabacum* to find out whether the mutational changes occurred in the parasitic and non-parasitic plants.

The *rbcL* gene encodes for subunit of a protein located in the chloroplast stroma that have been also evidenced in *Lathraea* amyloplasts [23]. In addition, since *Lathraea* plastids are devoid of lamellae system, we attempted to isolate and sequence a gene for a thylakoid membrane protein, the *atpB* gene. It could be believed that the selective pressure for the maintenance of this kind of gene has been greatly relaxed. In higher plant ptDNA the *atpB* gene for the β subunit of ATPsynthase is close to *rbcL* [8]. Consequently, the work described here has been carried out to analyse the nucleotide sequences of *rbcL*, *atpB* and the untranslated spacer region between these divergently transcribed genes of *L. clandestina*.

Materials and methods

Material

Lathraea clandestina L., parasite of alder (*Alnus glutinosa* L.), *Digitalis purpurea* L. and *Melampyrum pratense* L. were collected near Nantes. Tobacco was grown in a greenhouse under 16 h per day illumination (200 μmol quanta $m^{-2} s^{-1}$).

DNA isolation

Plastid DNA was isolated according to a published procedure [1] with the exception of *Lathraea* amyloplasts which were prepared as follows: 50 to 100 g of scale leaves were blended at 4 °C in 300 ml of extraction buffer [1] containing 3 g of PVP40. The homogenate was filtered through cheesecloth and miracloth with 100 μm, 50 μm and 20 μm pore sizes, successively, then centrifuged at $40 \times g$ for 15 min and the supernatant recentrifuged at $1000 \times g$ for 15 min.

Polymerase chain reaction

DNA amplification was conducted as described previously [22]. Sequences of strongly conserved regions in the tobacco and rice *rbcL* coding parts were chosen to synthesize corresponding primers used for amplification (Table 1). An intergenic region between 5′ extremities of divergently transcribed *atpB* and *rbcL* genes was amplified using two primers which correspond to short sequences of the 5′-coding region of both genes [11, 17].

DNA cloning and sequencing

PCR-amplified products were purified from agarose gel with GeneClean II kit (Bio 101). The

Table 1. Oligonuceotide primers used in PCR experiments.

Region	Sequence
rbcL coding region	5′-GCTTATGTCACCACAAACAGA-3′ 5′-TTACTTATCCAAAACGTCCAC-3′
atpB-rbcL intergenic region	5′-CGTGGAAACCCCAGAACCA-3′ 5′-GGAGTTACTCGGAATGCTGCC-3′

DNA termini were filled in with Klenow fragment of *Escherichia coli* DNA Polymerase I and phosphorylated with T4 polynucleotide kinase. Ligation was carried out with T4 DNA polynucleotide ligase using equimolare quantities of filled-in PCR product and the *Eco* RV-digested and dephosphorylated pBluescript KS$^+$ vector. A ligated DNA was transformed into the competent cells of the *E. coli* JM101 strain, this was followed by selection of white antibiotic resistant colonies on L-agar supplied with X-gal, IPTG and ampicillin (50 μg/ml). Recombinant plasmid DNAs were purified from overnight cultures grown in L-broth with ampicillin using Qiagen plasmid kit (Qiagen). Three recombinant plasmids from independent clones for each plant were sequenced by chain termination method [15] using Sequenase kit for double-stranded DNA (US Biochemicals). A 5.2 kb *Bam* HI-digested fragment, which carries the *rbcL* and *atpB* corresponding portions of the *Lathraea* plastid DNA, was also cloned and sequenced. Assembly Lign and MacVector (IBI) software were used for DNA and protein sequence analyses.

Transcription analysis

Total cellular RNA was extracted according to a published procedure [7] from each analysed species. Various amounts of RNA (0.02 to 2.5 μg) were fixed on a nylon membrane (Boehringer Mannheim) and hybridized with *rbcL* and *atpB* specific gene probes from tobacco labelled with DIG DNA Labeling kit (Boehringer Mannheim) according to the manufacter's recommendations.

Results

L. clandestina *carries the entire sequence for the* rbcL *gene*

DNA amplification of the *rbcL* gene of tobacco and *Lathraea*, using primers described in Table 1, led in both cases to PCR products of ca. 1.4 kb, as can be seen on agarose gel. A 1.4 kb PCR-amplified DNA was inserted into the pBluescript KS$^+$ vector digested with *Eco* RV endonuclease. The cloned fragment was sequenced from three plasmids and compared to prove their identity. In addition the *rbcL* gene was cloned as a 5.2 kb *Bam* HI restriction fragment from the ptDNA of *L. clandestina*. The analysed sequences of the PCR product and the corresponding region of the *Bam* HI fragment were found to be identical. The nucleotide sequence and the primer localization are reported in Fig. 1.

Neither premature stop codon nor frameshift were found in the *L. clandestina* gene sequence as compared with the *rbcL* gene of *N. tabacum* (Fig. 1). The coding parts of the *rbcL* gene from both sources share 94.5% of homology. In the *Lathraea* gene mostly single, and a few double and triple nucleotide substitutions, were detected. The majority of these substitutions were located along the 5′-terminal and internal parts and only two substitutions were found close to the 3′-terminal part of the *rbcL* gene. Almost 65% of the substitutions occurred in the third (wobble) codon position and they do not affect the coded amino acids of the large subunit of Rubisco. Consequently, the corresponding proteins from *N. tabacum* and *L. clandestina* have 95.6% identical amino acids (Fig. 2).

A 758 nt intergenic region was found between the *L. clandestina* divergently transcribed *atpB* and *rbcL* genes. Although the region is shorter in comparison with the 823 nt intergenic region of *N. tabacum* [17], it retains the continuous conservative DNA portions. The *Lathraea rbcL* promoter region has 82% of homology with that of tobacco, including well conserved −10 and −35 promoter sites and a 18 nucleotide spacer between them as well as the Shine-Dalgarno site.

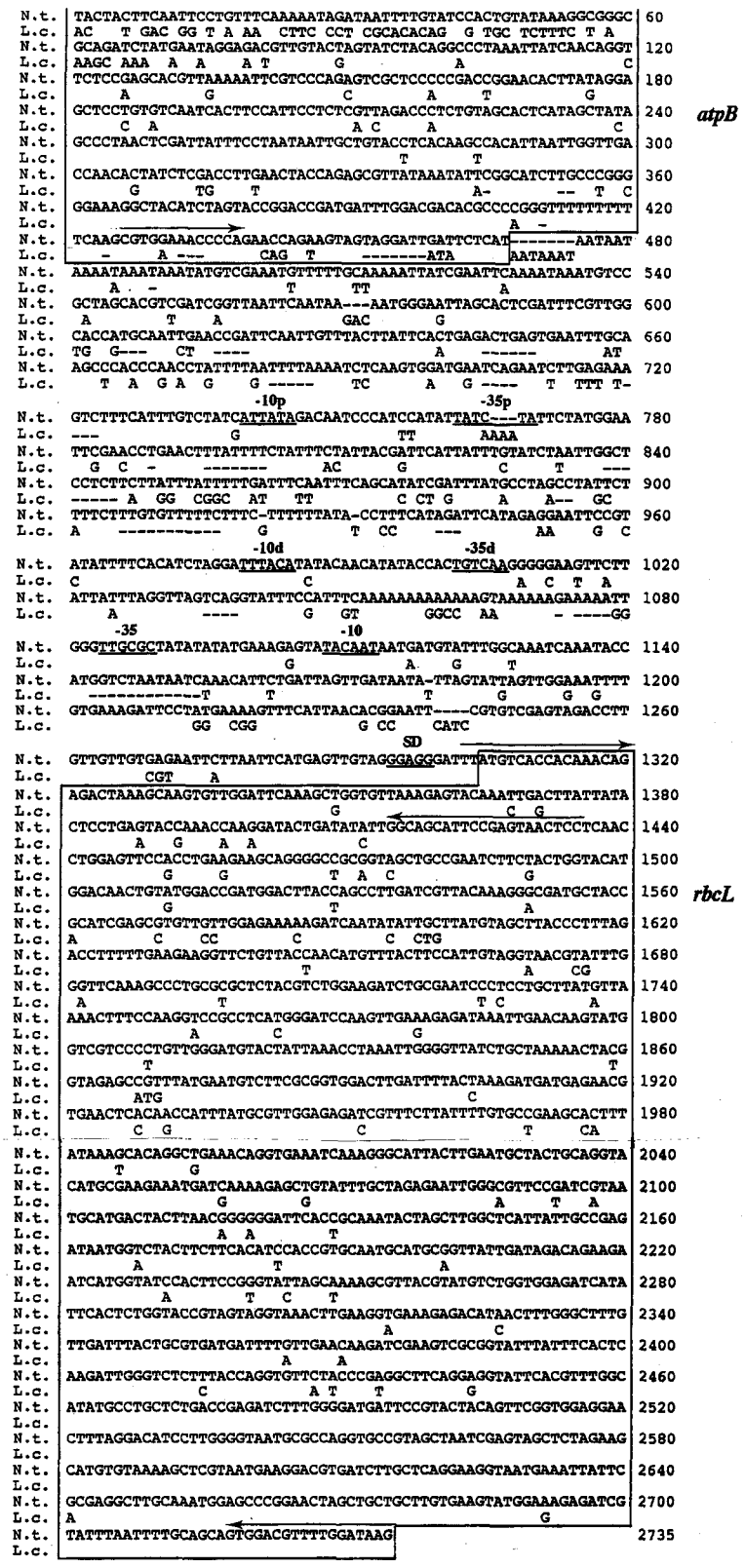

Fig. 1. Nucleotide sequences of the *rbcL* and *atpB* genes region of *Nicotiana tabacum* (N;t.) and *Lathraea clandestina* (L.c.). Coding regions are boxed. Promoter (−35, −10) and Shine-Dalgarno (SD) sequences are underlined. For *atpB* gene d and p indicate putative distal and proximal promoters, respectively [13]. Only nucleotide substitutions are shown. Deleted regions are indicated as dashed lines. Primer used in PCR experiments are indicated by arrows.

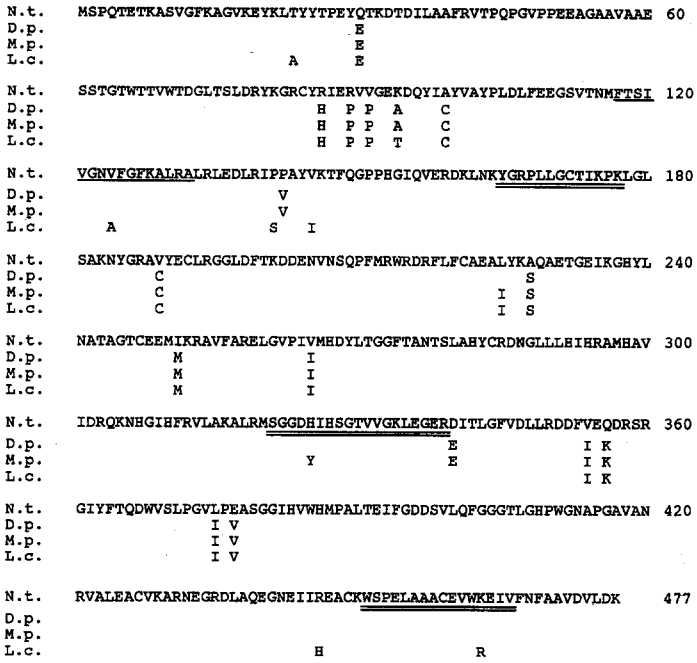

Fig. 2. Alignment of amino acid sequences of the plastid large subunit of Rubisco of *N. tabacum* (N.t.), *D. purpurea* (D.p.), *M. pratense* (M;P;) and *L. clandestina* (L.c.). The SS-binding and active site regions are single and double underlined respectively [16]. Only amino acid substitutions are shown.

Several nucleotide substitutions (most of them being replacements of A by G), as well as two small insertions (4 nt and 1 nt) and one 12 nt deletion, were detected downstream of the −10 site of the *L. clandestina rbcL* gene (see Fig. 1).

Sequence analysis show that the entire sequence for the *rbcL* gene is present in the holoparasite *L. clandestina* and this gene can be potentially expressed in plant cells using own transcriptional and translational signals.

atpB *is a truncated pseudogene*

The 5.2 kb *Bam* HI restriction fragment of *Lathraea* ptDNA that hybridizes with an heterologous *atpB* probe from tobacco was also used for sequencing the *atpB* gene. In comparison with tobacco there are several remarkable changes upstream the putative gene coding region (see Fig. 1), particularly in the −35 sequence of the proximal promoter (two promoters were found upstream of the *atpB* gene in photosynthetic plants [13]). The 5′ terminal part of the *atpB* gene shares a significant homology (89% in 388 nt DNA stretch) with its tobacco counterpart. However three deletions followed by a premature stop codon are found in the *Lathraea* gene sequence very close to its 5′ terminus. Consequently the deduced amino acid sequence differs from that of tobacco and should only be as long as 11 amino acids (Fig. 3). In addition, a sequence with rather different coding capacity is followed by stop codons at a distal part of the analysed DNA. From these data we conclude that the corresponding *atpB* sequence in the *Lathraea* ptDNA belongs, in fact, to a pseudogene.

The rbcL *gene is transcribed in* L. clandestina

Total cellular RNA was extracted from *L. clandestina* scale leaves and used in dot-blot hybridization experiments with the probes specific for tobacco *atpB* and *rbcL* genes. No positive signal was detected using the *atpB* gene probe with the

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N.t. MRINPTTSGSGVSTLEKKNPGRVVQIIGPVLDVAFPPGKMPNIYNALVVQGRDSVGQPIN  60
L.c. M YLLLCL  RZ----  K                    DAY-       K   T

N.t. VACEVQQLLGNNRVRAIAMSATEGLTRGMEVIDTGAPISVPVGGATLGRIFNVLGEPVDN 120
L.c.  T              V     D  M

N.t. LGPVDTSTTSPIHRSAPAFIQLDTKLSYFETGIKVVD                        157
L.c.       R  F FYFCLLTKEASTVCEGEVLZACQZVL
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Fig. 3. Comparison of the β subunit of ATP synthase from *N. tabacum* (N.t.) with deduced amino acid sequence corresponding to the *atpB* of *L. clandestina* (L.c.). Only amino acid substitutions are shown. Deleted regions are indicated as dashed lines; Z is a stop codon.

Lathraea RNA pattern in contrast with *Melampyrum* and *Digitalis* (Fig. 4) whereas it was previously shown that this probe hybridizes with *Lathraea* DNA. This fact confirms that the corresponding *atpB* pseudogene is not transcribed in *Lathraea* tissues. On the other hand the *Lathraea* RNA pattern gives a clear signal with the *rbcL* probe. However the amount of *rbcL* transcripts from *L. clandestina* was relatively low; it does not represent more than 5% of a signal detected with RNA patterns from *Melampyrum* or *Digitalis*.

The rbcL *gene is highly conserved among members of Scrophulariaceae*

The same oligonucleotide primers were supplied to amplify the *rbcL* gene from ptDNA of *M. pratense* and *D. purpurea*, hemiparasite and autotroph members of the Scrophulariaceae family, respectively. The obtained PCR products were of the same length as for *L. clandestina* and tobacco, as can be seen from their migration in agarose gel. Moreover, analysis of the sequenced DNA segments showed a presence of an open reading frame for 1431 nucleotides which is of the same size as found for the *rbcL* gene of *N. tabacum* and *L. clandestina*. The *rbcL* gene of *N. tabacum* shares 94.8% of homology with coding part of the gene both from *Melampyrum* and *Digitalis*. Homology was noticeably higher between the *Lathraea*, *Melampyrum* and *Digitalis* genes themselves: up to 97.8% between *Lathraea* and *Digitalis* and up to 97.5% between *Lathraea* and *Melampyrum*. Moreover, generally, the same mutations occurred in all of three plants studied.

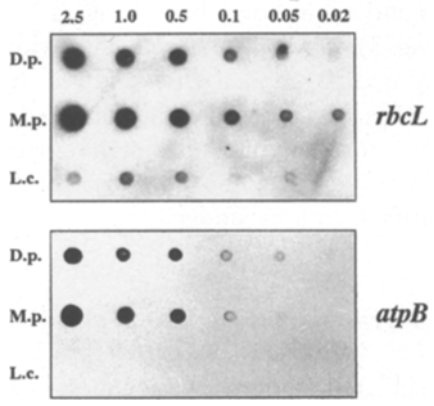

Fig. 4. Dot-blot hybridization of total RNA of *Digitalis purpurea* (D.p.), *Melampyrum pratense* (M.p.) and *Lathraea clandestina* (L.c.) with *rbcL* and *atpB* gene probes. Amounts of RNA (in μg) are indicated on top.

Amino acid sequence alignment of the *Lathraea*, *Melampyrum*, *Digitalis* and *Nicotiana* proteins for large subunit of Rubisco revealed that they have continuous conservative regions covering catalytic and SS binding (for small subunit [16]) regions (Fig. 2). However two synonymous replacements, of valine by alanine and of lysine by arginine were found in SS-binding and catalytic regions at 124 and 463 positions of the *Lathraea* protein, respectively. One more replacement, histidine by tyrosine, was observed at 325 position of *Melampyrum* protein. The most remarkable difference from tobacco protein primary structure involves five amino acid substitutions, including alanine by cysteine replacement in a short region (between 85 and 100 amino acids positions) of the proteins for all of studied members of Scrophulariaceae.

Discussion

The data presented here prove that *atpB* has evolved as a pseudogene in the holoparasitic flowering plant *Lathraea* and is not transcribed in tissues in spite of the presence of potential transcriptional signals. This failure is in agreement with ultrastructural observation of the *Lathraea* amyloplasts which are devoid of thylakoid membrane. The same was found for the nonphotosynthetic parasite *Epifagus* whose photosynthetic genes had been deleted or retained as pseudogenes [5]. However *atpB* gene is conserved and functional in *Cuscuta reflexa* [9], a stem parasite with a reduced plastid lamellae system and low level of chlorophylls [12]. It is therefore tempting to wonder whether a loss of the functional *atpB* gene is related to subterranean parasitism.

In contrast to *atpB*, the *rbcL* gene displays an entire nucleotide sequence. Both coding and upstream regions of the gene share extensive homology with those of the photoautotrophic higher plants *Nicotiana* and *Digitalis*. *rbcL* is weakly transcribed in *Lathraea* in comparison with *Digitalis* and *Melampyrum* genes. The low Rubisco activity found earlier in crude extracts of *Lathraea* [3], compared to the high activity of this carboxylase in *Digitalis* and *Melampyrum*, would seem to confirm these results. Moreover, both subunits of the *Lathraea* Rubisco protein were identified by western blotting using antibodies against Rubisco [23]. It appears that a low Rubisco activity in *Lathraea* is not related to the primary structure of the large subunit of Rubisco since virtually the same mutational changes appeared in the low-expressed *Lathraea* and highly expressed *Melampyrum* and *Digitalis* genes. A weak gene expression can not be explained by sequence rearrangements upstream of the *rbcL*-coding part, since they do not affect the transcriptional and translational signals. In any case a demand for even low functionality of *rbcL* gene in the holoparasite *Lathraea* is obscure. Several reasons for the low transcription level of *rbcL* in *Lathraea* might be put forward, including lack or low functionality of *rpo* genes which remain to be establish. However it seems likely that this gene is not actively transcribed as is the case in subterranean plants and plant organs such as spinach root [4].

Our results show that holoparasite *Lathraea* differs from root holoparasite members of Orobanchaceae family which retained *rbcL* as a truncated plastid gene in *Epifagus virginiana* [5] or as a pseudogene in *Orobanche hederae* [24]. In *Cuscuta reflexa*, the relatively unaltered ptDNA [2, 10] carries the *rbcL* gene with 3′-terminal extension for 54 nucleotides as compared to other higher plants [9]. Although the *C. reflexa rbcL* gene is 94% identical to its tobacco homologue, its expression was severely reduced at transcriptional level as compared to that in photoautothrophic higher plants [9]. A low functional state of the *rbcL* gene in both *Cuscuta* and *Lathraea* parasites raises the question of its role in parasitic plants.

The transcribed *rbcL* gene was also found in the nonphotosynthetic alga *Astasia longa* which is morphologically close to the photosynthetic *Euglena gracilis* [19]. *Astasia* has some characteristics resembling *Lathraea* since its reduced plastid genome has lost most of its bioenergetic genes [18] but has largely preserved a translational machinery [20, 21]. Besides both *Astasia* and *Lathraea* retain an intact *rbcL* which is expressed in their paramylon bodies and amyloplasts, respectively. However a more precise study of the organization of the *Lathraea* plastid genome, including the translational and transcriptional genes should be undertaken in order to establish the convergent evolution of the plastid DNA in two morphologically different heterotrophic organisms. Our data also prove a close relation of *rbcL* genes from *Lathraea*, *Melampyrum* and *Digitalis*. This shows that the phylogenetic position of these species is still conspicuous since homology between *Digitalis* autotroph and *Lathraea* is above 97%. Consequently, large subunits of Rubisco in the three Scrophulariaceae studied share some specific characteristics such as two cysteine residues more than their tobacco homolog. These features lead us to suggest that a study of the effect of parasitism on ptDNA should also focus on genome organization and gene content in addition to the sequence analysis of a

maintained and expressed gene like *rbcL* in *Lathraea* whose sequence is constrained by natural selection. In *Lathraea*, *rbcL* is a strong candidate for the focus of the selective pressure that maintains a functional genome. However the function of Rubisco remains unclear and we only can hypothesize that it may be related to internal CO_2 recycling, as PEPc does at higher rate [14], or storage and hydrolysis of starch [19]. It is concievable that other genes not known to have genetic functions remain in *Lathraea* ptDNA as in *Epifagus* with *clpP*, *accD*, *orf1738* and *orf2216* [27].

References

1. Bookjans G, Stummann BM, Henningsen KW: Preparation of chloroplast DNA from pea plastids isolated in a medium of high ionic strengh. Anal Biochem 141: 244–247 (1984).
2. Bömmer D, Haberhausen G, Zetsche K: A large deletion in the plastid DNA of the holoparasite flowering plant *Cuscuta reflexa* concerning two ribosomal proteins (*rpl2*, *rpl23*), one transfert RNA (*trnI*) and an ORF 2280 homologue. Curr Genet 24: 171–176 (1993).
3. Bricaud CH, Thalouarn P, Renaudin S: Ribulose-1,5-bisphosphate carboxylase activity in the holoparasite *Lathraea clandestina* L. J Plant Physiol 125: 367–370 (1986).
4. Deng XW, Gruissem W: Constitutive transcription and regulation of gene expression in non-photosynthetic plastids of higher plants. EMBO J 7: 3301–3308 (1988).
5. dePamphilis CW, Palmer JD: Loss of photosynthetic and chlororespiratory genes from the plastid genome of a parasitic flowering plant. Nature 348: 337–339 (1990).
6. Dodge JD, Lawes GB: Plastid ultrastructure in some parasitic and semi-parasitic plants. Cytobiology 9: 1–19 (1974).
7. Dunsmuir P, Bond D, Lee K, Gidoni D, Townsend J: Stability of introduced genes and stability in expression. In: Gelvin SB, Schilperoort RA, Verma DPS (eds) Plant Molecular Biology Manual, pp. C1/1–C1/17. Kluwer Academic Publishers, Dordrecht (1988).
8. Haberhausen G, Zetsche K: Nucleotide sequence of the *rbcL* gene and the intergenic promoter region between the divergently transcribed *rbcL* and *atpB* genes of *Ipomea purpurea* (L.). Plant Mol Biol 18: 823–825 (1992).
9. Haberhausen G, Valentin K, Zetsche K: Organization and sequence of photosynthetic genes from the plastid genome of the holoparasitic flowering plant *Cuscuta reflexa*. Mol Gen Genet 232: 154–161 (1992).
10. Haberhausen G, Zetsche K: Functional loss of all *ndh* genes in an otherwise relatively unaltered plastid genome of the holoparasitic flowering plant *Cuscuta reflexa*. Plant Mol Biol 24: 217–222 (1994).
11. Hiratsuka J, Shimada H, Whittier R, Ishibashi T, Sakamoto M, Mori M, Kondo C, Honji Y, Sun CR, Meng BY, Li YQ, Kanno A, Nishizawa Y, Hirai A, Shinozaki K, Sugiura M: The complete sequence of the rice (*Oryza sativa*) chloroplast genome: intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of the cereals. Mol Gen Genet 217: 185–194 (1989).
12. Machado MA, Zetsche K: A structural, functional and molecular analysis of plastids of the holoparasites *Cuscuta reflexa* and *Cuscuta europaea*. Planta 181: 91–96 (1990).
13. Manen JF, Savolainen V, Simon P: The *atpB* and *rbcL* promoters in plastid DNAs of a wide dicot range. J Mol Evol 38: 577–582 (1994).
14. Renaudin S, Thalouarn P, Rey L, Vidal J, Larher F: Phosphoenol pyruvate carboxylase in parasitic plants: further characterization in various species and localization at the level of cells and tissues in *Lathraea clandestina* L. J Plant Physiol 116: 455–465 (1984).
15. Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74: 5463–5467 (1977).
16. Shinozaki K, Sugiura M: The nucleotide sequence of the tobacco chloroplast gene for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase. Gene 20: 91–102 (1982).
17. Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chunwongse J, Obokata J, Shinozaki KY, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada H, Sugiura M: The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J 5: 2043–2049 (1986).
18. Siemeister G, Hachtel W: A circular 73 kb DNA from the colourless flagellate *Astasia longa* that resembles the chloroplast DNA of *Euglena gracilis*: restriction and gene map. Curr Genet 15: 435–441 (1989).
19. Siemeister G, Hachtel W: Structure and expression of a gene encoding the large subunit of ribulose-1,5-bisphosphate carboxylase (*rbcL*) in the colourless euglenoid flagellate *Astasia longa*. Plant Mol Biol 14: 825–833 (1990).
20. Siemeister G, Hachtel W: Organization and nucleotide sequence of ribosomal RNA genes on a circular 73 kbp DNA from the colourless flagellate *Astasia longa*. Curr Genet 17: 433–438 (1990).
21. Siemeister G, Buchholz C, Hachtel W: Genes for the plastid elongation factor Tu and ribosomal protein S7 and six tRNA genes on the 73 kb DNA from *Astasia longa* that resembles the chloroplast DNA of *Euglena*. Mol Gen Genet 220: 425–432 (1990).

22. Thalouarn P, Renaudin S: Polymerase chain reaction evidence of the *rbcL* gene in the Scrophulariaceae holoparasite *Lathraea clandestina* L. Comparison with the autotroph *Digitalis purpurea* L. and hemiparasite *Melampyrum pratense* L. CR Acad Sci Paris 313: 381–387 (1991).
23. Thalouarn P, Arnaud MC, Renaudin S: Evidence of ribulose-bisphosphate in the Scrophulariaceae holoparasite *Lathraea clandestina* L. CR Acad Sci Paris 309: 275–280 (1989).
24. Thalouarn P, Theodet C, Russo N, Delavault P: The reduced plastid genome of a non-photosynthetic angiosperm *Orobanche hederae* has retained the *rbcL* gene. Plant Physiol Biochem 32: 233–242 (1994).
25. Wimpee CF, Wrobel RL, Gravin DK: A divergent plastid genome in *Conopholis americana*, an achlorophyllous parasitic plant. Plant Mol Biol 17: 161–166 (1991).
26. Wolfe KH, Katz-Downie DS, Morden CW, Palmer JD: Evolution of the plastid ribosomal RNA operon in a non-green parasitic plant: accelerated sequence evolution, altered promoter structure and tRNA pseudogenes. Plant Mol Biol 18: 1037–1048 (1992).
27. Wolfe KH, Morden CW, Palmer JD: Function and evolution of a minimal plastid genome from a non-photosynthetic parasitic plant. Proc Natl Acad Sci USA 89: 10648–10652 (1992).
28. Wolfe KH, Morden CW, Palmer JD: Small single-copy region of plastid DNA in the non-photosynthetic angiosperm *Epifagus virginiana* contains only two genes. Differences among dicots, monocots and bryophytes in gene organization at a non-bioenergetic locus. J Mol Biol 223: 95–104 (1992).
29. Wolfe KH, Morden CW, Ems SC, Palmer JD: Rapid evolution of the plastid translational apparatus in a non-photosynthetic plant: loss or accelerated sequence evolution of tRNA and ribosomal protein genes. J Mol Evol 35: 304–317 (1992).