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Evolution

Occurrence of plastid RNA editing in all major lineages of land plants

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ABSTRACT **RNA editing changes posttranscriptionally single nucleotides in chloroplast-encoded transcripts. Although much work has been done on mechanistic and functional aspects of plastid editing, little is known about evolutionary aspects of this RNA processing step. To gain a better understanding of the evolution of RNA editing in plastids, we have investigated the editing patterns in *ndh*B and *rbc*L transcripts from various species comprising all major groups of land plants. Our results indicate that RNA editing occurs in plastids of bryophytes, fern allies, true ferns, gymnosperms, and angiosperms. Both editing frequencies and editing patterns show a remarkable degree of interspecies variation. Furthermore, we have found that neither plastid editing frequencies nor the editing pattern of a specific transcript correlate with the phylogenetic tree of the plant kingdom. The poor evolutionary conservation of editing sites among closely related species as well as the occurrence of single species-specific editing sites suggest that the differences in the editing patterns and editing frequencies are probably due both to independent loss and to gain of editing sites. In addition, our results indicate that RNA editing is a relatively ancient process that probably predates the evolution of land plants. This supposition is in good agreement with the phylogenetic data obtained for plant mitochondrial RNA editing, thus providing additional evidence for common evolutionary roots of the two plant organellar editing systems.**

RNA editing is one of the processes involved in transcript maturation in certain genetic systems. Depending on the nature of the alteration, the different types of RNA editing can be roughly subdivided into insertion/deletion and conversion editing. The first type was originally reported for kinetoplast DNA-encoded transcripts of trypanosomes. In this system, insertion or deletion of U residues is directed by small trans-acting RNA molecules termed guide RNAs (for reviews, see refs. 1 and 2). RNA editing in plant mitochondria (3–5) and chloroplasts (6, 7) belongs to the conversion type of editing. The vast majority of editing events in both chloroplasts and plant mitochondria are C-to-U transitions. Only few cases of reverse editing, the conversion of a U into a C, have been described for plant mitochondria and a single case for chloroplasts (8–10). A second characteristic feature shared between plastid and plant mitochondrial editing is the preference for second codon positions and the bias toward certain codon transitions. In both organelles the most frequent amino acid changes are Pro to Leu, Ser to Leu, and Ser to Phe (for a review, see refs. 11 and 12). The major difference between the two plant organellar editing systems lies in the editing frequency. While more than 1,000 editing sites were estimated for the *Oenothera* mitochondrial genome (13), only 27 editing sites were identified in the maize plastome (14).

RNA editing in chloroplasts has been reported for a limited number of angiosperm species, including the monocotyledons maize, rice, and barley (15–20) and the dicotyledons tobacco, spinach, bell pepper, and snapdragon (21–24). More recently, editing events were also described for the hornwort *Anthoceros formosae* (10) and for the gymnosperm *Pinus thunbergii* (25).

The relative abundance of editing events in transcripts of the *ndh*B gene (16, 20) encoding a subunit of a putative chloroplast NADH dehydrogenase (26) renders this gene as a suitable candidate for studying the structural and functional conservation of chloroplast editing sites within the plant kingdom. We have therefore investigated the occurrence of editing sites in *ndh*B transcripts from various species representing all major groups of land plants. For a comparison of the editing patterns in different genes, we have also examined transcripts of the *rbc*L gene encoding the large subunit of ribulose bisphosphate carboxylase.

MATERIALS AND METHODS

Plant Material. Green leaf tissue from the plant species examined was obtained from the Freiburg Botanical Garden, purchased from local markets or collected in the Black Forest.

Isolation of Nucleic Acids. Total cellular nucleic acids were isolated from 0.2–5 g plant tissue by different methods. The procedures described by Dellaporta *et al.* (27) and a cetyltrimethylammoniumbromide (CTAB)-based method (28) were used to prepare nucleic acids from angiosperms and gymnosperms, respectively. Nucleic acids from ferns and bryophytes were purified on anion exchange columns (Qiagen, Hilden, Germany). Aliquots of the nucleic acid preparations were treated separately for DNA and RNA analyses. The two samples were digested with either RNase A or DNase I (Boehringer Mannheim) to obtain pure DNA and RNA, respectively.

Reverse Transcription of RNA and Amplification of cDNA and DNA Samples by the PCR. RNA was reverse transcribed with a random primer mixture using RNase H^- Moloney murine leukemia virus reverse transcriptase (Superscript; GIBCO/BRL) according to the manufacturer's instructions. Total DNA and first strand cDNA were amplified by 40 cycles of 40 s at 93°C, 1.5 min at 55°C, and 2 min at 72°C with a 3-min extension of the first cycle at 93°C and a 10-min extension of the last cycle at 72°C. cDNA and DNA amplification products were purified for sequencing by electrophoresis on 1% agarose gels and subsequent extraction using the QIAEX II gel extraction kit (Qiagen).

Direct Sequencing of Amplification Products. Sequencing with nonfluorescent primers was performed by a modified chain termination method described by Bachmann *et al.* (29). The United States Biochemical cycle sequencing kit was used for sequencing with 3′ fluorescein-labeled primers (30).

List of Oligonucleotides. Oligonucleotides used for PCR and/or for sequencing were synthesized on a DNA synthesizer (model 394; Applied Biosystems): nb12, 5′-GGIGGIATGT-TTTTATGTGGIGCIAA-3′; nb13, 5′-GCIAGCATICGTT-TCATGCTIGT-3′; nb14, 5′-TA(T/C)GGI(T/C)(T/C)ITCI-

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. Z80856–Z80874).

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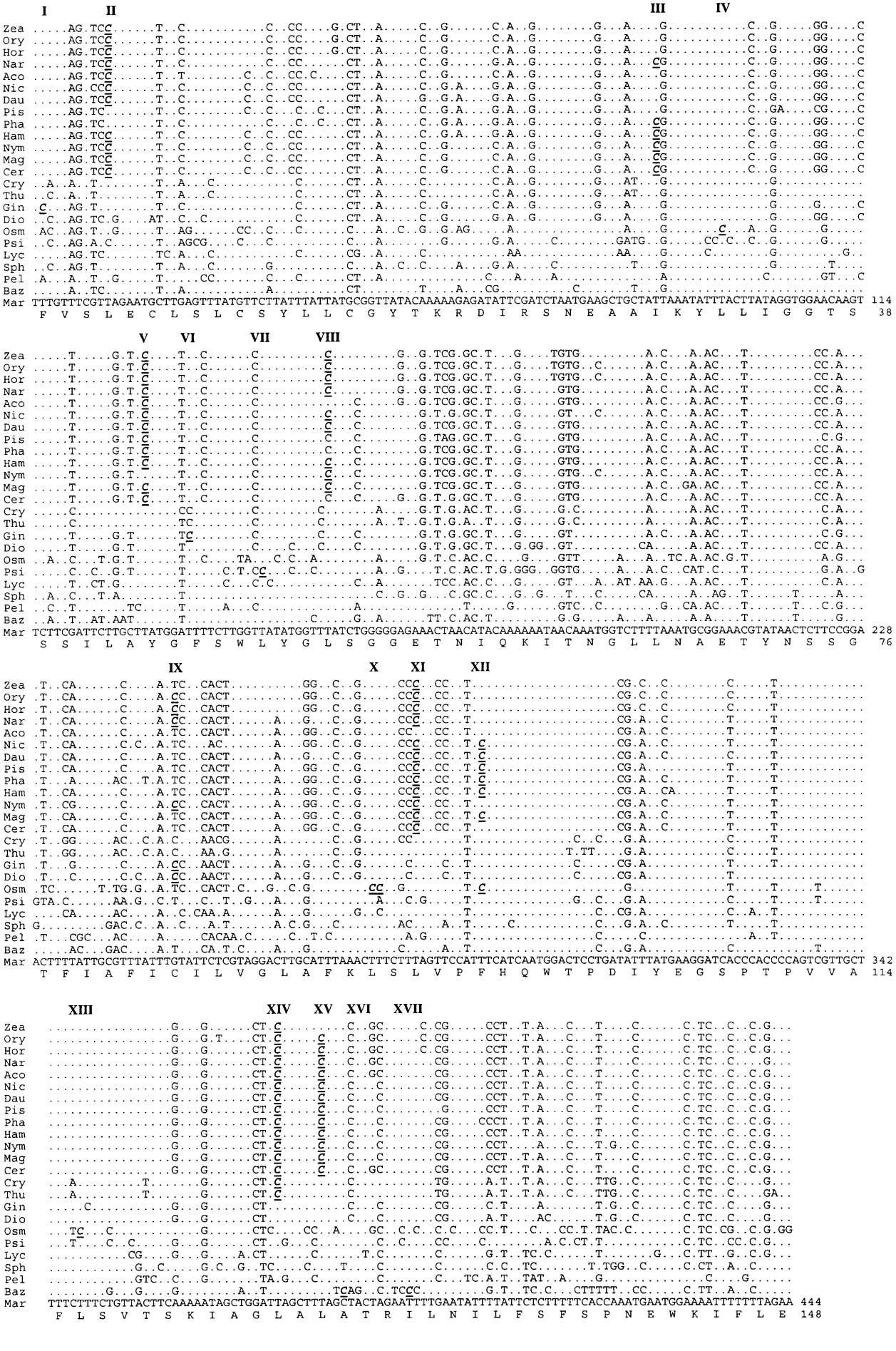
I
II
III
IV
Zea
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Hor
Nar
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Nic
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Pis
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Mag
Cer
Cry
Thu
Gin
Dio
Osm
Psi
Lyc
Sph
Pel
Baz
Mar TTTGTTTCGTTAGAATGCTTGAGTTTATGTTCTTATTTATTATGCGGTTATACAAAAAGAGATATTCGATCTAATGAAGCTGCTATTAAATATTTACTTATAGGTGGAACAAGT 114
F V S L E C L S L C S Y L L C G Y T K R D I R S N E A A I K Y L L I G G T S 38
V
VI
VII
VIII
Mar TCTTCGATTCTTGCTTATGGATTTTCTTGGTTATATGGTTTATCTGGGGGAGAAACTAACATACAAAAAATAACAAATGGTCTTTTAAATGCGGAAACGTATAACTCTTCCGGA 228
S S I L A Y G F S W L Y G L S G G E T N I Q K I T N G L L N A E T Y N S S G 76
IX
X
XI
XII
Mar ACTTTTATTGCGTTTATTTGTATTCTCGTAGGACTTGCATTTAAACTTTCTTTAGTTCCATTTCATCAATGGACTCCTGATATTTATGAAGGATCACCCACCCCAGTCGTTGCT 342
T F I A F I C I L V G L A F K L S L V P F H Q W T P D I Y E G S P T P V V A 114
XIII
XIV
XV
XVI
XVII
Mar TTTCTTTCTGTTACTTCAAAAATAGCTGGATTAGCTTTAGCTACTAGAATTTTGAATATTTTATTCTCTTTTTCACCAAATGAATGGAAAATTTTTTTAGAA 444
F L S V T S K I A G L A L A T R I L N I L F S F S P N E W K I F L E 148

GGIGGIGA-3′; cl1, 5′-ATGTC(A/G)CCACA(A/G)AC(G/C)GA(G/A)AC-3′; cl2, 5′-TC(T/A)C(G/T)(G/A)GC(T/A)AG(G/A)TC(A/G)CG(G/T)CCU-F-3′; cl3, 5′-TGGAT-(C/A)CC(T/A)TG(G/A)GG(C/T)GG(G/A)CCU-F-3′; cl4, 5′-TTCTT(A/G)TT(C/T)GTAGC(A/T)GA(A/G)GCU-F-3′; cl5, 5′-GAATCTTCIACIGGIACITGGAC(T/C)ACU-F-3′; cl6, 5′-TT(A/G)ATTTCTTTCCAIACTTC(G/A)CA-(T/A)GC-3′ (where I = inosine and F = fluorescence label).

RESULTS AND DISCUSSION

Species and Gene Selection. To investigate the extent and distribution of RNA editing and to compare the editing frequencies and editing patterns of plastid transcripts we have analyzed genomic DNA and cDNA of two different chloroplast genes. The analysis of editing in *ndh*B transcripts included members of the Spermatophyta, Pteridophyta, and Bryophyta. Within the Spermatophyta, two members of the monocots (*Narcissus pseudonarcissus* and *Acorus calamus*) and seven members of the dicots (*Daucus carota*, *Pisum sativum*, *Phaseolus vulgaris*, *Hamamelis mollis*, *Nymphaea caerula*, *Magnolia grandiflora*, and *Ceratophyllum demersum*) were selected. Data for four additional species belonging to the Spermatophyta were taken from the literature (16, 20). *Cryptomeria japonica*, *Thujopsis dolabrata*, *Ginkgo biloba*, and *Dioon edule* were analyzed as representatives of the gymnosperms. One fern (*Osmunda claytoniana*) and two fern allies (*Psilotum nudum* and *Lycopodium obscurum*) were chosen as representatives of the Pteridophyta. From the bryophytes, three members were included: the moss *Sphagnum palustre* and two liverworts, *Pellia epiphylla* and *Bazzania trilobata*.

Furthermore, a systematic search for editing sites within the *rbc*L gene was performed using published DNA sequences from the above mentioned or closely related species (for details see legend of Fig. 3).

Identification Of Editing Sites in *ndh*B-Encoded Transcripts. The *ndh*B-encoded transcript is probably the most frequently edited chloroplast RNA. Six editing sites have been reported in maize (16), eight in rice, and nine in barley and tobacco (20). We have therefore chosen this transcript to study the distribution of chloroplast editing within the plant kingdom. Interestingly, all but two editing sites are clustered in a region comprising about one-third of the coding region of the gene. Two conserved regions in the open reading frame were used to derive oligonucleotide primers. Amplification with this primer pair yielded for all plants studied here a cDNA product of 560 bp and a DNA product of about 1,250 bp (depending on the length of the intron), indicating a universal distribution of the group II intron of *ndh*B (data not shown). These amplification products contain 8 of the altogether 10 editing sites observed in the *ndh*B transcripts of barley and tobacco (20).

Three bryophytes were included in our analyses of *ndh*B cDNA sequences. Fig. 1 shows an alignment of the *ndh*B sequences obtained by direct sequencing of the amplification products. Neither in the moss *Sphagnum* nor in the liverwort *Pellia* was editing of *ndh*B transcripts observed. However, RNA editing is detected in the liverwort species *Bazzania trilobata*. Two C-to-U editing events which are strictly species-specific are observed in *Bazzania*. Both occur at a second codon position. No reverse (i.e., U-to-C) editing, as recently reported for the hornwort *Anthoceros* (10) could be found.

The origin of most of the editing sites can easily be explained with restoration of single T-to-C mutations which occurred at the DNA level either at first or second codon position. Exceptions are the two *Bazzania*-specific editing sites (Fig. 1). At site XVI, most of the examined species contain a GCC/T alanine codon. *Bazzania* contains a TCA serine codon at this site that is converted through C-to-U transition to a UUA leucine codon at the RNA level. At site XVII, where a CCC proline codon is edited to a CUC leucine codon, most of the species contain an ATT isoleucine codon. Interestingly, *Osmunda* and *Psilotum* also possess a leucine codon (CTC/T) which is, however, already encoded at the DNA level.

Sequence analysis of *ndh*B-derived cDNAs from a fern and the two fern allies *Psilotum* and *Lycopodium* led to the identification of four C-to-U transitions in the transcripts of the fern *Osmunda*, and of a single editing site in *Psilotum*. No editing site was detected in transcripts from *Lycopodium*. Editing site XII in *Osmunda* is also found in some dicot species, but not in monocots. The other three editing sites (IV, X, and XIII) appear to be restricted to *Osmunda*. The single editing site (VII) found in *Psilotum* also seems to be specific to this species.

All C-to-U transitions observed in *ndh*B transcripts occur at either second or first codon position (Figs. 1 and 2), editing at the *Osmunda*-specific site X occurs at two consecutive C residues. While editing at the second codon position is complete, partial editing was observed in the third codon position. At this site, only about 50% of the C residues are converted to U at the RNA level. Remarkably, at editing site XVII which occurs only in *Bazzania*, editing of the CCC codon is restricted to the second codon position and no partial editing occurs in third codon position. Whereas silent editing in plant mitochondria amounts to approximately 14% of all editing events (11), plastid editing in third codon position was described so far only for a CUC serine codon in the *atp*A mRNA from tobacco (31) and for an ACC threonine codon in the *rbc*L mRNA from the hornwort *Anthoceros* (10).

In *Psilotum*, editing site II is nonfunctional (Fig. 1). Such a "silenced" editing site was previously observed in the *rpo*B transcripts of barley (18) and of other closely related monocotyledonous species (P. Zeltz and H.K., unpublished data).

The loss of the capacity to edit this site in *Psilotum* is accompanied with a 5′ C-to-T point mutation, which converts a CCA proline codon into a TCA serine codon (Fig. 1). This suggests that loss of editing at this site may be caused by the point mutation at the first position of the edited codon. The importance of the 5′ upstream nucleotide for editing was recently tested by Bock *et al*. (32). Changing the T upstream of an editing site into a G drastically reduces editing efficiency. Thus mutation of the 5′-neighboring nucleotide may be one evolutionary mechanism for silencing editing sites. Alternatively, editing at site II could be of late origin—i.e., site II has never been an active site in *Psilotum*. In this scenario, the C at this position in *Psilotum* could represent an evolutionary intermediate creating the selective pressure that eventually resulted in the acquisition of an editing activity for this site.

The examination of RNA editing in *ndh*B transcripts of four gymnosperm species revealed that the editing frequency is relatively low as compared with the angiosperms (Figs. 1 and 2). *Ginkgo* contains three editing sites (I, VI, and IX). Only one editing site is found in *Dioon* (IX) as well as in two other

FIG. 1. Alignment of the analyzed region of plastid *ndh*B genes. Positions identical to the *M. polymorpha* (Mar) reference sequence are denoted by dots. The amino acid sequence derived from the *Marchantia ndh*B gene is given below using the one letter code. Nucleotides subject to RNA editing (sites I–XVII) are marked by bold letters. The insertion site of the single group II intron interrupting the *ndh*B reading frame in all species is located between positions 321 and 322. Abbreviations and database accession numbers for the nucleotide sequences are as follows: Zea (*Zea mays*, X86563), Ory (*Oryza sativa*, X15901), Hor (*Hordeum vulgare*, X90650), Nar (*Narcissus pseudonarcissus*, Z80865), Aco (*Acorus calamus*, Z80856), Nic (*Nicotiana tabacum*, Z00044), Dau (*Daucus carota*, Z80859), Pis (*Pisum sativum*, Z80872), Pha (*Phaseolus vulgaris*, Z80873), Ham (*Hamamelis mollis*, Z80862), Nym (*Nymphaea caerula*, Z80866), Mag (*Magnolia grandiflora*, Z80864), Cer (*Ceratophyllum demersum*, Z80857), Cry (*Cryptomeria japonica*, Z80858), Thu (*Thujopsis dolabrata*, Z80870), Gin (*Ginkgo biloba*, Z80861), Dio (*Dioon edule*, Z80860), Osm (*Osmunda claytoniana*, Z80867), Psi (*Psilotum nudum*, Z80871), Lyc (*Lycopodium obscurum*, Z80863), Sph (*Sphagnum palustre*, Z80869), Pel (*Pellia epiphylla*, Z80868), and Baz (*Bazzania trilobata*, Z80874).

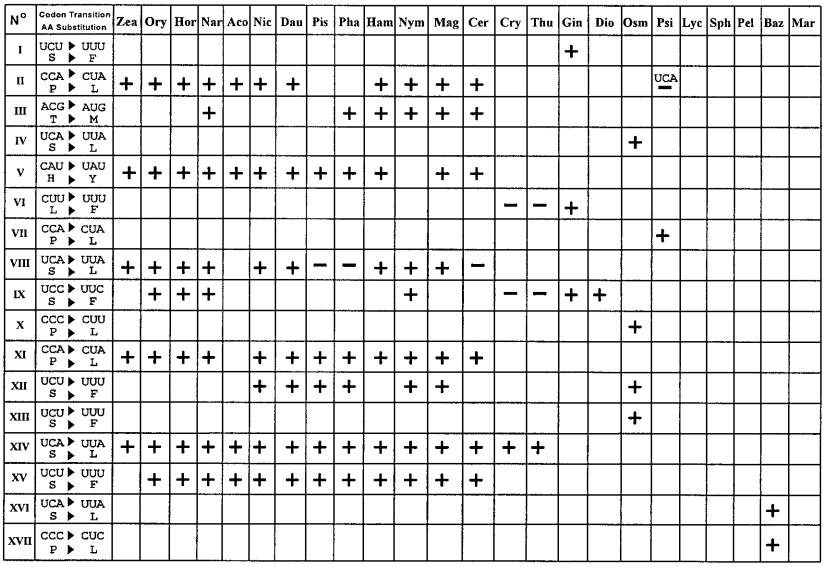

FIG. 2. Editing sites encoded in *ndh*B sequences from various plant species. Codon transitions and amino acid substitutions introduced by RNA editing are given. +, Editing events occurring in the *ndh*B-encoded transcripts; −, nonfunctional editing sites. Empty boxes indicate that the edited codon is present already at the DNA level. For abbreviations, see Fig. 1 legend.

gymnosperms, *Cryptomeria* and *Thujopsis* (XIV). Interestingly, this single editing site is also found in all of the other angiosperms examined. Whereas site IX is functional in *Ginkgo*, *Dioon* and in about one-third of the angiosperms, the very same site as well as an additional one (VI) are nonfunctional in *Cryptomeria* and *Thujopsis*.

Comparison with the homologous sequence from *Ginkgo* reveals two T-to-G mutations close to the nonfunctional editing site IX in the two gymnosperm species *Thujopsis* and *Cryptomeria*. The 5′ mutation lies 14 nt upstream, and the 3′ mutation 7 nt downstream of the editing site. The nonfunctional editing site VI is flanked by a T-to-C mutation immediately upstream of the nonfunctional editing site in *Cryptomeria*. In *Thujopsis*, a G-to-T mutation is found 10 nt upstream of site VI. Both species show an additional C-to-T mutation 21 nt downstream of this nonfunctional editing site. Direct evidence for the loss of an editing site being accompanied by the loss of the capacity to edit this site has come from a transgenic study (33). Replacement of the *psb*F gene from tobacco by the homologous gene from spinach, and thus introducing a heterologous editing site (the homologous position is already "edited" at the DNA level in tobacco), revealed that the *psb*F mRNA cannot be edited in tobacco.

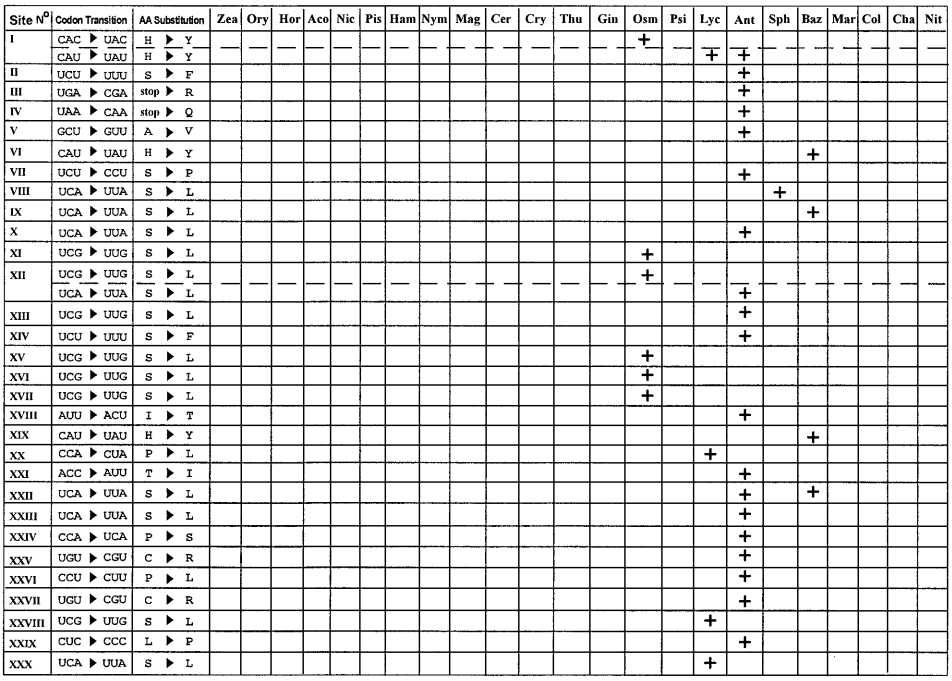

FIG. 3. Phylogenetic distribution of editing sites identified in *rbc*L-encoded transcripts. Symbols are used as in Fig. 2. The accession numbers are as follows: *Zea mays* (X86563), *Oryza sativa* (X15901), *Hordeum vulgare* (X00630), *Acorus calamus* (M91625), *Nicotiana tabacum* (Z00044), *Pisum sativum* (X03853), *Nymphaea odorata* (M77034), *Hamamelis mollis* (L01922), *Magnolia macrophylla* (X54345), *Ceratophyllum demersum* (M77030), *Cryptomeria japonica* (L25751), *Thujopsis dolabrata* (L12577), *Ginkgo biloba* (D10733), *Osmunda cinnamomea* (D14882), *Psilotum nudum* (L11059), *Lycopodium digitatum* (L11055), *Anthoceros formosae* (Ant; D43696), *Sphagnum palustre* (L13485), *Bazzania trilobata* (L11056), *Marchantia polymorpha* (X04465), *Coleochaete orbicularis* (L13477), *Chara connivens* (L13476), and *Nitella translucens* (L13482).

The editing frequencies in *ndh*B transcripts of angiosperms range in the monocots from four editing sites in *Acorus* to eight editing positions found in *Narcissus*. In dicots, only five sites are observed in *Pisum* while eight editing sites are found in *Hamamelis* and *Magnolia* (Figs. 1 and 2). The additional editing site III found in *Narcissus* was also found in five of the eight dicotyledonous plants studied. Only about one-half of the editing sites (II, V, and XIV) is conserved in all of the monocots examined. In contrast, editing sites III, VIII, IX, XI, XII, and XV show species-specific divergence. Moreover, in the dicotyledonous branch of the angiosperms, only four of eight species show an identical number and distribution of the editing sites: *Nicotiana* and *Daucus* which both possess seven editing sites and *Hamamelis* and *Magnolia* both containing eight sites. *Phaseolus* and *Ceratophyllum* although both containing six sites exhibit different editing patterns.

The latter two species as well as *Pisum* encode a nonfunctional editing site in *ndh*B (VIII). The most interesting feature of this nonfunctional site is that it occurs in species that are not close phylogenetic relatives. Phylogenetic analysis of nucleotide sequences from the plastid *rbc*L gene revealed that the aquatic plant *Ceratophyllum* represents an early sister group of the flowering plants, whereas *Pisum* belongs to a relatively recent branch in angiosperm evolution (34).

In contrast to the nonfunctional editing sites in gymnosperms, silencing of editing site VIII is not accompanied by point mutations. For example, in *Ceratophyllum*, a region of more than 100 nt upstream and over 100 nt downstream of this editing site are identical with the homologous region in *Narcissus* where this site is efficiently edited.

This finding demonstrates that the silencing of an editing site is not necessarily accompanied with a sequence divergence in close proximity to the editing site. Alternatively, silencing of editing sites in *ndh*B transcripts may be caused by the loss of a site-specific recognition factor. In general, silencing suggests that editing may no longer be necessary with respect to protein function, possibly because of compensatory mutations somewhere else in the protein.

Editing Patterns in *rbc*L Transcripts. To compare the evolution of editing patterns of two different genes, we have also searched for editing sites in the *rbc*L gene. Candidate RNA editing sites were identified based on *rbc*L amino acid and nucleotide sequence alignments using published sequences from the species listed in the legend of Fig. 3. Amino acid substitutions affecting otherwise conserved positions were tested for potential restoration of the conserved amino acid by editing at the first or second codon position. In none of the examined gymnosperm species could potential editing sites be detected. A single possible editing site corresponding to amino acid position 418 was identified in the three monocot species maize, rice, and barley. In this position, a GCA alanine codon could potentially be changed to a conserved GUA valine codon. However, sequencing of the cDNA amplification products revealed no C-to-U transition at this position (data not shown). This may indicate that replacement of one aliphatic amino acid residue by another at this position is compatible with protein function.

It was rather surprising that editing sites were found in *rbc*L transcripts of the two lower plants *Lycopodium* and *Sphagnum*. In Fig. 4, the editing sites observed for the different species including the hornwort *Anthoceros formosae* (10) are presented. This comparison shows that the *rbc*L gene of *Anthoceros* contains significantly more editing sites than the other species examined. Only three (I, XII, and XXII) of the altogether 30 editing positions are conserved between different plant species. Editing site I is found in *Osmunda*, *Lycopodium*, and *Anthoceros*, while the other two sites are shared by only two of the examined plants. Interestingly, in two of the three cases the codons containing the editing sites I and XII are not conserved. While editing at position I occurs in a CAC codon in *Osmunda*, *Lycopodium*, and *Anthoceros* possess a CAU codon at this position. At editing site XII, *Osmunda* contains a UCG codon while *Anthoceros* possesses a UCA codon. No editing sites were found in the primitive psilotopsid *Psilotum* (Fig. 3). Another rather unexpected finding was the detection of a single editing site in *rbc*L transcripts of the moss *Sphagnum*. The liverwort *Bazzania* shows editing at four positions whereas no editing sites are found in the liverwort *Marchantia*. Also no editing sites could be detected in the three green algae studied: *Coleochaete*, *Chara* and *Nitella*.

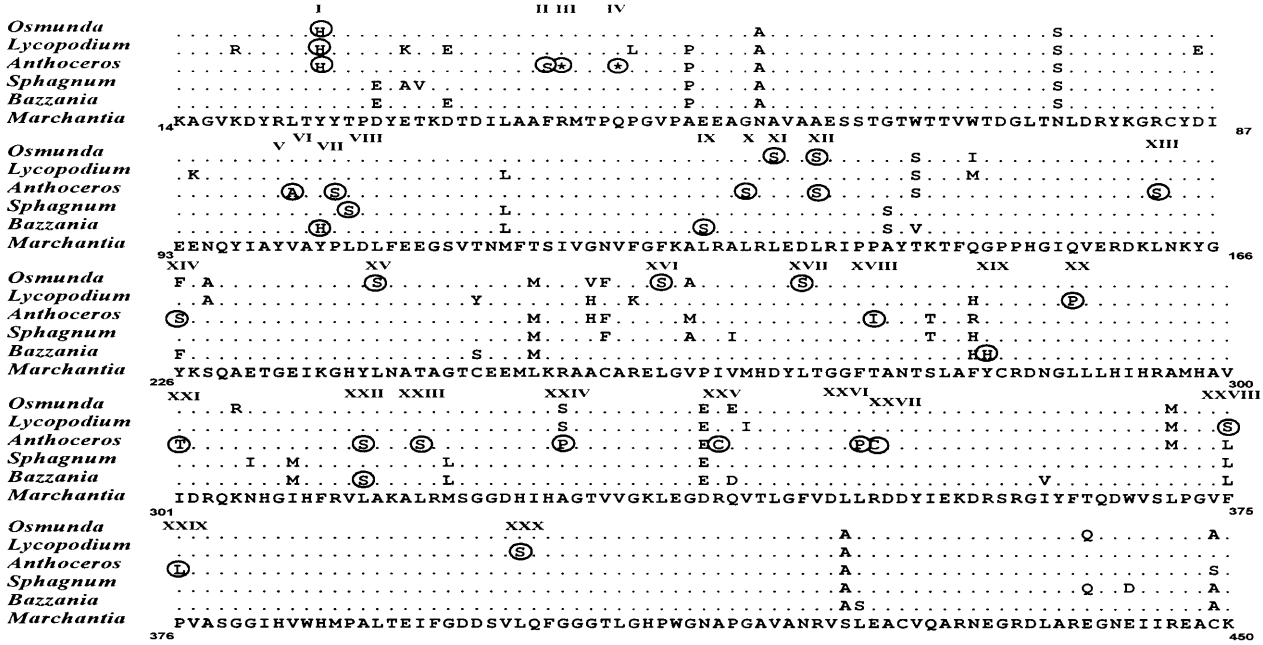

FIG. 4. Amino acid sequence alignment of *rbc*L-encoded polypeptides. The amino acid sequence as determined for the *Marchantia rbc*L gene is aligned with homologous sequences from the five species indicated by their genus name. Positions deviating from the *M. polymorpha* sequence are shown in the single letter code. Editing positions (I–XXX) are denoted by circling of the amino acid reflecting the unedited codon. The two nonsense codons at which editing occurs in *A. formosae* (10) are indicated by asterisks.

In *rbc*L transcripts of the bryophyte *Anthoceros* seven reverse editing sites have been observed (10). These U-to-C transitions are essential to restore codons for conserved amino acids and to eliminate two stop codons (Figs. 3 and 4). Interestingly, neither in *ndh*B nor in *rbc*L reverse editing sites were found in the other species examined. This clearly demonstrates that reverse editing is a rather rare event, which may have evolved independently of the conventional C-to-U editing. The recent finding that the chloroplast *chl*B gene sequences from the fern *Nephrolepsis exaltata* and from the cycad *Stangeria eriopus* contain stop codons suggests that reverse editing is not restricted to *Anthoceros* (35).

RNA Editing Frequencies Do Not Correlate with the Phylogenetic Position. Comparison of editing frequencies and editing patterns shows that RNA editing is a transcript- and species-specific process. Despite the occurrence of editing in members of all major groups of land plants, the editing pattern of a given transcript does not correlate with the phylogenetic position of the species. While the examined angiosperms encode numerous editing sites in *ndh*B, no editing sites were found in *rbc*L transcripts. Gymnosperms show a somewhat lower editing frequency in *ndh*B and also no editing in *rbc*L. The lower plants *Sphagnum* and *Lycopodium*, however, differ markedly from the spermatophyte plants. They contain no editing sites in *ndh*B transcripts, but one (*Sphagnum*) or four (*Lycopodium*) editing sites in *rbc*L. Only two species, the fern *Osmunda* and the liverwort *Bazzania*, exhibit RNA editing in transcripts of both of the examined genes.

Analysis of the editing patterns in the *ndh*B gene reveals that, surprisingly, the species-specific divergence is even more extensive among closely related species than between monocots and dicots. For example, seven of the eight editing sites found in *Magnolia ndh*B also exist in the monocot plant *Narcissus*. Maize and barley, which are both members of the same family (*Poaceae*), share only five of seven editing sites. Even more dramatic are the differences between the two liverworts *Bazzania* and *Marchantia*. While *Bazzania* contains two editing sites in *ndh*B and four editing sites in *rbc*L, no editing at all was found in *Marchantia*. The species specificity of the editing frequencies as well as the gene-specific editing patterns suggest more than one independent loss and/or gain of editing at a specific site. We propose that the different editing patterns were caused by both loss of existing editing sites and by acquisition of new sites. There are two observations providing evidence for the loss of editing sites: (*i*) there is poor evolutionary conservation of editing sites among closely related species (for example, see the remarkable differences in *ndh*B editing patterns of maize, rice, and barley), and (*ii*) there are editing sites that are absent from only one of the examined species (for example, editing sites V and XV that occur in all angiosperm plants except for *Nymphaea* and maize). While such a loss of editing sites can be easily explained by C-to-T reversion at the DNA level, the independent gain of one and the same editing site would require convergent evolution by independent creation of identical new sites accompanied with acquisition of the respective specificity factor(s).

Evolution of Plant Organellar Editing. Striking parallels become evident upon comparison of the distribution of editing in the two plant organelles, plant mitochondria and chloroplasts. As in plastids, RNA editing could be found in mitochondria of all major groups of land plants including the three classes of bryophytes (36, 37). The apparent absence of editing from both plastids and mitochondria of green algae and the liverwort *Marchantia polymorpha* may suggest a common evolutionary origin of the RNA editing activities in both of the organelles. The presence of editing in other Bryophytes may suggest that gain or secondary loss of the RNA editing activity occurred after the branching of the common ancestor group into different lineages of Bryophytes, possibly with the divergence of the two liverwort orders Jungermanniales (*Bazzania*) and Marchantiales (*Marchantia*).

The similar evolutionary distribution of RNA editing may also suggest that the two plant organelles share common components of the editing machinery that may have even existed in the common ancestor of land plants. The components required to determine the site-specificity of the editing reaction in the different compartments and transcripts may have subsequently evolved in an organelle- and gene-specific manner, probably as a result of the accumulation of T-to-C mutations at the DNA level. The lower nucleotide substitution rate in plant mitochondrial DNA as compared with plastid DNA (38) results in a lower probability of C-to-T reversions, and may thus be linked to the much higher editing frequencies of plant mitochondrial transcripts as compared with plastid mRNAs.

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