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RESEARCH ARTICLE

Variation in frequency of plastid RNA editing within *Adiantum* implies rapid evolution in fern plastomes

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PREMISE: Recent studies of plant RNA editing have demonstrated that the number of editing sites can vary widely among large taxonomic groups (orders, families). Yet, very little is known about intrageneric variation in frequency of plant RNA editing, and no study has been conducted in ferns.

METHODS: We determined plastid RNA-editing counts for two species of *Adiantum* (Pteridaceae), *A. shastense* and *A. aleuticum*, by implementing a pipeline that integrated read-mapping and SNP-calling software to identify RNA-editing sites. We then compared the edits found in *A. aleuticum* and *A. shastense* with previously published edits from *A. capillus-veneris* by generating alignments for each plastid gene.

RESULTS: We found direct evidence for 505 plastid RNA-editing sites in *A. aleuticum* and 509 in *A. shastense*, compared with 350 sites in *A. capillus-veneris*. We observed striking variation in the number and location of the RNA-editing sites among the three species, with reverse (U-to-C) editing sites showing a higher degree of conservation than forward (C-to-U) sites. Additionally, sites involving start and stop codons were highly conserved.

CONCLUSIONS: Variation in the frequency of RNA editing within *Adiantum* implies that RNA-editing sites can be rapidly gained or lost throughout evolution. However, varying degrees of conservation between both C-to-U and U-to-C sites and sites in start or stop codons, versus other codons, hints at the likely independent origin of both types of edits and a potential selective advantage conferred by RNA editing.

KEY WORDS bioinformatics; ferns; genomics; Pteridaceae; RNA editing; transcriptomics.

RNA editing is a vital step in the RNA maturation process of plastid and mitochondrial transcripts in nearly all land plants (Knoop, 2011). The majority of RNA-editing events in organellar mRNA act as a correcting mechanism that restores evolutionarily conserved amino acid sequences. RNA editing also occurs in tRNAs (Binder et al., 1994; Grewe et al., 2009), noncoding regions of the mRNA, including introns (Castandet et al., 2010; Oldenkott et al., 2014) and untranslated regions (UTRs) (Schuster et al., 1990), and rRNAs (Hecht et al., 2011), where it operates to restore base pairing that is involved in secondary or tertiary structures of RNA molecules. Therefore, RNA editing is critical to ensuring the accurate biological function of both coding and noncoding RNAs.

Between transcription and translation, RNA editing converts specific cytidines to uridines (C-to-U) or uridines to cytidines (U-to-C), as reviewed by Chateigner-Boutin and Small (2011) and Ichinose and Sugita, (2017). Forward (C-to-U) RNA editing is prevalent in the organellar genomes of all land plants, with one known exception: the marchantiid liverworts, for which a secondary loss has been inferred (Rüdinger et al., 2008). In contrast, reverse (U-to-C) RNA editing is apparently restricted to hornworts (Kugita et al., 2003), some lycophytes (Grewe et al., 2011), and ferns (Wolf et al., 2004; Guo et al., 2015), but is absent in seed plants (Tillich et al., 2006). Although a few studies have proposed that U-to-C editing may occur in angiosperm mitochondria at low frequency (Gualberto et al., 1990; Ong and Palmer, 2006; Picardi et al., 2010), others have more recently challenged the notion that U-to-C editing occurs in seed plants at all (Guo et al., 2015).

Several RNA-editing studies have exposed striking variation in the extent of RNA editing among different groups of land plants and also between the two organellar genomes. In angiosperms, the number of RNA-editing sites in plastids ranges from 23 in *Cucumis melo* (Guzowska-Nowowiejska et al., 2009) to 184 in *Amborella trichopoda* (Ishibashi et al., 2019) and from 313 in *Populus* to 755 in *Liriodendron* (Edera et al., 2018) in mitochondria. In seed-free plants, however, the range of variation in RNA-editing sites is even more remarkable. In plastids, the number of C-to-U edits varies from none in *Equisetum hyemale* (Knie et al.,

2016) and marchantiid liverworts (Rüdinger et al., 2008) to more than 3400 in *Selaginella uncinata* (Oldenkott et al., 2014); in mitochondria, limited data suggest that the total number of edits (C-to-U and U-to-C) ranges from 885 in *Salvinia cucullata* (Li et al., 2018) to 2139 in *Selaginella moellendorffii* (Hecht et al., 2011). With approximately 10,000 species (PPG I, 2016), ferns are the most diverse lineage of plants with both C-to-U and U-to-C RNA editing. Guo et al. (2015) detected remarkable variation in the number of plastid edits across three distantly related ferns (one leptosporangiate fern, *Adiantum capillus-veneris*, and two eusporangiate ferns, *Ophioglossum californicum* and *Psilotum nudum*) and more recently, Li et al. (2018) recovered substantial variation between two heterosporous leptosporangiate fern genera, *Azolla* and *Salvinia*, with only about 22% of edited sites conserved between them.

Less is currently known about the extent of variation in the number of RNA-editing sites at more shallow taxonomic levels (i.e., among congeneric species or across multiple genera belonging to the same family). Recent studies by Kawabe et al. (2019) and Smith (2020) demonstrated significant variation in the number of C-to-U plastid RNA-editing sites among three species (and one subspecies) of *Arabidopsis* and across three *Selaginella* species, respectively. In the present study, we examined *Adiantum* L. (Pteridaceae) to assess the amount of variation in RNA-editing sites within a single fern genus. We directly compared genomic DNA and transcriptome sequences to determine the number of RNA-editing sites across the complete plastomes of three distantly related species within *Adiantum*: *A. aleucitum* (Rupr.) C.A.Paris, *A. shastense* Huiet & A.R.Smith, and *A. capillus-veneris* L. (Huiet et al., 2018; Regalado et al., 2018). The first two species are only known as diploids, and although *A. capillus-veneris* is both diploid (Old World) and tetraploid (New World), the voucher used by Wolf et al. (2004) is from Japan and also presumed to be diploid. We generated novel RNA-editing estimates for *A. aleuticum* and *A. shastense* to compare with the previously published edit estimate for *A. capillus-veneris* (Wolf et al., 2004). Regalado et al. (2018) estimated that *A. aleuticum* and *A. shastense* together diverged from *A. capillus-veneris* approximately 60 million years ago (Ma), whereas *A. aleuticum* and *A. shastense* diverged from one another about 20 Ma (Fig. 1). We report striking variation in the number and location of RNA-editing sites across all three species and a high degree of conservation among certain editing sites, particularly those involving start and stop codons.

MATERIALS AND METHODS

Data acquisition

Raw Illumina RNA reads for the *A. shastense* and *A. aleuticum* plastomes were obtained from Song et al. (2018) and the 1000 plant transcriptome project (Carpenter et al., 2019), respectively. The raw reads were uploaded from the NCBI Sequence Read Archive to the Minnesota Supercomputing Institute's High Performance Computing cluster (MSI HPC) using the sratoolkit programs prefetch and fastq-dump (Leionen et al., 2011). The RNA reads for both *A. shastense* and *A. aleuticum* were sequenced on the Illumina HiSeq platform (Song et al., 2018; Carpenter et al., 2019). Assembled and annotated DNA plastomes for *A. shastense* (Song et al., 2018) (GenBank accession MG432483) and *A. aleuticum* (Robison et al., 2018) (GenBank accession MH173079) came from the same voucher specimens as the RNA data used in this study and were also transferred from GenBank to the MSI HPC. The plastomes for *A. shastense* and *A. aleuticum* were both assembled using NOVOPlasty (Dierckxsens et al., 2016), whereas the *A. capillus-veneris* plastome was assembled manually (Wolf, 2003).

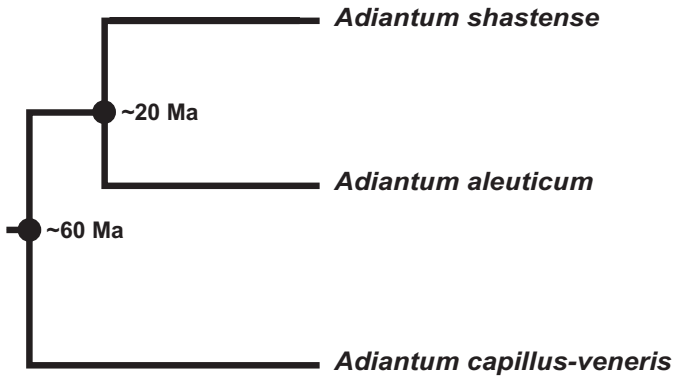

FIGURE 1. Divergence date estimates for the three *Adiantum* species in this study. Node ages based on Regalado et al. (2018).

RNA-editing analysis

Adapters and low-quality sequences were trimmed from the transcriptomic reads of *A. aleuticum* and *A. shastense* with Trimmomatic version 0.33 (Bolger et al., 2014) using the following parameters: ILLUMINACLIP:TruSeq3-PE.fa:2:30:10:2:true–SLIDINGWINDOW:4:20–MINLEN:70. Bowtie2 indices were built for the *A. aleuticum* and *A. shastense* plastomes using the bowtie2-build command in Bowtie2 version 2.3.4.1 (Langmead and Salzburg, 2012). RNA reads from each species were then mapped to their respective plastomes using Tophat v. 2.0.13 (Trapnell et al., 2009) with the following parameters used in the editing pipeline by Guo et al. (2015): –N ––4 read-gap-length ––3 ––read-edit-dist 5 –I 5000 ––no-coverage-search. The resulting BAM file was then used to call DNA/RNA variants. First, the FASTA files for the plastomes of *A. aleuticum* and *A. shastense* were indexed using the faidx program in the samtools version 1.9 program suite (Li et al., 2009). The BAM output from Tophat was then used in combination with the indexed reference plastome sequences to find DNA/RNA variants using the mpileup and call features of the bcftools version 1.9 program suite (Narasimhan et al., 2016) to generate a variant call format (VCF) file containing all DNA/RNA variants detected by bcftools.

The resulting VCF file was filtered using a custom script to include only DNA/RNA variants representing likely RNA edits. The script was modified from the edit detection pipeline used in Guo et al. (2015). For putative C-to-U edit sites, only C:T and A:G variants were accepted (for sense and antisense strands, respectively). Similarly, only T:C and G:A variants were accepted to represent putative U-to-C edit sites. At each variant site, read depth and the percentage of RNA reads showing the putative edit were calculated. To account for possible sequencing errors, variants with less than 3× read depth or variants that were supported in fewer than 5% of the RNA reads mapped to that particular site were discarded; only variants meeting this threshold were considered to be putative edit sites. Edits were then manually inspected in Geneious version 10.2.6 (Kearse et al., 2012) and characterized based on the amino acid change produced and their location in the plastomes (i.e., what gene/tRNA/UTR/intron the edit affected).

Comparing RNA-editing sites

RNA-editing sites were compared across each of the three *Adiantum* species by individually aligning each plastid gene that underwent RNA editing using Geneious version 10.2.6 (Kearse et al., 2012) to first extract the gene sequences subject to RNA editing using the Extract Annotation tool, then to align DNA for each plastid gene from each of the three species that underwent a putative RNA edit, enabling us to accurately assess the homology of each edited site. RNA-editing sites occurring in the recently discovered *ycf94* gene (Song et al., 2018) were excluded from comparison due to a lack of transcriptomic data from *A. capillus-veneris*. Additionally, the second exon of *ndhB* was also excluded from comparison due to a lack of sufficient read depth in *A. aleuticum* transcriptomic data. These were the only RNA-editing sites not included in the analyses.

RESULTS

RNA-editing analyses from our study revealed 509 plastid RNA-editing sites in *A. shastense* and 505 in *A. aleuticum* (Table 1, Fig. 2). Wolf et al. (2004) documented 350 RNA-editing sites present in the *A. capillus-veneris* plastome. Of the RNA-editing sites that we compared among the three species, 234 were found in all three plastomes, and an additional 177 were found in *A. aleuticum* and *A. shastense,* but not in *A. capillus-veneris* (Fig. 3). *Adiantum capillus-veneris* exclusively shared only 22 editing sites with *A. aleuticum* and 24 with *A. shastense* (Fig. 3). The number of RNA-editing sites found to be exclusive to *A. shastense*, *A. aleuticum*, and *A. capillus-veneris* were 70, 68, and 58, respectively (Fig. 3). In total, 653 distinct RNA-editing sites were found in the three *Adiantum* plastomes examined.

Overall, U-to-C RNA-editing sites were more conserved than C-to-U sites (Fig. 4A, B). Of the 51 distinct U-to-C RNA-editing sites found, 32 (62.7%) were present in all three species (Fig. 4B), whereas, of the 602 distinct C-to-U RNA editing sites documented, only 202 (33.6%) were shared (Fig. 4A). We also compared the conservation of editing sites between nonsynonymous and synonymous edits of the coding regions. Of the nonsynonymous edits, 220 of a total 592 distinct nonsynonymous edits (37.2%) were present in each species (Fig. 4C); in contrast, only 3 of the 76 distinct synonymous edits (3.9%) were shared by all species (Fig. 4D). Finally, edits involved in creating or removing start or stop codons showed elevated levels of conservation, with 29 (69%) of the 42 total RNA-editing sites associated with stop codons found in all three species (Fig. 4E), and an additional nine editing sites shared between *A. shastense* and *A. aleuticum*. Only *A. aleuticum* possessed distinct RNA-editing sites (4) that removed premature stop codons (Fig. 4E). Of the 23 edits involved in creating start codons, 19 (82.6%) appeared in all three species (Fig. 4F).

We found the majority of RNA-editing sites in all three species at the first and second codon positions, with U-to-C edits almost exclusively at the first codon position (Fig. 5). Therefore, the bulk (nearly 75%) of edits in coding regions for each species results in a nonsynonymous amino acid change (Fig. 6), with less than 10% of edits being synonymous in any species (Fig. 6). A small proportion of edits were also found in noncoding regions (UTRs and introns) and tRNAs (Fig. 5, Table 1).

In addition, changes in the hydrophobicity of the encoded proteins before and after RNA editing was investigated. The vast majority of nonsynonymous RNA edits convert a codon for a hydrophilic amino acid to one that is hydrophobic (Fig. 7A). A smaller proportion convert between two different hydrophobic amino acid codons, and still fewer convert hydrophobic amino acid codons to hydrophilic amino acid codons (Fig. 7A). This trend was also examined within three specific plastid protein classes: cytosolic proteins (proteins in the stroma or lumen), extrinsic proteins (those partially spanning a membrane), and integral proteins (those fully imbedded in the membrane). We found that similar patterns exist in all three protein classes, namely, that an increase in hydrophobicity is brought on by RNA editing (Fig. 7B). No C-to-U or U-to-C changes are capable of producing a change between two hydrophilic amino acid codons; therefore, these types of changes were not considered.

TABLE 1. Summary of plastid RNA editing frequencies for three *Adiantum* species.

Plastome location	Editing event	*A. shastense*	Percent of edits	*A. aleuticum*	Percent of edits	*A. capillus-veneris*	Percent of edits
Total		**509**		**505**		**350**	
	C-to-U	468	91.93	457	90.50	315	90.00
	U-to-C	41	8.07	48	9.50	35	10.00
Protein coding		**481**		**480**		**332**	
	1st codon position	122	25.36	131	27.29	86	25.90
	2nd codon position	308	64.03	308	63.96	226	68.07
	3rd codon position	51	10.60	42	8.75	20	6.02
	Start codon creation	21	4.37	23	4.58	20	6.02
	Stop codon creation	6	1.25	7	1.46	3	0.90
	Stop codon removal	32	6.65	35	7.29	26	7.83
tRNA		2	0.39	1	0.20	1	0.29
Noncoding regions		25	4.92	25	4.95	17	4.86
	Intron	13	2.56	9	1.78	6	1.71
	UTRs	12	2.36	14	2.97	11	3.14

The bold values are from where the percentages are derived. The second set of bold values in the "protein coding" row are from where the percentages for the 6 rows below come from.

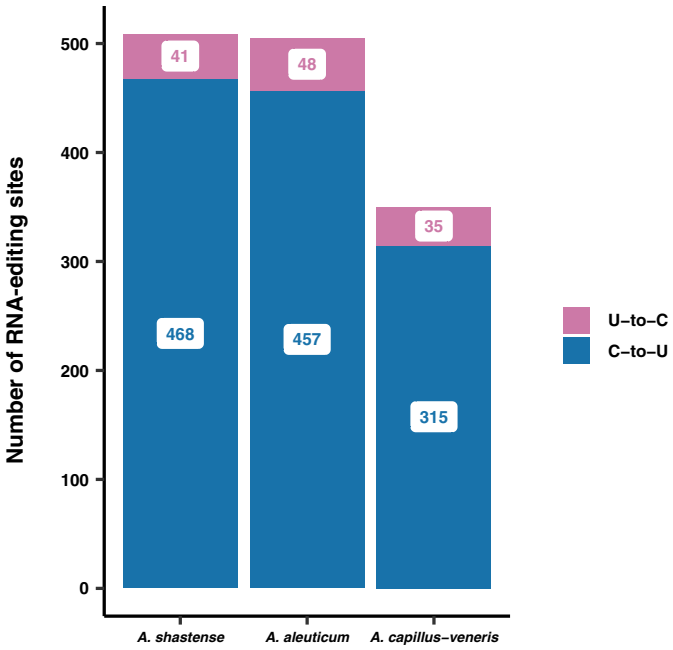

FIGURE 2. Frequency of C-to-U and U-to-C RNA-editing sites for each *Adiantum* species in this study.

DISCUSSION

Ferns are an appealing focal group for studies of RNA editing—they are one of the only major plant lineages to exhibit both forward (C-to-U) and reverse (U-to-C) RNA editing, and they are rich in species diversity. *Adiantum*, in particular, has a wealth of genomic and transcriptomic resources available, making it an excellent model genus for the first infrageneric comparison of the plastid editome in ferns.

Our analyses revealed striking variation in the number of RNA-editing sites in the plastomes of *Adiantum* (Table 1, Fig. 2). The total number of plastid RNA-editing sites ranged from 509 in *A. shastense* to 350 in *A. capillus-veneris*, a range of more than 150 sites. To the best of our knowledge, other than the extreme examples documented from *Selaginella* (Smith, 2020), this infrageneric

FIGURE 3. Venn diagram of shared and exclusive RNA-editing sites identified among the three *Adiantum* species in this study.

difference in chloroplast RNA-editing sites is the largest reported for land plants. We found no evidence to suggest that differences between the Sanger sequencing method used by Wolf et al. (2004) to generate the *A. capillus-veneris* data set and the Illumina sequencing method used for *A. shastense* and *A. aleuticum* contributed to the observed differences in RNA-editing frequency. In almost all cases where an edit was present in *A. shastense* and *A. aleuticum*, the nucleotide produced by RNA editing was already present in the genomic sequence of *A. capillus-veneris* (i.e., if *A. shastense* and *A. aleuticum* shared a cytosine [C] position that underwent C-to-U RNA editing, a thymine [T] was almost always present at that position in the plastome of *A. capillus-veneris*).

In total, we recorded 653 distinct plastid RNA-editing sites (C-to-U and U-to C) across three species of *Adiantum* (Fig. 3). Of these, 602 are the C-to-U type, and 51 are U-to-C edits (Fig. 4 A, B). The number of distinct C-to-U RNA-editing sites detected in sampled members of the genus *Adiantum* (602) is almost three times more than the 227 C-to-U plastid RNA-editing sites reported so far across all angiosperms (Ishibashi et al., 2019). This result is particularly striking considering that the length of time since these three species of *Adiantum* shared a common ancestor (ca. 60 Myr, Fig. 1) is less than half the estimated divergence time that spans the angiosperm clade (ca. 140 Myr, Magallón et al., 2015). A point of focus for further research will be to investigate why *Adiantum*, and likely ferns as a whole, possess such a diverse repertoire of plastid RNA-editing sites, especially when compared to angiosperms.

Although the number of RNA-editing sites is highly variable in *Adiantum*, similar patterns emerge in the editomes of the three species. For example, the majority of edits are concentrated at the first and second codon positions (Fig. 5, Table 1), reflecting the role of RNA editing in restoring function to conserved amino acid codons. More specifically, U-to-C edits in each species are almost exclusively found at the first codon position, whereas C-to-U edits are most often at the second codon position (Fig. 5). For each species, roughly 75% of the RNA-editing events result in a nonsynonymous amino acid change that does not involve a start or stop codon (Fig. 6). Furthermore, we found that each species studied here has similar proportions of RNA-editing sites associated with noncoding regions (UTRs and introns), tRNAs, as well as edits that created stop codons (Fig. 6, Table 1).

One marked difference we observed, however, was that the plastid editome of *A. capillus-veneris* was characterized by a relatively lower proportion of synonymous edits (Fig. 6). This result is likely coupled with the fact that we observed nearly half as many edits at the third codon position of *A. capillus-veneris* (Table 1). Another difference we noted was that *A. capillus-veneris* has a slightly higher proportion of editing sites that result in the removal of internal stop codons and the creation of start codons than the other two species (Fig. 6, Table 1). This result makes sense given the apparently high degree of conservation of RNA editing sites associated with start or stop codons (Fig. 4E, F), and the significantly lower number of total plastid edits in *A. capillus-veneris* (Fig. 2, Table 1). While other RNA-editing sites have been lost in *A. capillus-veneris* (or gained in *A. shastense* and *A. aleuticum*), the RNA-editing sites associated with start and stop codons persist, suggesting they may confer an evolutionary advantage.

C-to-U and U-to-C RNA-editing sites show opposing patterns of conservation. While C-to-U sites are generally not well conserved (Fig. 4A), U-to-C sites show a high degree of conservation

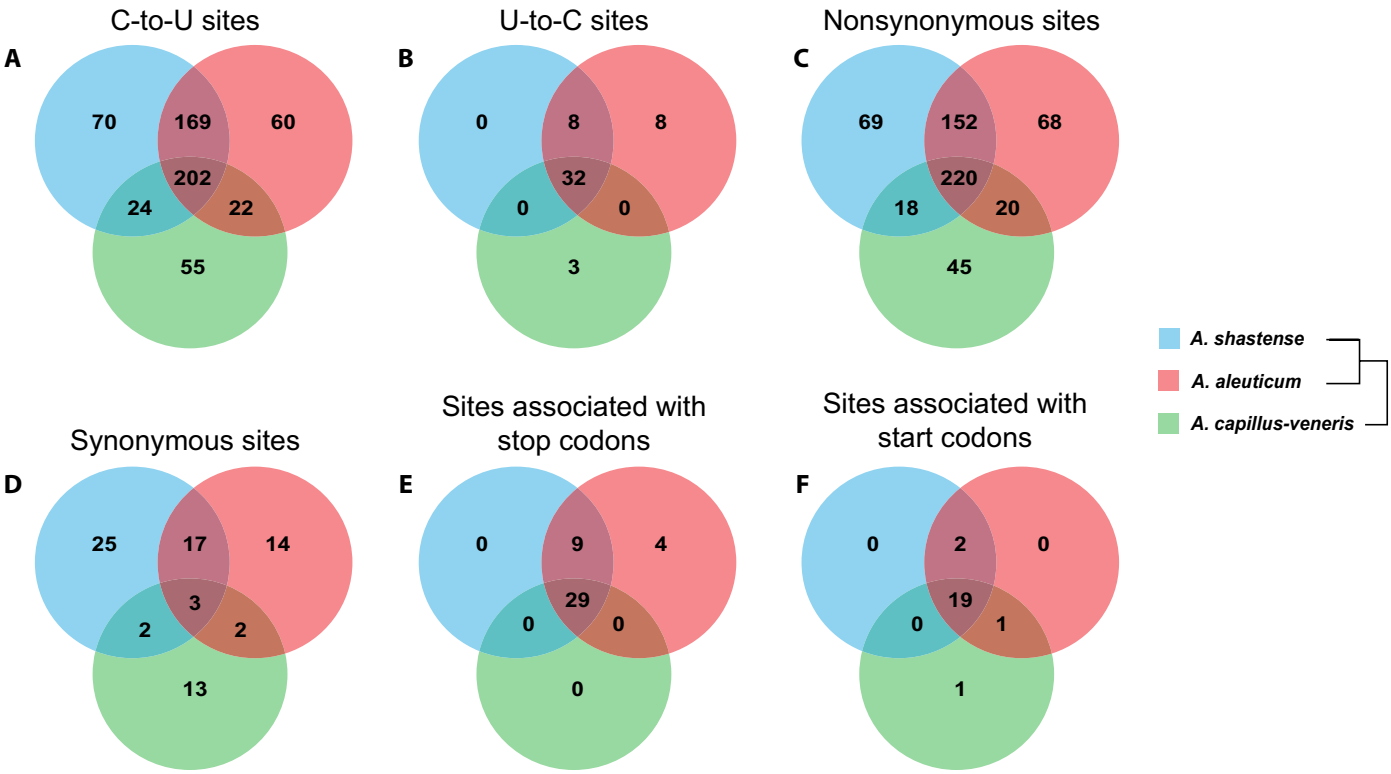

FIGURE 4. Venn diagram depicting the conservation of specific types of RNA-editing sites among the three *Adiantum* species in this study. (A) C-to-U RNA-editing sites. (B) U-to-C RNA-editing sites. (C) Nonsynonymous RNA-editing sites. (D) Synonymous RNA-editing sites. (E) RNA-editing sites that remove or create stop codons. (F) RNA-editing sites that create start codons.

(Fig. 4B), probably because they are mostly involved in correcting internal stop codons—a group of edits also showing a high degree of conservation (Fig. 4E). This result suggests that harboring internal stop codons in the plastomes of these three *Adiantum* species and editing them after transcription may in some way be adaptive, possibly by adding an extra layer of control on gene expression as suggested by Li et al. (2018). The possibility that RNA editing could be advantageous is further supported by our findings that C-to-U

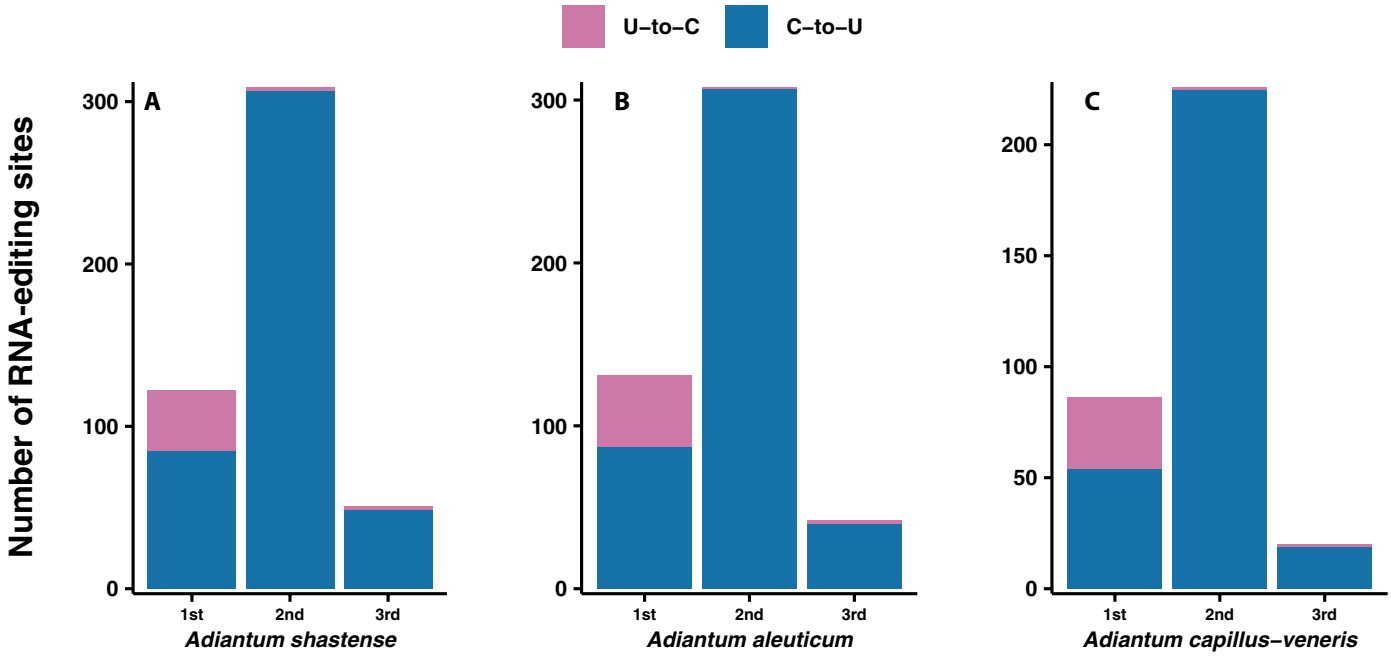

FIGURE 5. Number of RNA-editing sites per codon position for each *Adiantum* species in this study. (A) *Adiantum shastense*. (B) *Adiantum aleuticum*. (C) *Adiantum capillus-veneris*.

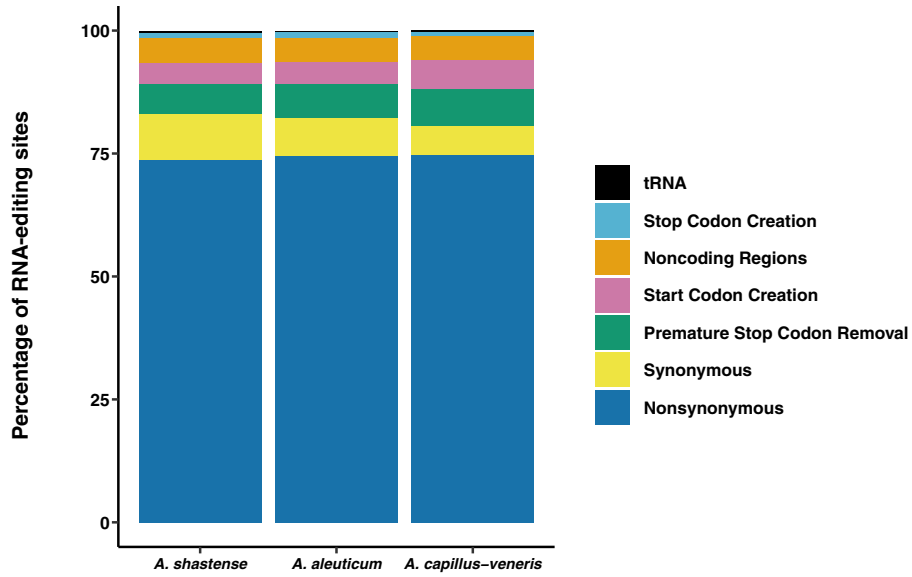

FIGURE 6. Percentage of specific sequence modifications produced by RNA editing in sampled taxa. Histograms represent 100% of RNA edits detected.

editing sites that create start codons (Fig. 4F) are also much more highly conserved than other C-to-U sites (Fig. 4A) and that editing events resulting in nonsynonymous amino acid changes (Fig. 4C) are more conserved than those leading to synonymous changes (Fig. 4D). While it has been suggested that creating start codons and removing stop codons via RNA editing may be used by certain plants (ferns, hornworts, and lycophytes) to control organellar transcript translation, these notions have not been tested empirically (Li et al., 2018). Why there appears to be a preference for creating or removing start and stop codons by RNA editing, rather than encoding them at the DNA level, remains an area worthy of further consideration.

Another trend we observed for each *Adiantum* species, was that RNA editing greatly increased the hydrophobicity of the proteins arising from edited codons (Fig. 7A). This trend is also seen in the plastomes of other plant groups, such as angiosperms (Ishibashi et al., 2019) and the hornwort *Anthoceros formosae* (Kugita et al., 2003) and in the mitogenome of *Arabidopsis thaliana* (Giege and Brennicke, 1999). Plant organellar genomes contain a significantly large proportion of genes encoding membrane-bound proteins that are rich in hydrophobic amino acids (Covello and Gray, 1993; Gray et al., 1999). Increased hydrophobicity in the plastid-encoded membrane-bound proteins of *Adiantum* likely reflects the conservation of these hydrophobic residues in membrane-bound proteins, supporting the notion that RNA editing confers a selective advantage by maintaining gene functionality in the plastomes. However, we found the

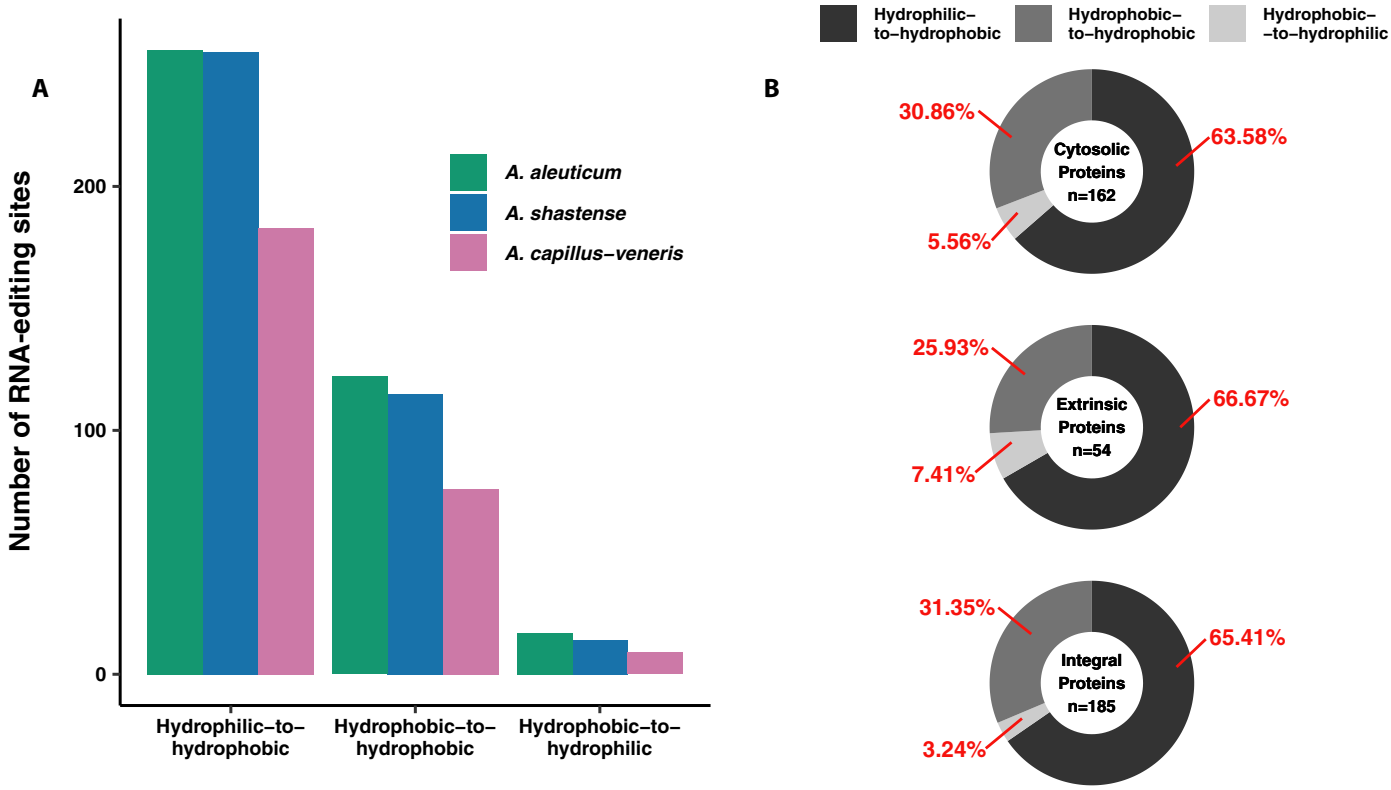

FIGURE 7. (A) Comparison of edit sites that lead to a change in hydrophobicity/hydrophilicity of the resulting amino acid via nonsynonymous RNA editing. (B) Percentage of nonsynonymous RNA-editing sites leading to each type of change in hydrophobicity in three protein classes; "n" is the number of nonsynonymous RNA editing sites in each particular protein class.

trend of increased hydrophobicity extends to cytosolic proteins as well (Fig. 7B). Furthermore, these findings corroborate those from a comparative study by Jobson and Qiu (2008) who demonstrated that hydrophobicity was also increased as a result of RNA editing in both the plastid editome of *Anthoceros formosae* and the mitochondrial editome of *Beta vulgaris*.

Our study provides a first glimpse at the variation in frequency of plastid RNA editing in *Adiantum*. By comparing plastid RNA-editing frequencies among three *Adiantum* species, we revealed a difference of >150 edits between two *Adiantum* lineages. By comparing the location of these editing sites, we demonstrated that there are nearly three times as many distinct C-to-U RNA-editing sites in *Adiantum* than there are across all of angiosperms. Our observation that RNA-editing sites associated with start and stop codons show a much higher degree of conservation than do other sites implies that RNA editing is not only an integral step in gene expression for plastid-derived transcripts, but also that the RNA-editing process is likely under selection and is not evolutionarily neutral.

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AUTHOR CONTRIBUTIONS

B.D.F., E.M.S., and A.L.G. conceived the research idea. B.D.F. performed all analyses. B.D.F., E.M.S., K.M.P., and A.L.G. contributed to writing the paper.

DATA AVAILABILITY STATEMENT

All data sets are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.g1jwstqp3 (Fauskee et al., 2021).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Number of RNA-editing sites per plastid gene for three *Adiantum* species.

LITERATURE CITED

Binder, S., A. Marchfelder, and A. Brennicke. 1994. RNA editing of tRNA(Phe) and tRNA(Cys) in mitochondria of *Oenothera berteriana* is initiated in precursor molecules. *Molecular and General Genetics* 244: 67–74.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120.

Carpenter, E. J., N. Matasci, S. Ayyampalayam, S. Wu, J. Sun, J. Yu, F. R. Jimenez Vieira, et al. 2019. Access to RNA-sequencing data from 1,173 plant species: the 1000 plant transcriptomes initiative (1KP). *Gigascience* 8: giz126.

Castandet, B., D. Choury, D. Bégu, X. Jordana, and A. Araya. 2010. Intron RNA editing is essential for splicing in plant mitochondria. *Nucleic Acids Research* 38: 7112–7121.

Chateigner-Boutin, A. L., and I. Small. 2011. Organellar RNA editing. *Wiley Interdisciplinary Reviews: RNA* 2: 493–506.

Covello, P. S., and M. W. Gray. 1993. On the evolution of RNA editing. *Trends in Genetics* 9: 265–268.

Dierckxsens, N., P. Mardulyn, and G. Smits. 2016. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *Nucleic Acids Research* 45: e18.

Edera, A. A., C. L. Gandini, and M. V. Sanchez-Puerta. 2018. Towards a comprehensive picture of C-to-U RNA editing sites in angiosperm mitochondria. *Plant Molecular Biology* 97: 215–231.

Fauskee, B. D., E. M. Sigel, K. M. Pryer, and A. L. Grusz. 2021. Data from: Variation in frequency of plastid RNA editing within *Adiantum* implies rapid evolution in fern plastomes. *Dryad Digital Repository*. https://doi.org/10.5061/dryad.g1jwstqp3.

Giege, P., and A. Brennicke. 1999. RNA editing in Arabidopsis mitochondria effects 441 C to U changes in ORFs. *Proceedings of the National Academy of Sciences* 96: 15324–15329.

Gray, M. W., G. Burger, and B. F. Lang. 1999. Mitochondrial evolution. *Science* 283: 1476–1481.

Grewe, F., P. Viehoever, B. Weisshaar, and V. Knoop. 2009. A *trans*-splicing group I intron and tRNA-hyperediting in the mitochondrial genome of the lycophyte *Isoetes engelmannii*. *Nucleic Acids Research* 37: 5093–5104.

Grewe, F., S. Herres, P. Viehöver, M. Polsakiewicz, B. Weisshaar, and V. Knoop. 2011. A unique transcriptome: 1782 positions of RNA editing alter 1406 codon identities in mitochondrial mRNAs of the lycophyte *Isoetes engelmannii*. *Nucleic Acids Research* 39: 2890–2902.

Gualberto, J. M., J. H. Weil, and J. M. Grenenberger. 1990. Editing of the wheat *cox*III transcript: evidence for twelve C to U and one U to C conversions and for sequence similarities around editing sites. *Nucleic Acids Research* 18: 3771–3776.

Guo, W., F. Grewe, and J. P. Mower. 2015. Variable frequency of plastid RNA editing among ferns and repeated loss of uridine-to-cytidine editing from vascular plants. *PLoS One* 10: e0117075.

Guzowska-Nowowiejska, M., E. Fiedorowicz, and W. Pląder. 2009. Cucumber, melon, pumpkin, and squash: Are rules of RNA editing in flowering plants chloroplast genes so well known indeed? *Gene* 434: 1–8.

Hecht, J., F. Grewe, and V. Knoop. 2011. Extreme RNA editing in coding islands and abundant microsatellites in repeat sequences of *Selaginella moellendorffii* mitochondria: the root of frequent plant mtDNA recombination in early tracheophytes. *Genome Biology and Evolution* 3: 344–358.

Huiet, L., F.-W. Li, T.-T. Kao, J. Prado, E. Schuettpelz, and K. M. Pryer. 2018. A worldwide phylogeny of *Adiantum* (Pteridaceae) reveals remarkable convergent evolution in leaf blade architecture. *Taxon* 67: 488–502.

Ichinose, M., and M. Sugita. 2017. RNA editing and its molecular mechanism in plant organelles. *Genes* 8: 5.

Ishibashi, K., I. Small, and T. Shikanai. 2019. Evolutionary model of plastidial RNA editing in angiosperms presumed from genome-wide analysis of *Amborella trichopoda*. *Plant and Cell Physiology* 60: 2141–2151.

Jobson, R. W., and Y. L. Qui. 2008. Did RNA editing in plant organellar genomes originate under natural selection or through genetic drift? *Biology Direct* 3: 43.

Kawabe, A., H. Y. Furihata, Y. Tsujino, T. Kawanabe, S. Fujii, and T. Yoshida. 2019. Divergence of RNA editing among *Arabidopsis* species. *Plant Science* 280: 241–247.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.

Knie, N., F. Grewe, S. Fischer, and V. Knoop. 2016. Reverse U-to-C RNA editing exceeds C-to-U RNA editing in some ferns—a monilophyte-wide comparison of chloroplast and mitochondrial RNA editing suggest independent evolution of the two processes in both organelles. *BMC Evolutionary Biology* 16: 134.

Knoop, V. 2011. When you can't trust the DNA: RNA editing changes transcript sequences. *Cellular and Molecular Life Sciences* 68: 567–586.

Kugita, M., Y. Yamamoto, T. Fujikawa, T. Matsumoto, and L. Yoshinaga. 2003. RNA editing in hornwort chloroplasts makes more than half the genes functional. *Nucleic Acids Research* 31: 2417–2423.

Langmead, B., and S. L. Salzburg. 2012. Fast gapped-read alignment with Bowtie 2. *Nature Methods* 9: 357–359.

Leinonen, R., H. Sugawara, M. Shumway, and International Nucleotide Sequence Database Collaboration. 2011. The sequence read archive. *Nucleic Acids Research* 39: D19–D21.

Li, F.-W., P. Brouwer, L. Carretero-Paulet, S. Cheng, J. De Vries, P. M. Delaux, A. Eily, et al. 2018. Fern genomes elucidate land plant evolution and cyanobacterial symbioses. *Nature Plants* 4: 460–472.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, et al. 2009. The sequence alignment/map format and SAMtools. *Bioinformatics* 25: 2078–2079.

Magallón, S., S. Gómez-Acevedo, L. L. Sánchez-Reyes, and T. Hernández-Hernández. 2015. A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. *New Phytologist* 207: 437–453.

Narasimhan, V., P. Danecek, A. Scally, Y. Xue, C. Tyler-Smith, and R. Durbin. 2016. BCFtools/RoH: a hidden Markov model approach for detecting autozygosity from next-generation sequencing data. *Bioinformatics* 32: 1749–1751.

Oldenkott, B., K. Yamaguchi, S. Tsuji-Tsukinoki, N. Knie, and V. Knoop. 2014. Chloroplast RNA editing going extreme: more than 3400 events of C-to-U editing in the chloroplast transcriptome of the lycophyte *Selaginella uncinata*. *RNA* 20: 1499–1506.

Ong, H. C., and J. D. Palmer. 2006. Pervasive survival of expressed mitochondrial *rps14* pseudogenes in grasses and their relatives for 80 million years following three functional transfers to the nucleus. *BMC Evolutionary Biology* 6: 55.

Picardi, E., D. S. Horner, M. Chiara, R. Schiavon, G. Valle, and G. Pesole. 2010. Large-scale detection and analysis of RNA editing in grape mtDNA by RNA deep-sequencing. *Nucleic Acids Research.* 38: 4755–4767.

PPG I. 2016. A community-derived classification for extant lycophytes and ferns. *Journal of Systematics and Evolution* 54: 563–603.

Regalado, L., J. Lóriga, J. Bechteler, A. Beck, H. Schneider, and J. Heinrichs. 2018. Phylogenetic biogeography reveals the timing and source areas of the *Adiantum* species (Pteridaceae) in the West Indies with a specific focus on Cuba. *Journal of Biogeography* 45: 541–551.

Robison, T. A., A. L. Grusz, P. G. Wolf, J. P. Mower, B. D. Fauskee, K. Sosa, and E. Schuettpelz. 2018. Mobile elements shape plastomes evolution in ferns. *Genome Biology and Evolution* 10: 2558–2571.

Rüdinger, M., M. Polsakiewicz, and V. Knoop. 2008. Organellar RNA editing and plant-specific extensions of pentatricopeptide repeat proteins in jungermanniid but not marchantiid liverworts. *Molecular Biology and Evolution* 25: 1405–1414.

Schuster, W., R. Hiesel, B. Wissinger, and A. Brennicke. 1990. RNA editing in the cytochrome *b* locus of the higher plant *Oenothera berteriana* includes a U-to-C transition. *Molecular and Cellular Biology* 10: 2428–2431.

Smith, D. R. 2020. Unparalleled variation in RNA editing amount *Selaginella* plastomes. *Plant Physiology* 182: 12–14.

Song, M., L. Y. Kuo, L. Huiet, K. M. Pryer, C. J. Rothfels, and F. W. Li. 2018. A novel chloroplast gene reported for flagellate plants. *American Journal of Botany* 105: 117–121.

Tillich, M., P. Lehwark, B. R. Morton, and U. G. Maier. 2006. The evolution of chloroplast RNA editing. *Molecular Biology and Evolution* 23: 1912–1921.

Trapnell, C., L. Pachter, and S. L. Salzburg. 2009. TopHat: discovering splice junctions with RNA-seq. *Bioinformatics* 25: 1105–1111.

Wolf, P. G. 2003. Complete nucleotide sequence of the chloroplast genome from a leptosporangiate fern, *Adiantum capillus-veneris* L. *DNA Research* 10: 59–65.

Wolf, P. G., C. A. Rowe, and M. Hasebe. 2004. High levels of RNA editing in a vascular plant chloroplast genome: analysis of transcripts from the fern *Adiantum capillus-veneris*. *Gene* 339: 89–97.