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Molecular Data Concerning Alloploid Character and the Origin of Chloroplast and Mitochondrial Genomes in the Liverwort Species *Pellia borealis*

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Abstract

The liverwort *Pellia borealis* is a diploid, monoecious, allopolypliod species (n=18) that as it was postulated, originated after hybridization and duplication of chromosome sets of two cryptic species: *Pellia epiphylla-species N* (n=9) and *Pellia epiphylla-species S* (n=9). Our recent results have supported the allopolyploid origin of *P. borealis*. We have shown that the nuclear genome of *P. borealis* consists of two nuclear genomes: one derived from *P. epiphylla-species N* and the other from *P. epiphylla-species S*. In this paper we show the origin of chloroplast and mitochondrial genomes in an allopolyploid species *P. borealis*. To our knowledge there is no information concerning the way of mitochondria and chloroplast inheritance in *Bryophyta*. Using an allopolyploid species of *P. borealis* as a model species we have decided to look into chloroplast and mitochondrial genomes of *P. borealis, P. epiphylla-species N* and *P. epiphylla-species S* for nucleotide sequences that would allow us to differentiate between both cryptic species and to identify the origin of organelle genomes in the alloploid species. We have amplified and sequenced a chloroplast $tRNA^{Leu}$ gene (anticodon UAA) containing an intron that has shown to be highly variable in a nucleotide sequence and used for plant population genetics. Unfortunately these sequences were identical in all three liverwort species tested. The analysis of the nucleotide sequence of chloroplast, an intron containing $tRNA^{Gly}$ (anticodon UCC) genes, gave expected results: the intron nucleotide sequence was identical in the case of both *P. borealis* and *P. epiphyllaspecies N*, while the sequence obtained from *P. epiphyllaspecies S* was different in several nucleotide positions. These results were confirmed by the nucleotide sequence of another chloroplast molecular marker the chloroplast, an intron-containing $tRNA^{Lys}$ gene (anticodon UUU). We have also sequenced mitochondrial, an intron-containing $tRNA^{Ser}$ gene (anticodon GCU) in all three liverwort species. In this case we found that, as in the case of the chloroplast genome, *P. borealis* mitochondrial genome was inherited from *P. epiphylla-species N*. On the basis of our results we claim that both organelle genomes of *P. borealis* derived from *P. epiphylla-species N*.

Introduction

Pellia borealis is a diploid species (n=18 in gametophyte) belonging to genus *Pellia*, order *Metzgeriales*, class *Hepaticae*, subdivision *Bryophyta*. This species originated, as postulated in earlier studies, via hybridization and duplication of chromosome sets of two cryptic species: *Pellia epiphylla-species N* (n=9) and *Pellia epiphylla-species S* (n=9) (Odrzykoski, 1996). *Pellia epiphylla-species N* and *Pellia epiphylla-species S* are allopatric species, at least in Poland. Our recent results (Fiedorow, 1998; Pacak, 1998) have contributed to the elucidation of an allopolyploid character of *P. borealis*. We have shown that the nuclear genome of *P. borealis* is an assembly of two genomes: one from *P. epiphylla-species N* and the oth-

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er from *P. epiphylla-species S*. We have studied the organization of nuclear $tRNA^{Leu}$ genes that are organized in tandems. The comparison of nucleotide sequences of tandemly repeated $tRNA^{Leu}$ genes in liverwort species has shown that the $tRNA^{Leu}$ genes are identical in a sequence. However, the nucleotide sequences of spacers joining tandemly organized tRNA genes differ between *P. epiphylla-species N* and *P. epiphylla-species S*. This feature could be explained by the phenomenon of concerted evolution. According to this theory, tandemly repeated gene families and tandemly repeated sequences have the same or almost the same nucleotide sequence of the repeated unit in one species but differ in a sequence when compared with even a closely related species. In the case of tandemly repeated $tRNA^{Leu}$ genes we do not observe interspecies nucleotide sequence differences between $tRNA^{Leu}$ genes as these are conservative, functional genes under selectional pressure. The observed interspecies differences in spacer nucleotide sequences represent probably mutations that are neutral.

In the case of *P. borealis* – the polyploid species – we have identified both types of spacer sequences: the one identical to that found in *P. epiphylla-species N* and the other found in *P. epiphylla-species S*. We have observed the same situation in the case of identical studies of ITS 1 (internal transcribed spacer 1) from tandemly repeated rDNA units. On the basis of our data and on enzymatic studies we claim that *P. borealis* represents an allopolyploid species that originated after hybridization and chromosome sets duplication of two cryptic species: *P. epiphylla-species N* and *P. epiphylla-species S*.

We have decided to solve the problem of mitochondria and chloroplast origin in an allopolyploid species *P. borealis*: Do they originate from *P. epiphylla-species N* or *P. epiphylla-species S*? Is it possible to answer this question, on the basis of nucleotide sequences from chloroplast and mitochondrial genomes of *P. borealis, P. epiphylla-species N* and *P. epiphylla-species S*? To our knowledge there is no information concerning the way of chloroplast and mitochondria inheritance in *Bryophyta*. Elucidation of this process, in an allopolyploid species *P. borealis* could contribute to the general knowledge about the chloroplast and mitochondria transmission in *Bryophyta*. In this paper we present data showing the inheritance of both organelles from only one parent *P. borealis*, namely *P. epiphylla-species N*.

Materials and Methods

In vitro culture of liverworts

Pellia species were taken from a greenhouse collection of liverworts kept by Department of Genetics, Adam Mickiewicz University, Poznań. Apical parts of thalli were sterilized in 0.5% sodium hypochlorite solution for 15 minutes. Sterilized sections of thallus were put on a special liverwort modified medium containing per one liter: NH_4NO_3 - 0.12g; KH_2PO_4 - 0.7g; $MgSO_4 \times 7H_2O$ - 0.246g; $CaCl_2$ - 0.02g; $FeCl_3$ - 0.03g; sucrose - 10g; agar - 10g. The agar medium was adjusted to pH 5.0 (Lukavsky, 1991). We also used 1/2×Murashige and Skoog medium pH 5.64 (Murashige, 1962) for liverworts *in vitro* culture.

Total genomic DNA isolation

Genomic DNA was extracted using procedure DNeasy Plant Mini Kit (QIAGEN). Total DNA was isolated from 100 mg of plant tissue from *in vitro* culture.

Polymerase chain reaction (PCR)

PCR mixture contained the following components for 10μL reactions: 7.5 ng of total DNA; 0.5μM primer A; 0.5μM primer B; TRIS/Cl; 1.5 mM $MgCl_2$; KCl; $(NH_4)_2SO_4$; 1 mM spermidine (Fiedorow, 1997); 200μM of each dNTP; 0.5 unit of *Taq* DNA polymerase (QIAGEN).

PCR was initiated by denaturation at 95℃ for 5 min. and followed by 30 cycles: denaturation 94℃ - 1 min, annealing 56℃ - 1 min, elongation 72℃ - 1 min.

The reaction was ended by elongation at 72℃ for 5 min.

Primers used for PCR reactions were based on complete mitochondrial genome (accession M 68929) and chloroplast genome (accession X 04465, Y 00686) from liverwort *Marchantia polymorpha* (Ohyama, 1996).

Primers for PCR amplification were as follows:
-for intron sequence of chloroplast $tRNA^{Leu}_{(UAA)}$ gene
A primer 5' GGG GGT ATG GCG AAA TTG G 3'
B primer 5' TGG GGG TAG AGG GAC TTG 3'
-for intron sequence of chloroplast $tRNA^{Gly}_{(UCC)}$ gene
A primer 5' ACC CGC ATC GTT AGC TTG 3'
B primer 5' GCG GGT ATA GTT TAG TGG 3'
-for intron sequence of chloroplast $tRNA^{Lys}_{(UUU)}$ gene
A primer 5' AAC TCA ATG GTA GAG TAC TC 3'
B primer 5' GGC TCG AAC CCG GAA CTC 3'
-for intron sequence of mitochondrial $tRNA^{Ser}_{(GCU)}$ gene
A primer 5' GGA GGT ATG GCT GAG TGG 3'
B primer 5' GAG GAA ATG GGA TTT GAA CC 3'

Products of PCR were separated on 0,8 % agarose gel in 1xTEB and isolated from agarose gel using QIAquick Gel Extraction Kit (QIAGEN).

Cloning and sequencing

PCR products were cloned using pGEM-T Easy Vector System (Promega).

DNA sequencing was carried out using fmoL DNA Cycle Sequencing System (Promega).

Sequence data analysis

Program Clustal X (1.64b) (Gibson, 1996) and Phylip package (Felsenstein, 1995) were used to construct and test trees.

Results

The search for nucleotide sequences of chloroplast and mitochondrial genomes that would differentiate between cryptic species and would thus allow identification of chloroplast and mitochondria genomes in an allopolyploid species *P. borealis* is a challenge for the following reasons: the rate of nucleotide substitutions in chloroplast genome is two times slower than in nuclear plant genome and the rate of nucleotide substitutions in plant mitochondrial genome is three times slower than that in chloroplast genome (Wolfe, 1997). One of the frequently used chloroplast sequence in plant systematic studies of closely related species is a $tRNA^{Leu}$ gene (anticodon UAA) containing a group I intron. This intron, containing several hundred nucleotides in length, exhibits great variability in nucleotide sequence when cyanobacteria strains or closely related plant species are compared (Meissner, 1998; Paulsrud, 1998). The chloroplast $tRNA^{Leu}$ gene is interrupted by the intron sequence between the first and the second anticodon nucleotide. On the basis of the exon nucleotide sequences of the $tRNA^{Leu}$ gene known from chloroplast genome of *Marchantia polymorpha* (its complete sequence is known), we have designed primers for PCR amplification of the $tRNA^{Leu}$ gene intron for all liverwort species from genus *Pellia*. As a result of PCR amplification we obtained products of 426 to 334 bp in length for *Pellia* liverworts and a product of 391 bp in length for the liverwort *Aneura pinguis* (a species from genus *Aneura* that is closely related to genus *Pellia*). PCR products were sequenced, both directly and after cloning in pGEM-T Easy Vector. Unfortunately, intron nucleotide sequences in the case of *P. epiphylla-species N, P. epiphylla-species S* and *P. borealis* were identical. However, the comparison of intron nucleotide sequences for all liverwort species from genus Pellia showed that it is possible to construct fenetic dendrogram for the genus, using *Aneura pinguis* intron nucleotide sequence as an outgroup (Figure 1).

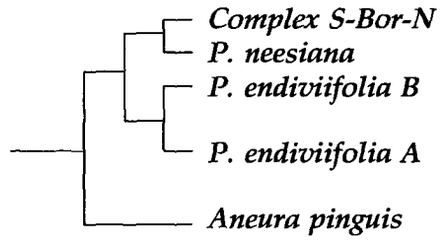

Figure 1. Fenetic dendrogram of liverworts from genus *Pellia* based on the chloroplast $tRNA^{Leu}$ (UAA) gene sequences (UPGMA method). Complex *S-Bor-N*: complex *Pellia epiphylla-species S - P. borealis - P. epiphylla-species N*

The dendrogram was identical to that obtained on the basis of nucleotide sequences derived from nuclear genomes of the liverwort from genus *Pellia* (in preparation) with the exception of *P. epiphylla-species N, P. epiphylla-species S* and *P. borealis* (in the case of nuclear genome sequences we were able to differentiate between all three species, which was not the case when using chloroplast $tRNA^{Leu}$ sequences). Figure nr 2 shows nucleotide sequence alignment for chloroplast $tRNA^{Leu}$ genes of all liverworts from genus *Pellia* and from *Aneura pinguis*.

The next chloroplast gene we have decided to sequence in order to elucidate the origin of the chloroplast in an allopolyploid species *P. borealis* was a $tRNA^{Gly}$ gene (anticodon UCC) containing group II intron. Transfer RNA^{Gly} gene is interrupted by the intron sequence in the region of DHU arm of mature $tRNA^{Gly}$. PCR primers for the amplification of the chloroplast $tRNA^{Gly}$ gene were designed on the basis of the nucleotide sequence of $tRNA^{Gly}$ gene from *M. polymorpha*. PCR products were about 800 bp in length. Fragments of PCR products obtained from total DNA of *P. epiphylla-species N, P. epiphylla-species S* and *P. borealis* were sequenced directly and after cloning in pGEM-T Easy vector. The comparison of nucleotide sequences of fragments of the chloroplast $tRNA^{Gly}$ intron in three liverwort species studied gave the expected results. The fragments of the nucleotide sequence of $tRNA^{Gly}$ from *P. epiphylla-species S* were different from the identical nucleotide sequences of $tRNA^{Gly}$ gene introns from *P. epiphylla-species N* and *P. borealis*. We have found six nucleotide substitutions, one nucleotide insertions and one deletion. We have analyzed two geographically distant populations for each species and obtained identical results. Figure 3 shows the comparison of $tRNA^{Gly}$ gene intron sequences obtained for three liverwort species studied.

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1.Complex S-B-N   AATCCGATTGAGCCTTAGTGGAGAAATCGACTAAGTGATTGTTTCCACATTCAGGGAAAC
2.P. neesiana     AATCCGATTGAGCCTTAGTAGAGAAATCGACTAAGTGATTGTTTCCACATTCGGGGAAAC
3.P. endivii.-B   AATCCAATTGAGCCTTGGTAGAGAAATCGACTAAGTGATTGTTTCCACATTCAGGGAAAC
4.P. endivii.-A   AATCTAATTGAGCCTTGGTGGAGAAATCGACTAAGTGATTGTTTCCACATTCAGGGAAAC
5.A. pinguis      AATTTGATCGAGCCTCAGTGGAGAAATCGACTGAGTGATTGTTCCCATATGCAGGGAAAC
                  ***    ** ******  ** ************  ********** *** **  * *******

1.                CTAGGTTGAAGAAGGAAGATACACTAGGTAATCCTGAGCCAAATCTCGTTCACGAAATAG
2.                CTAGGTTGAAGAAGGAAGATACACTAGGTAATCCTGAGCCAAATCTCGTTCACGAAATAG
3.                CTAGGTT--AGCTGAAACATA-TTAGGTAATCCTGAGCCAAAATTCGTTTACGAAATAG
4.                CTAGGTT--AGCTGAAACATA--TTAGGTAATCCTGAGCCAAAATTTGTTTACGAAATAG
5.                CTAGGTT-----AGAAACATA--TTAGGCAATCCTGAGCCAACTTTCGATTATGAAATAG
                  *******       * ** ***   **** *************   * * * * *******

1.                GTGCAGAGACTCAAAGGAAACTATCCCGATGAAAGAATTTTTCGATCCTTCTGGTTACGA
2.                GTGCAGAGACTCAAAGGAAACTATCCCGATGAAAAAAATTTTTGATCCTTCTGGTTACGA
3.                GTGCAGAGACTCGAAGGAAACTATCCCAACGAGATCAAATTA-GATGTTTTTAGTTACCA
4.                GTGCAGAGACTCGAAGGAAACTATCCCAACGAGATCAAATTA-GATGTTTTTAGTTA---
5.                GTGCAGAGACTCAAAGGGAACCATCCTAACCAAA--AAATTA-GTAATCACTGACAAATA
                  ************ **** *** ****  *  * *  *  **  *       *    *

1.                AGCT----ACCCCGTA-----CCCTACAACTGTGGTGGTAGCGACACAGTATCGAAA---
2.                CGCT----ACCCCATA-----TCCTACAACTGTGGTGGTAGTGACAAAGTATCGAAA---
3.                TTCATTGGACCCAGCAGTAACTCTGATAGCGATAATGGTATCCA-ATAGTAAAAATAAAT
4.                ----------------------------------------TAGTTA--CCGTAT--------
5.                TTTAAATAATCCTATTA-------TCTGGTGAAATTGGTAGTCA---GGGATTCGAA---
                                                          **   *    * *

1.                ----GCAGTAGCAATAAT-----ATAGAC-------------GAGGATAAAGATAGAGTC
2.                ----GCAGTAGCAATAAT-----ATAGAC-------------GAGGATAAAGATAGAGTC
3.                GCTTGTATTTTCATTGTTTCGTAATAGACAATCATCACGTATGAGGATAAAGATAGAGTC
4.                ----------------------------------------GAGGATAAAGATAGAGTC
5.                ----GATGGAGATGAAGAG----AGAGTCCGTTCTCAC---TGGTCATATATTTATATTC
                                                         *   *** *   ** * **

1.                CTTTCTTACGAGCCATA---GGCGGCGATGCAAATCGCGGTAAAAAG
2.                CTTTCTTACGAGCCATA---GGCGGCGATGCAAATCGCGGTAGAAAG
3.                CTTTTCTACGAGCTATATTTGGCAGCGTTGTAAATCGTAGTAGAAAG
4.                CTTTTCTACAAGCTGTATTTGGCAGCGGTGTAAATCGTGGTAGAAAG
5.                ATTATAT-----CTATT--CGGCCGCGGTGCAAAGCGTCGTGGGAAG
                   **   *     * *    *** *** ** *** **  **   ***
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Figure 2. Alignment of chloroplat $tRNA^{Leu}$ (UAA) gene sequences using Clustal X (1.64b) program. Complex *S-B-N - P. epiphylla-species S, P. borealis, P. epiphylla-species N; P. endivii.-B P. endiviifolia-species B; P. endivii.-A P. endiviifolia-species A*

Another chloroplast DNA sequence we decided to study was the sequence of the $tRNA^{Lys}$ gene (anticodon UUU). In *M. polymorpha* chloroplast genome this gene contains a 2111 bp long group II intron. PCR products, obtained for three liverwort species under investigation, were partially sequenced using primers designed according to $tRNA^{Lys}$ gene exons from *M. polymorpha*. The length of the $tRNA^{Lys}$ introns, calculated from the length of PCR products, is about 2300 bp. Again, the comparison of partially sequenced $tRNA^{Lys}$ gene introns shows differences: *P. epiphylla-species S* sequence is different from identical sequences for *P. epiphylla- species N* and *P. borealis*. In this case we identified five nucleotide substitutions and one deletion in P. epiphylla-species S intron fragments. Figure 4 shows the comparison between partially sequenced $tRNA^{Lys}$ gene introns for *P. epiphylla-species N, P. epiphylla-species S* and *P. borealis*. According to our results, obtained from chloroplast $tRNA^{Gly}$ and $tRNA^{Lys}$ gene sequences, *P. epiphylla-species N* is a donor of chloroplast genome for *P. borealis*.

```
1.P. epiphylla-N  GTGCGATTCGTTTCCGGACCGGGAGGAGGAAAAAGTTGAGGGATCATAATATCACATATA
2.P. borealis     GTGCGATTCGTTTCCGGACCGGGAGGAGGAAAAAGTTGAGGGATCATAATATCACATATA
3.P. epiphylla-S  ------------------------------------------------------------
                                                        i
1.                CACACACGTATATATATATGTATATACGTGTATATAATATGTATCCCCC-TCCCCCTCCA
2.                CACACACGTATATATATATGTATATACGTGTATATAATATGTATCCCCC-TCCCCCTCCA
3.                ----------------------------GTGTATATAATATGTATCCCCCCTCCCCCTCCA
                                              ********************** **********
                                                                 s       s
1.                AATCTCATTTCGTGAAGAGTTCGACTGAATCTCTCCCACCTCAATGCTATGATGGGAAAA
2.                AATCTCATTTCGTGAAGAGTTCGACTGAATCTCTCCCACCTCAATGCTATGATGGGAAAA
3.                AATCTCATTTCGTGAAGAGTTCGACTGAATCTCTCCCACCTCAATGCTGTGATGGGGAAA
                  ********************************************** ******* ***
                   s
1.                GTGTCATTTCCTCAATCACTATTCTCCAGTGACTGAAGAAGCGGAATGTTTCGAATTTTT
2.                GTGTCATTTCCTCAATCACTATTCTCCAGTGACTGAAGAAGCGGAATGTTTCGAATTTTT
3.                GCGTCATTTCCTCAA-----------------------------------------------
                  * *************

1.                TCGATCCGCAGAAAATCCAGGGAAAGGAATCAAAAAACGTAACTCTTCATCGTAACTGAA
2.                TCGATCCGCAGAAAATCCAGGGAAAGGAATCAAAAAACGTAACTCTTCATCGTAACTGAA
3.                ------------------------------------------------------------

1.                TCATCGACCGTATGGAACCAAATACATACGATGCTGGGTAGTTAGAGATAGAGTAACTCT
2.                TCATCGACCGTATGGAACCAAATACATACGATGCTGGGTAGTTAGAGATAGAGTAACTCT
3.                ------------------------------------------------------------

1.                AGTTTGCTAGAGATACAAGCATTTATTCTTGCTCGGTGGATAATATTTTCCAAAAGATAC
2.                AGTTTGCTAGAGATACAAGCATTTATTCTTGCTCGGTGGATAATATTTTCCAAAAGATAC
3.                ------------------------------------------------------------

1.                AGAATCAATGAAATGGGGAACGAAGTAATTGGAAAGTTCTATAGTTTTCTCTCAACTATAT
2.                AGAATCAATGAAATGGGGAACGAAGTAATTGGAAAGTTCTATAGTTTTCTCTCAACTATAT
3.                ----------------------------------------------CTCTCAACTATAT
                                                                 ************
                   d
1.                TTATTTTTCCGATAGGATCATCTAATAAAGTATTTTTTTACTCTCAGCAACTGAATCAGT
2.                TTATTTTTCCGATAGGATCATCTAATAAAGTATTTTTTTACTCTCAGCAACTGAATCAGT
3.                ----TTTTCCGATAGGATCATCTAATAAAGTATTTTTTTACTCTCAGCAACTGAATCAGT
                      ************************************************************
                   s
1.                AGGAGAAGAAACTTACATAGATGCTATGGATATATCTAAATAATTTTGATAATCGAGTAT
2.                AGGAGAAGAAACTTACATAGATGCTATGGATATATCTAAATAATTTTGATAATCGAGTAT
3.                AAGAGAAGAAACTTACATAGATGCTATGGATATATCTAAATAATTTTGATAATCGAGTAT
                  * ************************************************************
                                                          s
1.                AGCATAGTCGAAATATTAGCTATTCTATTTACCGGTTTCTTACGAATCGTGGTGTATATT
2.                AGCATAGTCGAAATATTAGCTATTCTATTTACCGGTTTCTTACGAATCGTGGTGTATATT
3.                AGCATAGTCGAAATATTAGCTATTCTATTTACCGGTTTCTTACGAATCGTAGTGTATATT
                  ************************************************ **********
                                                            s
1.                CATAAAGGAGCCGAACGGAGAGAAATTTCCATATTCGGTTCTGGATTAGAGGCGTTCGTA
2.                CATAAAGGAGCCGAACGGAGAGAAATTTCCATATTCGGTTCTGGATTAGAGGCGTTCGTA
3.                CATAAAGGAGCCGAACGGAGAGAAATTTCCATATTCGGTTCTGAATTAGAGGCGTTCGTA
                  ******************************************** ***************

1.                TCGAGCGTCGACTATGAC
2.                TCGAGCGTCGACTATGAC
3.                TCGAGCGTCGACTATGAC
                  ******************
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Figure 3. Alignment of chloroplast $tRNA^{Gly}$ (UCC) gene intron sequences. The alignment shows a complete sequence of *P. borealis* and *P. epiphylla – species N* intron and partially sequenced (5', 3' fragments) intron from and *P. epiphylla – species S*. The alignment was carried out using ClustalX (1.64b) program. d-deletion, i-insertion, s-substitution

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1.P.borealis      TCCATCCATACCATTGGCGAGGTTTGCGACACTACGACTAATCCCAGAGTAAAACTTTTC
2.P.epiphylla-N   TCCATCCATACCATTGGCGAGGTTTGCGACACTACGACTAATCCCAGAGTAAAACTTTTC
3.P.epiphylla-S   TCCATCCATACCATTGGCGAGGTTTGCGACACTACGACTAATCCCAGAGTAAAACTTTTC
                  ************************************************************

1.                GGAGAAAATAGCATGTCGTTTATTTCTTCCTTTCCCTCCCATCGTCAGGAAGGAAAAGAA
2.                GGAGAAAATAGCATGTCGTTTATTTCTTCCTTTCCCTCCCATCGTCAGGAAGGAAAAGAA
3.                GGAGAAAATAGCATGTCGTTTATTTCTTCCTTTCCCTCCCATCGTCAGGAAGGAAAAGAA
                  ************************************************************
                     ss     d          s
1.                CAATATATTAATTATATTCTCTAGTCGATTGGAGTTAATGGATAAAGCTAAATCGCTTGA
2.                CAATATATTAATTATATTCTCTAGTCGATTGGAGTTAATGGATAAAGCTAAATCGCTTGA
3.                CATAATATT----ATATTCTCCAGTCGATTGGAGTTAATGGATAAAGCTAAATCGCTTGA
                  **  *****     ******** ************************************
                       s
1.                ATCATAGTCGGAGAAAGAACCAGCTTCTGTTTCTCGTTCATCGAAACATTATCCTCTCCA
2.                ATCATAGTCGGAGAAAGAACCAGCTTCTGTTTCTCGTTCATCGAAACATTATCCTCTCCA
3.                ATCATGGTCGGAGAAAGAACCAGCTTCTGTTTCTCGTTCATCGAAACATTATCCTCTCCA
                  ***** ****************************************************
                                                            s
1.                TCAAATTTATAAGTTGTTAGAAGCTTTTTCATATAGTCAATACA
2.                TCAAATTTATAAGTTGTTAGAAGCTTTTTCATATAGTCAATACA
3.                TCAAATTTATAAGTTGTTAGAAGCTTTTTCATATAGTCAATGCA
                  ***************************************** **
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Figure 4. Alignment of chloroplast $tRNA^{Lys}$ (UUU) gene, part of intron sequences. The alignment shows partial intron sequences of *P. borealis, P. epiphylla – species N and P. epiphylla – species S.* The alignment was carried out using Clustal X (1.64b) program. d-deletion, s-substitution

```
                        i
1.P.epiphylla-N   GAACAG----CTACTATTGCCTATTAACACGGGTAGCCGCCACAGGTTAGAGTGTTTACA
2.P.borealis      GAACAG----CTACTATTGCCTATTAACACGGGTAGCCGCCACAGGTTAGAGTGTTTACA
3.P.epiphylla-S   GAACAGATGGCTACTATTGCCTATTAACACGGGTAGCCGCCACAGGTTAGAGTGTTTACA
                  ******    ***********************************************

1.                CATGGAAGTTCTAAATGACTCGACAGTTGCTTGAGCCGTATGCGGGGAAACTCGCACGTA
2.                CATGGAAGTTCTAAATGACTCGACAGTTGCTTGAGCCGTATGCGGGGAAACTCGCACGTA
3.                CATGGAAGTTCTAAATGACTCGACAGTTGCTTGAGCCGTATGCGGGGAAACTCGCACGTA
                  ************************************************************

1.                CGGTTCTTAGGGGGGAAAGAA
2.                CGGTTCTTAGGGGGGAAAGAA
3.                CGGTTCTTAGGGGGGAAAGAA
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Figure 5. Alignment of mitochondrial $tRNA^{Ser}$ (GCU) gene intron sequences. Alignment shows partial intron sequences of *P. borealis, P. epiphylla – species N* and *P. epiphylla – species S.* Alignment was carried out using Clustal X (1.64b) program. i-insertion

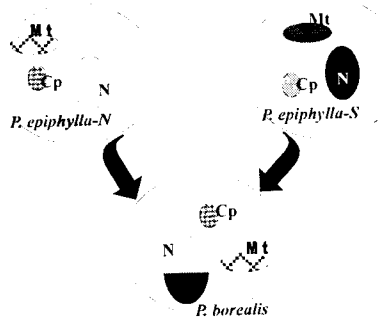
Similar studies concerning the origin of mitochondrial genome in allopolyploid species *P. borealis* have also been carried out. We have partially sequenced an intron of mitochondrial $tRNA^{Ser}$ gene (anticodon GCU) from *P. epiphylla-species N*, *P. epiphylla-species S* and *P. borealis*. In the case of mitochondrial $tRNA^{Ser}$ gene of *M. polymorpha* (the complete sequence of mitochondrial genome of *M. polymorpha* is known) there is a group II intron 991 bp in length. PCR products obtained using primers designed on the basis of *M. polymorpha* $tRNA^{Ser}$ gene exon sequences were partially sequenced. The length of introns of $tRNA^{Ser}$ mitochondrial gene in three liverwort species studied was estimated for 1400 bp. The comparison of partial intron sequences revealed that nucleotide sequences of *P. eipihylla-species N* and *P. borealis* are identical while the sequence derived from *P. epiphylla – species S* is different. We have identified one four-nucleotide insertion in the case of *P. epiphylla – species S*. Figure 5 presents the comparison of partial $tRNA^{Ser}$ gene intron sequences from three liverwort species studied.

Discussion

Organelle transmission has been studied in many plants. In angiosperms, the pattern of plastid transmission has been documented in >50 genera of monocotyledons and dicotyledons. Generally the plastid transmission is maternal. However, biparental transmission has been observed in about 20 genera. Among these, crosses of certain species yielded >5% of the progeny possessing paternal plastids (Gillham, 1994). Mitochondria transmission in angiosperms is generally maternal (Small, 1999; Gillham, 1994; Rajora, 1992). However, in this case paternal inheritance has been observed as well (Erickson, 1990). Among gymnosperms the majority of observations has been done on conifers. In this case transmission of plastids and mitochondria is generally paternal (Mogensen, 1996; Dong, 1994), but in the largest conifer family, *Pinaceae*, the pattern of mitochondria transmission is maternal (Mogensen, 1996). In the fern *Pteris vittata* and *Marsilea* maternal inheritance of organelles has been discovered. Some results concerning the organelles transmission in algae have been published: in the case of algae that produce isogametes, organelle transmission is uniparental and in the case of anisogamous and oogamous algae organelle inheritance is maternal (Kuroiwa, 1991).

To our knowledge there is no information concerning mitochondria and chloroplast inheritance in *Bryophyta*. Some information could be deduced from the structure and behaviour of a liverwort male gamete. It is composed of three parts: the apical part containing anchor of flagellum, the central part containing nucleus and distal part containing chloroplast and usually one mitochondrion. The distal part of a male gamete is rejected before fertilization, which suggests maternal transmission of organelles in liverwort species (Szweykowska, 1993). Our results confirm this pattern of mitochondria and chloroplast transmission, at least in the case of allpolyploid species *P. borealis*. We have found that organelle inheritance in *P. borealis* is uniparental (Figure 6).

Although our results do not show whether the organelle inheritance is maternal or paternal, we can assume, taking into account the structure and behaviour of a liverwort male gamete, that *P. epiphylla-species N* was probably the donor of a female gamete during the hybridization process between *P. epiphylla-species N* and *P. epiphylla-species S* that gave rise to *P. borealis*.



Cp-chloroplast, Mt-mitochondrion, N-nucleus

Figure 6. The origin of chloroplasts and mitochondria in an allopolyploid *Pellia borealis* (based on the analysis of cptRNA-Gly, cptRNA-Lys and mttRNA-Ser introns).

The recently published results by Boisselier-Dubayle et al. (Boisselier-Dubayle, 1998) have shown that a liverwort species *Porella baueri* is an allopolyploid species that originated after hybridization and duplication of chromosome sets of two closely related species: *P. platyphylla* and *P. cordaeana*. Moreover, it has been discovered that the event of hybridization and polyploidization that gave rise to *P. baueri* had place several times in Europe. In the next experiments we intend to study many different Polish *P. borealis* populations and look for the organelle pattern of inheritance. If the hybridization of *P. epiphylla-species N* and *P. epiphylla-species S* has occurred several times there is a hope of finding it by looking for the organelle origin in *P. borealis*.

Our investigations have enabled us to reconstruct

unknown historical events concerning the origin of *P. borealis* species. The data concerning allopolyploidal origin of many liverwort species has been accumulated. There is a possibility of studying the origin of organelles in these species and generalizing the pattern of mitochondria and chloroplast transmission in liverworts. Although liverworts have limited economical importance, the fact that they are identified as the earliest land plants make them an interesting scientific object (Qiu, 1998). They may combine early primitive features (similar features to algae) and advanced properties of vascular plants.

Footnote

Accession numbers of intron sequences (deposited in the GenBank):

1. Chloroplast $tRNA^{Leu}_{(UAA)}$ intron

Aneura pinguis-AF 244085, *P. endiviifolia-species A*-AF 241223, *P. endiviifolia-species B*-AF 244087, *P. neesiana*-AF 180683, *P. borealis*-AF 244086

P. epiphylla-species S and *P. epiphylla-species N* have an identical intron sequence as *P. borealis.*

2. Chloroplast $tRNA^{Gly}_{(UCC)}$ intron

P. epiphylla-species S-AF 240161 and AF 240162, *P. borealis*-AF 240473, *P. epiphylla-species N*-AF 217210

3. Chloroplast $tRNA^{Lys}_{(UUU)}$ intron

P. epiphylla-species S-AF 238497, *P. borealis*-AF 238498, *P. epiphylla-species N*-AF 238496

4. Mitochondrial $tRNA^{Ser}_{(GCU)}$ intron

P. epiphylla-species S-AF 242357, *P. borealis*-AF 244576, *P. epiphylla-species N*-AF 242358

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