Uniparental Inheritance of Chloroplast DNA Is Strict in the Isogamous Volvocalean \textit{Gonium}

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Abstract

\textbf{Background:} A problem has remained unresolved regarding the exceptions to the unilateral inheritance of chloroplast DNA (cpDNA) from \textit{MT}+/female in \textit{Chlamydomonas} and other volvocaleans demonstrated by the previous genetic analyses. For identification of the parental types of cpDNA, these studies used parents that have differences in restriction fragment length polymorphisms and exhibit partial sexual incompatibility.

\textbf{Methodology/Principal Findings:} In the present study, we used sexually compatible parents of the isogamous colonial volvocalean \textit{Gonium maiaprilis} that seemed an ideal species to identify the pattern of cpDNA inheritance based on the length difference in the putative group I intron interrupted in the Rubisco large subunit gene and objective identification of mating types by the presence or absence of the minus-dominance (MID) gene. We examined patterns of inheritance of cpDNA and presence/absence of a MID ortholog (GmMID) in 107 F\textsubscript{1} progeny of \textit{G. maiaprilis} that were obtained by inducing germination of separated single zygotes. The results demonstrated no exception of the uniparental inheritance of cpDNA from the \textit{MT}+ parent (lacking GmMID) in sexually compatible or genetically less divergent strains of \textit{G. maiaprilis}.

\textbf{Conclusions/Significance:} The present data suggest that the uniparental inheritance of cpDNA is likely more strict in crossings of less diverged strains or sexually compatible parental volvocaleans, and some genetic inconsistency between the parents may cause exceptional uniparental inheritance of cpDNA.

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Introduction

Chloroplast DNA (cpDNA) in the volvocalean algae is predominantly transmitted from only one of the two parental mating types to the progeny; from mating type plus (\textit{MT}+) in the isogamous species \textit{Chlamydomonas reinhardtii} [1] and \textit{Gonium pectorale} [2] or from female in the oogamous \textit{Volvox carteri} [3]. However, these studies showed that 2–8\% of the F\textsubscript{1} progeny have an exceptional pattern of uniparental inheritance of cpDNA (cpDNA) [1–3], i.e. they inherit cpDNA from the \textit{MT}−/male. For identification of the parental types of cpDNA, these studies used strains of complementary mating types (sexes) that have differences in restriction fragment length polymorphisms (RFLPs) and exhibit partial sexual incompatibility [2–4].

Studies of intra/interspecific crossings in mouse demonstrated that paternal mitochondrial DNA (mtDNA) is selectively eliminated during early embryogenesis in intra-specific crossings, whereas 50\% of paternal mtDNA are transmitted to progeny in interspecific crossings [5,6]. Thus, crossings between pairs with partial sexual isolation or between genetically differentiated entities in the volvocaleans may also increase the exceptional rate of uniparental inheritance of organelle DNA when compared with intra-specific crossings.

\textit{Gonium maiaprilis} is an isogamous colonial volvocalean that exhibits heterothallic sexuality [7] (Figure 1). The mating type (\textit{MT}−-)determining minus dominance gene, \textit{MID} [8], was recently identified in the closely related species \textit{G. pectorale} [2]. In addition, our preliminary comparison of cpDNA sequences including a putative group I intron in the Rubisco large subunit (\textit{rbcL}) genes indicated a difference in length of the introns among the \textit{G. maiaprilis} strains. Thus, \textit{G. maiaprilis} seems an ideal species to identify the pattern of cpDNA inheritance based on the difference in the group I intron and objective identification of mating types by the presence or absence of the \textit{MID} gene [2].

In this study, we examined patterns of inheritance of cpDNA in 107 F\textsubscript{1} progeny of \textit{G. maiaprilis}. The results demonstrated no exception of the uniparental inheritance of cpDNA from the \textit{MT}+ parent in sexually compatible strains of \textit{G. maiaprilis}.

Results

One hundred and thirty-three gone colonies, each representing a separate meiotic product, were isolated from 44 germinating zygotes of \textit{G. maiaprilis} Asa041901 x Asa041903 to establish F\textsubscript{1} strains (Figure 1). Ultimately, 77\% (103/133) of the gone colonies became actively growing cultures. Based on backcrossing, 58 of the
103 exhibit a minus mating phenotype and the remaining 45 a plus mating phenotype (Table 1).

To determine the presence or absence of the MID gene, the MID orthologue (GmMID) was isolated from G. maiaprilis and characterized (Figures S1, S2, S3). Genomic PCR using GmMID-specific primers demonstrated that all 60 F1 strains (including additional two F1 strains previously established [7]) with minus mating phenotype have GmMID whereas all 47 F1 strains (including additional two F1 strains previously established [7]) with plus mating phenotype lack this gene (Figures 2 and S4). On the other hand, all 107 F1 strains had cpDNA of the Asa041901 (MT+) type based on genomic PCR using rbcL group I intron-specific primers (Figures 2, 3, S4 and Table 1).

The secondary structures of the nuclear ribosomal DNA internal transcribed spaces 1 and 2 (ITS-1 and ITS-2) contain single base substitutions in four positions between G. maiaprilis Asa041901 and Asa041903 (Figure S5). These substitutions did not correspond to compensatory base change (CBC), suggesting that the strains fall within a range of an interfertile entity or a biological species [7,9]. In G. pectorale Mongolia1 and Mongolia4, seven single base substitutions were detected in the ITS secondary structures (Table 2) although no CBC was recognized (Figure S6). Furthermore, the nucleotide sequences of the rbcL coding region (1128 bp) of the G. maiaprilis parents are exactly the same (GenBank/EMBL/DDBJ accession nos. AB520743-5, [7]) whereas one nucleotide substitution is present between the parents of G. pectorale (Table 2).

### Discussion

In 78 F1 strains of G. pectorale Mongolia1×Mongolia4, five exceptions of the uniparental inheritance of cpDNA were reported [2] (Table 2). In contrast, there were no exceptions of the uniparental inheritance of cpDNA among the 107 G. maiaprilis F1 strains (Table 2 and Figure S4). This difference in the rate of exceptional uniparental inheritance of cpDNA between G. maiaprilis and G. pectorale is significant (P = 0.0014<0.05) by Fisher’s exact test [10]. On the other hand, the survival rate of F1 progeny (77%) in G. maiaprilis is high as in other volvocalean (Table S1) [3,11,12]. In G. pectorale Mongolia1×Mongolia4, however, the survival of F1 progeny was poor thus obviating tetrad analysis [2]. In addition, genetic difference between G. maiaprilis Asa041901 and Asa041903 is smaller than that between G. pectorale Mongolia1 and Mongolia4 (Table 2). Therefore, reproductive/genetic isolation between G. maiaprilis Asa041901 and Asa041903 is apparently less than that between G. pectorale Mongolia1 and Mongolia4.

These results suggest that the uniparental inheritance of cpDNA may be more strict in crossings of less diverged strains or sexually compatible parental volvocalean parents, and some genetic inconsistency between the parents may cause exceptional uniparental inheritance of cpDNA. The difference in the rate of exceptional uniparental inheritance of cpDNA (Table 2) could be considered to result from the difference in maturation of zygotes prior to germination between G. maiaprilis and G. pectorale. The zygotes of

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**Table 1.** Mating phenotypes, presence/absence of GmMID and inheritance of cpDNA in F1 progeny of G. maiaprilis Asa041901×Asa041903.

| Mating phenotype | No. of F1 strains | Presence of GmMID | Absence of GmMID | cpDNA from Asa041901(+) | cpDNA from Asa041903(−) |
|------------------|------------------|------------------|------------------|------------------------|------------------------|
| Mating type −    | 60               | 60               | 0                | 60                     | 0                      |
| Mating type +    | 47               | 0                | 47               | 47                     | 0                      |
| Total            | 107              | 60               | 47               | 107                    | 0                      |

*a*Based on backcrossing.

*b*Based on genomic PCR (Figures 2 and S4).

**Figure 1.** Diagram of sexual reproduction in heterothallic Gonium maiaprilis. Based on Hayama et al. [7].

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G. maiaprilis were induce to germinate after six-week dark treatment while immature zygotes were used for germination in G. pectorale Mongolia1 × Mongolia4 [2]. However, determination of the uniparental inheritance or complete digestion of cpDNA from MT—occurs in the early stage of zygote formation or quadriflagellate zygotes in Chlamydomonas reinhardtii [13]. Thus, the uniparental inheritance of cpDNA in the volvocales may be based on a precision molecular system that requires interactions of alleles from both parental cells, of sex-related genes that may be evolving rapidly [14], although details of the molecular mechanism for uniparental inheritance in the Volvocales remain unresolved [15].

Exceptional cases of the uniparental inheritance of mutations to streptomycin resistance in Chlamydomonas reinhardtii [16,17] and the colonial volvocalean Eudorina elegans [18] were reported in classic genetic studies. However, these studies are based on crossings of UV-induced mutant strains that might have been affected by additional mutations causing confusion of the consortium of the parental cells for uniparental inheritance of the organelle DNAs.

Materials and Methods

Cultures and induction of sexual reproduction in Gonium maiaprilis

Two G. maiaprilis strains of complementary mating types (Asa041901 and Asa041903) were used in this study. These two strains are available from the Microbial Culture Collection at the National Institute for Environmental Studies (NIES-Collection [19]). The cultures were grown in screw-cap tubes (18 × 150 mm) containing about 10 mL VTAC or AF-6 medium modified by elimination of CaCO3 and addition of 400 mg L⁻¹ MES [19–21].

Figure 2. Mating phenotypes (MT) and results of genomic PCR for parental strains (Asa041901[01] and Asa041903 [03]) and 12 representative F₁ strains of Gonium maiaprilis. Presence/absence of GmMID and the length polymorphism within the cpDNA rbcL group I intron are assessed by gel electrophoreses. The nuclear gene EF-1α like serves as a control. The horizontal line over the F₁ progeny indicates F₁ strains originating from the same zygote.
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Figure 3. Diagram showing intron/exon structure and positions of specific primers (Table S2) in the rbcL genes from Gonium maiaprilis Asa041901 (01) and Asa041903 (03) (GenBank/EMBL/DBJ accession nos. AB520743 and AB520744). Thick bars represent exons interrupted by a putative group I intron between basepairs 462 and 463 of the sequence of the rbcL gene of Chlorella vulgaris [accession no. AB001684]. Numbers above the alignment indicate the nucleotide position within the intron.
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Cultures were grown at 20°C, on a 14:10 h light-dark cycle, under cool-white fluorescent lamps at 163–175 μmol m⁻² s⁻¹ intensity.

For induction of sexual reproduction, approximately 10 ml of a 14-day-old culture in VTAC medium were reduced to 1 mL by centrifugation. The concentrated cultures of the two complementary mating types were mixed in Petri dishes (60-mm diameter) with 5.0 ml mating medium [20]. These dishes were cultured at 25°C on a 14:10 h light-dark cycle, under cool-white fluorescent lamps at 163–175 μmol m⁻² s⁻¹ intensity. After 10–14 days under these conditions, zygotes were pipetted onto the surface of AF-6 medium solidified with 1% agar in Petri dishes (90-mm diameter). The dishes were placed in the dark by wrapping in a double layer of aluminum foil and maintained in darkness at 20°C for about 6 weeks, after which the matured, clumped zygotes were determined using AF-6 medium solidified with 1% agar in Petri dishes (90-mm diameter). The dishes were placed in the dark by wrapping in a double layer of aluminum foil and maintained in darkness at 20°C for about 6 weeks, after which the matured, clumped zygotes were determined using AF-6 medium solidified with 1% agar in Petri dishes (90-mm diameter). The dishes were placed in the dark by wrapping in a double layer of aluminum foil and maintained in darkness at 20°C for about 6 weeks, after which the matured, clumped zygotes were determined using AF-6 medium solidified with 1% agar in Petri dishes (90-mm diameter). The dishes were placed in the dark by wrapping in a double layer of aluminum foil and maintained in darkness at 20°C for about 6 weeks, after which the matured, clumped zygotes were determined using AF-6 medium solidified with 1% agar in Petri dishes (90-mm diameter). The dishes were placed in the dark by wrapping in a double layer of aluminum foil and maintained in darkness at 20°C for about 6 weeks, after which the matured, clumped zygotes were determined using AF-6 medium solidified with 1% agar in Petri dishes (90-mm diameter). The dishes were placed in the dark by wrapping in a double layer of aluminum foil and maintained in darkness at 20°C for about 6 weeks, after which the matured, clumped zygotes were determined using AF-6 medium solidified with 1% agar in Petri dishes (90-mm diameter). The dishes were placed in the dark by wrapping in a double layer of aluminum foil and maintained in darkness at 20°C for about 6 weeks, after which the matured, clumped zygotes were determined using AF-6 medium solidified with 1% agar in Petri dishes (90-mm diameter). The dishes were placed in the dark by wrapping in a double layer of aluminum foil and maintained in darkness at 20°C for about 6 weeks, after which the matured, clumped zygotes were determined using AF-6 medium solidified with 1% agar in Petri dishes (90-mm diameter).

Determination of the length polymorphism within the cpDNA rbcL group I intron of *Gonium maiaprilis*

The nucleotide sequence of the putative rbcL group I intron from *G. maiaprilis* Asa041901 and Asa041903 (GenBank/EMBL/DBJ accession nos. AB520743 and AB520745) was determined by direct sequencing [7] using two specific primers located in the adjoining rbcL coding regions (Table S2) and showed a 27 bp difference in sequence length between the two strains (Figure 3).

Genomic PCR for parental and F1 strains of *Gonium maiaprilis*

Presence/absence of GmMID and the length polymorphism within the cpDNA rbcL group I intron are assessed by gel electrophoreses. The nuclear gene EF-1alpha like of *G. maiaprilis* (GenBank/EMBL/DBJ accession nos. AB623051 and AB623052) was determined by direct sequencing [7] using specific primers (Table 2) and genomic DNA, and serves as a control. PCR was performed with two specific primers for each gene (Table S2) and TaKaRa LA Taq (Takara bio inc., Shiga, Japan), under the following conditions: 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 40 seconds, followed by 72°C for 7 minutes.

ITS-1 and ITS-2 secondary structures

The ITS-1 and ITS-2 sequences (GenBank/EMBL/DBJ accession nos. AB623040-2) were directly determined by the methods described in Hayama et al. [7] with primers for ITS regions (Table S2). The secondary structures of ITS-1 and ITS-2 were predicted using CentroidFold [29,30] and revise the secondary structure models of ITS-1 and ITS-2 from earlier studies [7,9,31–33].

Supporting Information

Figure S1 Comparison of exon-intron structure between *GmMID* and five other MID homologs. (TIF)

Figure S2 Alignment of six MID proteins from *Volvox carteri* (VcMID), *Platymonas reinhardtii* (PleMID), *Gonium pectorale* (GpMID), *G. maiaprilis* (GmMID), *Chlamydomonas reinhardtii* (CrMID), and *C. globosa* (previously misidentified as *C. incerta* [34]) (GiMID). Solid and shaded backgrounds indicate identity in 100% or in over 60% of the sequences aligned, respectively. Five amino acids composing a leucine zipper are marked with asterisks. A line above the
alignment marks the RWP-RK domain of 47 amino acids used for the phylogenetic analyses (Figure S3).

(TIF)

Figure S3 Maximum likelihood (ML) phylogenetic tree showing MID proteins from Volvox carteri (VcMID), Pleodorina starroii (PlestMID), Gonium maiaprilis (GmMID), G. pectorale (GpMID), Chlamydomonas reinhardtii (CrMID) and C. globsa (previously misidentified as C. incerta [34]) (CiMID). Other members of the RWP-RK family from Chlamydomonas and Volvox are included as outgroup. Numbers next to branch points are bootstrap values for ML/neighbour joining/maximum parsimony methods.

(TIF)

Figure S4 Summary of mating phenotypes (MT), presence (gray)/absence (white) of GmMID and types of cpDNA (rbcL group I intron) from parental strains [Asa041901[01] and Asa041903 [03]] and their 107 F1 strains of Gonium maiaprilis. White or gray box represents the same character as that of Asa041901 or Asa041903, respectively, for each of the three attributes. Each horizontal line indicates those F1 strains originating from the same germinating zygote. Isolation of progeny representing both mating types in the 3 and 4-membered tetrads indicate that these are meiotic products.

(TIF)

Figure S5 Secondary structures of the ITS-1 and ITS-2 DNA regions in the present study.

(TIF)

Table S1 Survival rates of F1 progeny from intra and interspecific crossing in various colonial volvocaceans.

(DOC)

Table S2 Primers used for amplifications and sequencing of four DNA regions in the present study.

(DOC)

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Author Contributions

Conceived and designed the experiments: YS TH HN. Analyzed the data: YS TH RM HN. Contributed reagents/materials/analysis tools: YS TH MH RM HN. Wrote the paper: YS HN.

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