J. Phycol. 26, 490–494 (1990)

UNIQUE CHLOROPLAST GENOME IN *SPIROGYRA MAXIMA* (CHLOROPHYTA) REVEALED BY PHYSICAL AND GENE MAPPING[1]

James R. Manhart[2]

Department of Biology, Texas A&M University, College Station, Texas 77843

Robert W. Hoshaw

Department of Ecology and Evolutionary Biology, The University of Arizona, Tucson, Arizona 85721

and

Jeffrey D. Palmer

Department of Biology, Indiana University, Bloomington, Indiana 47405

ABSTRACT

The chloroplast genome in Spirogyra maxima *(Hassall) Kützing is 130 kb in size and lacks large repeat units. The 16S and 23S rRNA genes are separated by approximately 44 kb, which makes co-transcription of these genes unlikely.* Spirogyra maxima *is unusual among green algae in that its chloroplast genome apparently does not contain the* tufA *gene, the absence of which is characteristic of land plant chloroplast DNAs. Apart from three groups of genes,* psaA-psaB, psbC-psbD, *and* atpA-atpF/H, *which are co-transcribed in land plants and also map together in* S. maxima *chloroplast DNA, the* S. maxima *gene order overall is unlike that of any other chloroplast genome.*

Key index words: chloroplast DNA; physical and gene maps; Spirogyra maxima

A comparison of green algal and land plant chloroplast DNAs (cpDNAs) reveals great diversity in the former and surprising uniformity in the latter. With few exceptions, the 500+ land plant cpDNAs examined thus far are 120–160 kb in size and contain a large inverted repeat (IR) that divides the circular chromosome into large single copy (LSC) and small single copy (SSC) regions (Palmer 1985a, b, Palmer et al. 1988). Each IR copy contains a complete set of co-transcribed rRNA genes. The most common gene order in land plants is exemplified by the order found in tobacco (Shinozaki et al. 1986). The exceptions to this typical gene order result primarily from one or a few inversions (reviewed in Palmer et al. 1988). Since land plant cpDNAs are so conserved with regard to gene order, the few changes that do occur have the potential to be useful as phylogenetic markers at broad taxonomic levels (Palmer et al. 1988).

In contrast to land plants, green algal cpDNAs show tremendous variation with regard to size, physical structure, and gene order. The size range is from 89 kb in *Codium fragile* (Suringar) Hariot (Hedberg et al. 1981, Manhart et al. 1989) to approximately 2000 kb in *Acetabularia* (Green 1976, Padmanabhan and Green 1978). Within the Charophyceae, which is thought to be closest to the land plant lineage (Bremer 1985, Mishler and Churchill 1985), the chloroplast genome sizes are 100 kb for *Coleochaete orbicularis* Pringsheim, 130 kb for *Spirogyra maxima* and *Sirogonium melanosporum* (Randhawa) Transeau, 160–180 kb for *Chara* spp. and 350 kb for *Nitella translucens* (Pers.) Ag. (Manhart and Palmer 1988). Genome size in these five charophyte genera varies more than in all 500+ land plant cpDNAs examined. The size of the *Spirogyra maxima* chloroplast genome, at 130 kb, makes it and *Sirogonium melanosporum* the only charophytes investigated to date with chloroplast genomes in the same size range as in the majority of land plants (Manhart and Palmer 1988).

Too few green algal cpDNAs have been characterized to determine if large IR's are normally part of the genome makeup, as in land plants. Four species of *Chlamydomonas* have IR's in their chloroplast genomes, but the genic content and relative positioning of the IR's in *Chlamydomonas* cpDNAs are different from those found in land plants (Rochaix 1978, Lemieux and Lemieux 1985, Lemieux et al. 1985, Palmer et al. 1985). *Chlorella ellipsoidea* Gerneck is the only green alga known to have an IR that divides the chromosome into LSC and SSC regions (Yamada 1983). *Codium fragile* (Manhart et al. 1989) does not have an IR, but this is not surprising since its genome is only 89 kb in size.

Chloroplast gene orders have been determined in three species of *Chlamydomonas* (Lemieux et al. 1985, Turmel et al. 1988) and *Codium fragile* (Manhart et al. 1989). *Chlamydomonas eugametos* Moewus and *C. moewusii* Gerloff have similar gene orders that are quite different from that of *C. reinhardtii* Dangeard (Lemieux et al. 1985). The *Codium* gene order does not resemble any known land plant or green algal gene order (Manhart et al. 1989). Unlike land plants, the few green algal cpDNAs for which gene orders have been determined are so divergent that it is not possible to interconvert them by a few discrete rear-

[1] Received 20 February 1990. Accepted 14 May 1990.

[2] Address for reprint requests.

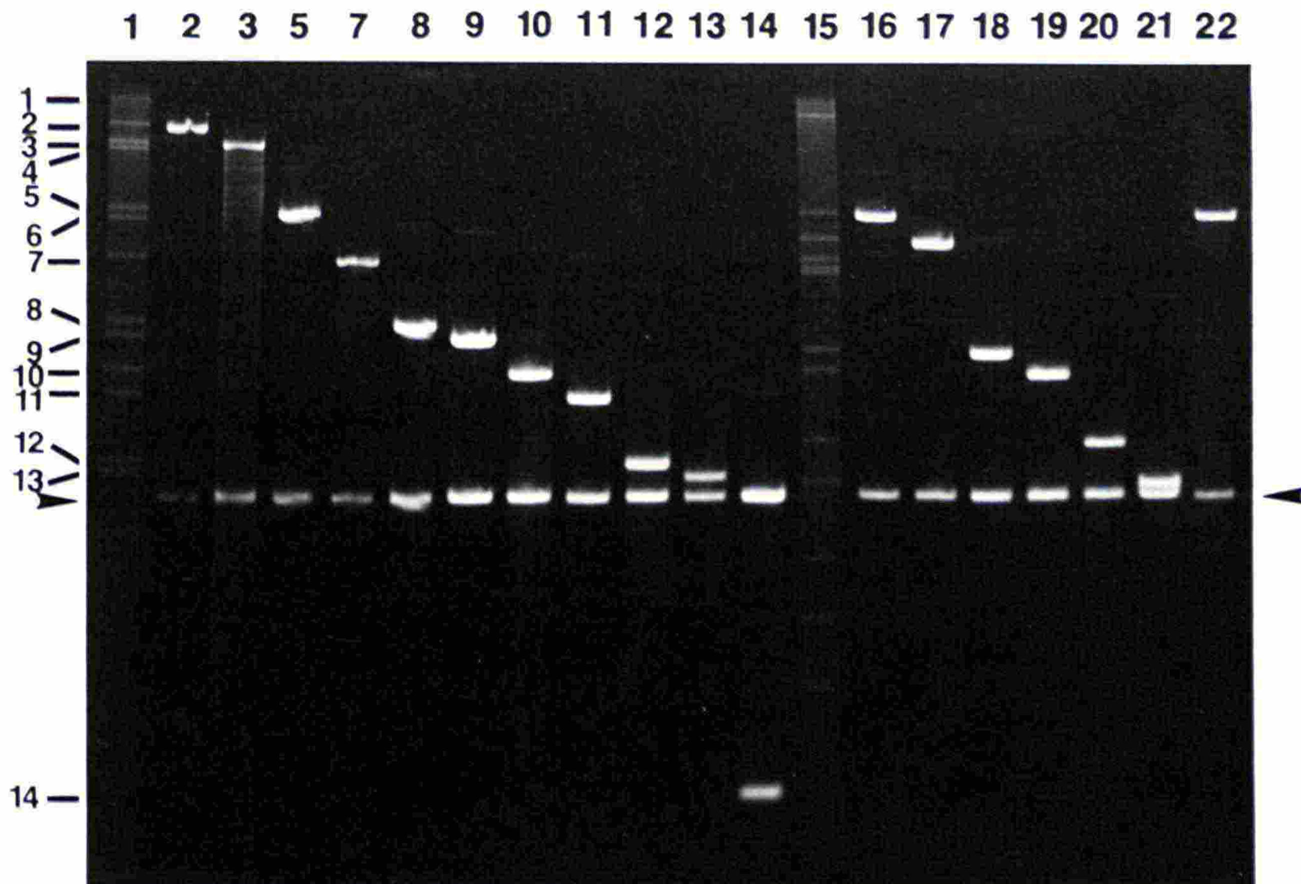

Fig. 1. Restriction endonuclease analysis of *Spirogyra maxima* total cpDNA and cpDNA clones. Lanes 1 and 15 contain total cpDNA cut with *Cla*I and *Pst*I, respectively. The sizes of *Cla*I fragments 1–15 are given in Fig. 2. Lanes 2–14 are cloned *Cla*I fragments as listed at the left of lane 1. Lanes 16–21 are cloned *Pst*I fragments of 9.0, 7.6, 4.8, 4.5, 3.5, and 3.1 kb in size, respectively. Lane 22 is a 9.2 kb *Cla*I + *Pst*I fragment. Arrowheads indicate pBS vector band of 3.0 kb.

rangements, much less to use genome rearrangements as phylogenetic characters.

The *Spirogyra maxima* chloroplast genome was characterized to provide detailed information about its physical structure and gene order. This information will expand our limited knowledge about green algal cpDNAs and possibly provide information about phylogenetic relationships among the green algae and between the green algae and land plants.

MATERIALS AND METHODS

Filaments of *Spirogyra maxima* were obtained from unialgal cultures grown in soil-water medium (Starr and Zeikus 1987) on a 16:8 h LD cycle at 20° C ± 2° C under fluorescent light at an irradiance of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This alga was used earlier for cytological studies following collection from a site near Tampa, Florida (Hoshaw et al. 1987). Filaments of 2-week-old cultures were harvested and washed prior to freezing in liquid nitrogen. Twenty grams of frozen filaments were ground in a coffee mill, further ground with a mortar and pestle in 100 mL 2× CTAB buffer (Doyle and Doyle 1987) and placed in a water bath at 60° C for 30 min. This material was extracted with 25 mL chloroform : isoamyl alcohol (24:1). The aqueous layer was removed, and the DNA was precipitated with 2/3 volume of ice-cold isopropanol. The precipitate was resuspended in TE buffer (10mM Tris, 1 mM EDTA, pH 7.4). CsCl was added to a final density of 1.55 $g \cdot mL^{-1}$ and 1 mg of EtBr was added. The solution was centrifuged at 55,000 rpm for 6 h in a Beckman VTi 65.2 vertical rotor. The banded DNA was removed, and EtBr was extracted with water and salt-saturated isopropanol. CsCl was added to bring the final density to 1.66 $g \cdot mL^{-1}$, and bisbenzimide H33258 was added to a final concentration of 0.04 $mg \cdot mL^{-1}$. This solution was spun as before, and the upper cpDNA band was removed. Bisbenzimide was removed as for EtBr, and the solution was dialyzed against TE to remove CsCl.

Spirogyra maxima cpDNA was cut with the restriction endonucleases *Cla*I, *Eco*RV, *Nco*I, and *Xba*I. Digests were electrophoresed through 1.0% agarose. Fragment sizes were determined by comparison with λ DNA cut with *Hind*III, *Sal*I, *Sma*I, *Eco*RI + *Hind*III, and *Eco*RI + *Kpn*I.

The restriction endonucleases *Cla*I and *Pst*I were used singly and in combination to clone the *Spirogyra maxima* chloroplast genome. The plasmid vector pBS (Stratagene) was used to transform *E. coli* strain DH5α, and white colonies were selected on ampicillin/X-gal plates.

Restriction site and gene maps were constructed by transfer of DNA fragments from agarose gels to Magnagraph nylon filters (Fisher Scientific Co.) and hybridization of ^{32}P-labelled probes to these filters. Homologous hybridization probes consisted of recombinant clones (Fig. 1) and a gel-isolated 8.6 kb *Cla*I fragment. Heterologous gene probes were produced from the recombinant plasmids listed in Table 1. Probes were labelled with ^{32}P by the random primer method following directions in the Boehringer Mannheim Random Primer DNA Labeling Kit. Filter hybridizations (65° C and 4× SSC for homologous probes and 60° C and

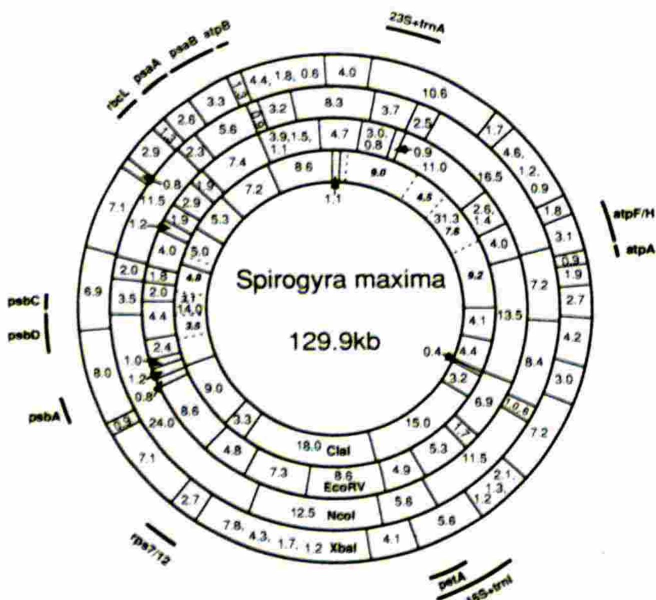

FIG. 2. Physical and gene maps of *Spirogyra maxima* cpDNA. Gene names are as in Table 1. The summations of fragment sizes for the four enzymes shown are 129.9 kb (*Cla*I), 130.0 kb (*Eco*RV), 130.8 kb (*Nco*I), and 128.8 kb (*Xba*I). *Pst*I and *Cla*I-*Pst*I fragments are indicated by dotted lines and bold, italicized numbers. The locations of the trnA and trnI genes were determined by nucleotide sequencing of cloned fragments using primers to conserved regions of the 23S and 16S rRNA genes, respectively (Manhart and Palmer 1990). The orientations of the 16S and 23S rRNA genes have not been determined. The loci indicated for *atp*F/H and *rps*7/12 may contain one or both of the genes.

4 × SSC for heterologous probes) and washes (65° C and 1 × SSC for homologous probes and 60° C and 1 × SSC for heterologous probes) were done as described in Palmer (1982, 1986).

RESULTS AND DISCUSSION

Chloroplast genome cloning. A total of 117.3 kb or 90.3% of the *Spirogyra* chloroplast genome was cloned. *Cla*I fragments of 18.0, 15.0, 9.0, 7.2, 5.3, 5.0, 4.4, 4.1, 3.3, 3.2, and 1.1 kb (Fig. 1) were obtained by screening 100 colonies. Of the 31.3 kb *Cla*I fragment, 30.3 kb was obtained by cloning a 9.2 kb *Cla*I-*Pst*I fragment and internal *Pst*I fragments of 7.6, 4.5, and 9.0 kb (Fig. 1). Of the 14 kb *Cla*I fragment, 10.3 kb was obtained by cloning a 4.8 *Pst*I fragment that overlapped with the adjacent 5.0 kb *Cla*I fragment and internal 3.1 and 3.5 kb *Pst*I fragments (Fig. 1). Repeated attempts to clone the 8.6 kb *Cla*I fragment and the remaining 3.7 kb of the 14.0 kb *Cla*I fragment, which contains a portion of the *psb*A gene (Fig. 2), were unsuccessful.

Physical structure. The mapping data indicate that the chloroplast genome of *S. maxima* consists of a circular chromosome 130 kb in size which does not contain any duplications or repeat units detectable under the described experimental conditions (Fig. 2). The genome size is in the same range as the majority of land plants (120–160 kb; Palmer 1985a, b). The absence of a large IR which contains rRNA genes, although very rare in land plants (Palmer et al. 1988), appears to be more common in the green algae, occurring in *Codium* (Manhart et al. 1989) and *S. maxima*.

TABLE 1. *Gene probes. Sources, except for 5′rps7-3′rps12,* atp*B/E,* psa*A-5′*psa*B, 3′*psa*B, 3′*atp*H-5′*atp*F, and* atp*A are described in Jansen and Palmer (1987) and Manhart et al. (1989).*

Gene designation	Plant source	Hybridization signal
5′*rps*7-3′*rps*12[a]	Tobacco	+
*rpo*A	Spinach	−
3′*pet*A	Pea	+
5′*pet*A	Pea	+
*rbc*L	Pea	+
*atp*E	Spinach	−
*atp*B/E[b]	Tobacco	+
*psa*A-5′*psa*B[c]	Spinach	+
3′*psa*B[d]	Spinach	+
3′*psb*C	Spinach	+
3′*psb*D	Pea	+
*rpo*B	Tobacco	−
3′*atp*H-5′*atp*F[e]	Pea	+
*atp*A[f]	Tobacco	+
16SrRNA	Tobacco	+
23rRNA	Tobacco	+
*tuf*A	*Chlamydomonas*	−

[a] 1226 bp *Bam*HI-*Bam*HI fragment containing 278 bp of 5′ end of *rps*7, and all of exons 3 (231 bp) and 2 (35 bp) of 3′*rps*12, and extending 101 bp before exon 2 (Shinozaki et al. 1986).

[b] 1051 bp *Spe*I-*Bam*HI fragment beginning 848 bp past initiation codon (IC) of *atp*B through termination codon (TC) (648 bp), containing all of *atp*E (401 bp) and ending 5 bp past *atp*B IC (Shinozaki et al. 1986).

[c] 2505 bp *Bam*HI-*Bam*HI fragment beginning 50 bp past IC of *psa*A and ending 277 bp past IC of *psa*B (Kirsch et al. 1986).

[d] 1683 bp *Bam*HI-*Bam*HI fragment beginning 598 bp past IC and ending 80 bp past TC (Kirsch et al. 1986).

[e] 767 bp *Pst*I-*Bam*HI fragment beginning 92 bp past IC of *atp*H and ending 111 bp past IC of *atp*F (Hudson et al. 1986).

[f] 1449 bp *Bcl*I-*Kpn*I fragment beginning 14 bp before IC and ending 80 bp from TC (Shinozaki et al. 1986).

Gene content. It is difficult to make absolute statements about gene content on the basis of Southern hybridizations using heterologous probes. The lack of autoradiographic signals for *atp*E, *rpo*A and *rpo*B (Table 1) can either be interpreted as indicative of gene absence or a high level of nucleotide sequence divergence. These particular gene probes also give either no autoradiographic signal or very weak signals with *Codium fragile* cpDNA under the described hybridization conditions (Manhart et al. 1989). In addition, with the exception of the *rps*12 gene, ribosomal protein gene probes from angiosperm cpDNA did not hybridize with *Codium fragile* cpDNA (Manhart et al. 1989). Strauss et al. (1988) noted the lack of hybridization of some angiosperm ribosomal protein genes to conifer cpDNAs. The ribosomal protein genes are relatively small and appear to evolve more rapidly than photosynthetic genes (Sugiura 1989).

The absence of any detectable hybridization of *Spirogyra maxima* cpDNA with the *tuf*A clone, which contains an internal, highly conserved region of *tuf*A from *Chlamydomonas* cpDNA (Table 1), is quite in-

triguing. This probe hybridizes with other green algal cpDNAs, with the exception of *Spirogyra maxima* and *Sirogonium melanosporum* (Baldauf et al. 1990). The absence of this gene has been noted in land plant cpDNAs (Sugiura 1989, Baldauf and Palmer 1990), and there is evidence for its transfer from the chloroplast to the nucleus in the green algal ancestor to land plants (Baldauf and Palmer 1990). Since *Coleochaete*, *Nitella*, and *Chara*, which have a *tuf*A gene in their cpDNAs, are more closely related to land plants than *Spirogyra maxima* and *Sirogonium melanosporum* (Bremer 1985, Manhart and Palmer 1990) the apparent absence of the *tuf*A gene from the cpDNAs of these two members of the Zygnematales is best treated as a loss that occurred in parallel with the complete loss of *tuf*A from land plant cpDNAs (Baldauf et al. 1990).

Gene order. The gene order of *Spirogyra maxima* cpDNA does not bear any marked resemblance to land plant cpDNAs or any other green algal cpDNA (Lemieux et al. 1985, Manhart et al. 1989). The only similarities are that the members of three pairs of functionally related genes, *psa*A-*psa*B, *psb*C-*psb*D, and *atp*A-*atp*F/H, that are known to be co-transcribed in land plant cpDNA, map near each other in *S. maxima* cpDNA. The *psa*-A-*psa*B and *psb*C-*psb*D pairings also occur in *Codium fragile* cpDNA (Manhart et al. 1989), and the *atp*A-*atp*F gene cluster occurs in *Chlamydomonas moewusii* (Turmel et al. 1988). The resemblance ends there, however, with the locations of the other genes mapped in *S. maxima* cpDNA quite different relative to land plants (Fig. 3) and other green algae.

In land plants the 16S and 23S rRNA genes are located in the IR and are separated by a spacer of about 2.5 kb in size that contains two tRNA genes, trnA(UGC) and trnI(GAU). The rRNA genes, including the spacer, are co-transcribed in tobacco (Shinozaki et al. 1986) and presumably all land plants. In *Spirogyra maxima* cpDNA, the 16S and 23S rRNA genes are approximately 44 kb apart (Figs. 2, 3), which is the greatest separation known for these two genes in any chloroplast genome. Since the rRNA genes in *S. maxima* cpDNA are so far apart and have additional genes between them, it is extremely unlikely they are co-transcribed. The only other known instances in bacteria and cpDNAs where the rRNA genes are probably not co-transcribed include *Leptospira interrogans* (Stimson) Wenyon, a gram-negative bacterium (Fukunaga and Mifuchi 1989), *Chlorella ellipsoidea* (Yamada and Shimaji 1986, 1987), and *Codium fragile* (Manhart et al. 1989). Nucleotide sequencing (Manhart and Palmer 1990) has shown that trnA is adjacent to the 23S rRNA gene and trnI is adjacent to the 16S rRNA gene in *S. maxima* cpDNA (Fig. 2). This is the same relative positioning found in the IR of land plants. Thus, the standard rRNA operon has been broken essentially in half in *S. maxima*, potentially by as few as one rearrangement.

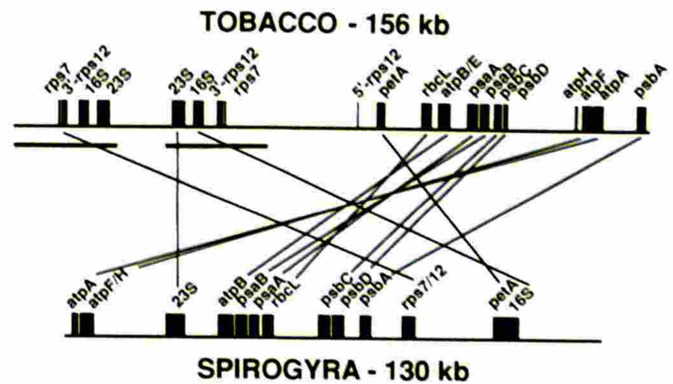

FIG. 3. Comparison of gene orders in tobacco and *Spirogyra* chloroplast DNAs. *Spirogyra* gene order is from Figure 2; tobacco gene order is from Shinozaki et al. (1986). Gene names are as in Table 1. Maps are drawn to same scale. Heavy lines in tobacco genome indicate locations of the inverted repeats.

Characterization of the cpDNAs of additional Zygnematalean taxa should provide information on possible modes of evolution in chloroplast genomes. For example, if the IR is absent in related taxa such as *Sirogonium*, *Zygnema*, and *Mougeotia*, we might expect the genomes to be rearranged relative to each other as in certain legume genera (Palmer et al. 1987) and conifers (Strauss et al. 1988) that have lost one of the repeats. This would provide a test of the hypothesis that the loss of the inverted repeat leads to an unstable genome that undergoes more frequent rearrangements than genomes with the IR (Palmer and Thompson 1982, Palmer 1985b, Strauss et al. 1988). The data may also prove useful in testing alternative phylogenetic hypotheses proposed for the Zygnemataceae on the basis of chloroplast form and growth habit (Hoshaw and McCourt 1988).

This research was supported in part by NSF grants BSR-8600167 and BSR-8906126 to JRM, NSF grant BSR-8996262 to JDP, and NSF grants BSR-8215730 and BSR-8516681 to RWH.

Baldauf, S. L., Manhart, J. R. & Palmer, J. D. 1990. Different fates of the chloroplast *tuf*A gene following its transfer to the nucleus in green algae. *Proc. Nat. Acad. Sci. U.S.A.* In press.

Baldauf, S. L. & Palmer, J. D. 1990. Evolutionary transfer of the chloroplast *tuf*A gene to the nucleus. *Nature (Lond.)* 344: 262–5.

Bremer, K. 1985. Summary of green plant phylogeny and classification. *Cladistics* 1:369–85.

Doyle, J. J. & Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11–5.

Fukunaga, M. & Mifuchi, I. 1989. Unique organization of *Leptospira interrogans* rRNA genes. *J. Bacteriol.* 171:5763–7.

Green, B. R. 1976. Covalently closed minicircular DNA associated with *Acetabularia* chloroplasts. *Biochem. Biophys. Acta* 447:156–66.

Hedberg, M. F., Huang, Y. S. & Hommersand, M. H. 1981. Size of the chloroplast genome in *Codium fragile*. *Science (Wash. D.C.)* 213:445–7.

Hoshaw, R. W. & McCourt, R. M. 1988. The Zygnemataceae (Chlorophyta): a twenty-year update of research. *Phycologia* 27:511–48.

Hoshaw, R. W., Wells, C. V. & McCourt, R. M. 1987. A polyploid species complex in *Spirogyra maxima* (Chlorophyta, Zygnemataceae), a species with large chromosomes. *J. Phycol.* 23: 267–73.

Hudson, S. H., Mason, J. G., Holton, T. A., Koller, B., Cox, G. B., Whitfeld, P. R. & Bottomley, W. 1986. A gene cluster in the spinach and pea chloroplast genomes encoding one CF_1 and three CF_0 subunits of the H+-ATP synthase complex and the ribosomal protein S2. *J. Mol. Biol.* 196:283–98.

Jansen, R. K. & Palmer, J. D. 1987. Chloroplast DNA from lettuce and *Barnadesia* (Asteraceae): structure, gene localization, and characterization of a large inversion. *Curr. Genet.* 11:553–64.

Kirsch, W., Seyer, P. & Herrmann, R. G. 1986. Nucleotide sequence of the clustered genes for two P_{700} chlorophyll *a* apoproteins of the photosystem I reaction center and the ribosomal protein S14 of the spinach plastid chromosome. *Curr. Genet.* 10:843–55.

Lemieux, B. & Lemieux, C. 1985. Extensive sequence rearrangements in the chloroplast genomes of the green algae *Chlamydomonas eugametos* and *Chlamydomonas reinhardtii*. *Curr. Genet.* 10:213–9.

Lemieux, B., Turmel, M. & Lemieux, C. 1985. Chloroplast DNA variation in *Chlamydomonas* and its potential application to the systematics of this genus. *Biosystems* 18:293–8.

Manhart, J. R., Kelly, K., Dudock, B. S. & Palmer, J. D. 1989. Unusual characteristics of *Codium fragile* chloroplast DNA revealed by physical and gene mapping. *Mol. Gen. Genet.* 216: 417–21.

Manhart, J. R. & Palmer, J. D. 1988. Variation in charophyte chloroplast DNAs. *J. Phycol.* 24(Suppl.):24.

——— 1990. The gain of two chloroplast tRNA introns marks the green algal ancestors of land plants. *Nature (Lond.)*. 345: 268–70.

Mishler, B. D. & Churchill, S. P. 1985. Transition to a land flora: phylogenetic relationships of the green algae and bryophytes. *Cladistics* 1:305–28.

Padmanabhan, U. & Green, B. R. 1978. The kinetic complexity of *Acetabularia* chloroplast DNA. *Biochem. Biophys. Acta* 521: 67–73.

Palmer, J. D. 1982. Physical and gene mapping of chloroplast DNA from *Atriplex triangularis* and *Cucumis sativa*. *Nucleic Acid Res.* 10:1593–605.

——— 1985a. Evolution of chloroplast and mitochondrial DNA in plants and algae. *In* R. J. MacIntyre [Ed.] *Monographs in Evolutionary Biology: Molecular Evolutionary Genetics*. Plenum Press, New York, pp. 131–240.

——— 1985b. Comparative organization of chloroplast genomes. *Annu. Rev. Genet.* 19:325–54.

——— 1986. Isolation and structural analysis of chloroplast DNA. *Meth. Enzymol.* 118:167–86.

Palmer, J. D., Boynton, J. E., Gillham, N. W. & Harris, E. H. 1985. Evolution and recombination of the large inverted repeat in *Chlamydomonas* chloroplast DNA. *In* Arntzen, C., Bogorad, L., Bonitz, S. & Steinback, K. [Eds.] *Molecular Biology of the Photosynthetic Apparatus*. Cold Spring Harbor Press, New York, pp. 269–778.

Palmer, J. D., Jansen, R. K., Michaels, H. J., Chase, M. W. & Manhart, J. R. 1988. Chloroplast DNA variation and plant phylogeny. *Ann. Mo. Bot. Gard.* 75:1180–206.

Palmer, J. D., Osorio, B., Aldrich, J. & Thompson, W. F. 1987. Chloroplast DNA evolution among legumes: loss of a large inverted repeat occurred prior to other sequence rearrangements. *Curr. Genet.* 11:275–86.

Palmer, J. D. & Thompson, W. F. 1982. Chloroplast DNA rearrangements are more frequent when a large inverted repeat sequence is lost. *Cell* 29:537–50.

Rochaix, J. D. 1978. Restriction endonuclease map of the chloroplast DNA of *Chlamydomonas reinhardtii*. *J. Mol. Biol.* 126: 597–617.

Shinozaki, K., Ohme, M., Tanaka, M., Wakagusi, T., Hayashida, N., Matsubayashi, T., Saita, N., Chunwongse, J., Ibokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torasawa, K., Meng, B. Y., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdoh, N., Shimada, H. & Sugiura, M. 1986. The complete nucleotide sequence of the tobacco chloroplast genome: its gene order and expression. *EMBO J.* 5:2043–9.

Starr, R. C. & Zeikus, J. A. 1987. UTEX—the culture collection of algae at the University of Texas at Austin. *J. Phycol.* 23(Suppl.):1–47.

Strauss, S. H., Palmer, J. D., Howe, G. T. & Doerksen, A. H. 1988. Chloroplast genomes of two conifers lack a large inverted repeat and are extensively rearranged. *Proc. Nat. Acad. Sci. U.S.A.* 85:3898–902.

Sugiura, M. 1989. Chloroplast chromosomes of land plants. *Annu. Rev. Cell Biol.* 5:51–70.

Turmel, M., Lemieux, B. & Lemieux, C. 1988. The chloroplast genome of the green alga *Chlamydomonas moewusii*: localization of protein-coding genes and transcriptionally active regions. *Mol. Gen. Genet.* 214:412–9.

Yamada, T. 1983. Characterization of inverted repeat sequences and ribosomal RNA genes of chloroplast DNA from *Chlorella ellipsoidea*. *Curr. Genet.* 7:481–7.

Yamada, T. & Shimaji, M. 1986. Peculiar features of rRNA genes of the *Chlorella* chloroplast DNA. *Nucleic Acid Res.* 14: 3827–39.

——— 1987. Splitting of the ribosomal RNA operon on chloroplast DNA from *Chlorella ellipsoidea*. *Mol. Gen. Genet.* 208: 377–83.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.