# Group I Introns within the Nuclear-encoded Small-Subunit rRNA Gene of Three Green Algae[1]

*Lee W. Wilcox,* Louise A. Lewis,*,[2] Paul A. Fuerst,† and Gary L. Floyd**

Departments of *Plant Biology and †Molecular Genetics, Ohio State University

Four group I introns from the nuclear-encoded (18S) rRNA genes of three chlorophycean green algae are described; two are in *Dunaliella parva,* and one each is in *D. salina* and *Characium saccatum.* The introns within the gene in the latter two organisms are located at the sites equivalent to the 5′ and 3′ introns of *D. parva,* respectively. All four introns lack open reading frames and are relatively small, 381–447 bp. Both primary- and secondary-structural features place these introns within subgroup IC1 described by Michel and Westhof. Phylogenetic relationships of the three intron-containing taxa and their relatives, as inferred from comparisons of 18S rDNA sequences, suggest that inheritance of the introns along with the gene can account for their present distribution. The discovery of these four introns, in addition to two others known to exist in other chlorophycean green algae, suggests that group I introns within the 18S rRNA gene may be relatively common in the green algae.

## Introduction

Group I introns have been shown to occur within a number of genes from a variety of organisms (see Cech 1988; Michel and Westhof 1990), including eubacteria (Kuhsel et al. 1990; Xu et al. 1990). The majority of group I introns in rRNA genes have been reported from the large-subunit (LSU) rRNA gene, both nuclear and organellar (Cech 1988; Michel and Westhof 1990). The occurrence of group I introns in small-subunit (SSU) rRNA genes appears to be more sporadic. Introns within the nuclear-encoded (18S) SSU rRNA gene may be rare, if not entirely lacking, in a number of major eukaryotic lineages. Despite widespread study of the gene, group I introns in 18S rRNA genes have only been reported from the fungus *Pneumocystis carinii* (Sogin and Edman 1989), the mycobiont of the lichen *Cladonia* (P. DePriest and M. Been, personal communication), and the green alga *Ankistrodesmus stipitatus* (Dávila-Aponte et al. 1991).

During the course of a study utilizing 18S rRNA gene sequences to address phylogenetic questions in the green algae, we have identified five taxa which possess group I introns within this gene: *Dunaliella parva, Characium saccatum, Dunaliella salina, Neochloris aquatica,* and *Neochloris* sp. The 18S introns of the first three organisms are quite similar to one another and are described here, while that in *N. aquatica* is distinct from these and, possibly, from all other group I introns (L. A. Lewis, unpub-

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2. Current address: Department of Botany, Duke University, Durham, North Carolina 27706.

Address for correspondence and reprints: Gary L. Floyd, Department of Plant Biology, The Ohio State University, 1735 Neil Avenue, Columbus, Ohio 43210.

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lished observations) and will be reported on elsewhere. The intron in *Neochloris* sp. [for information on this organism, see Lewis et al. (1991)] remains to be fully characterized. It would appear that the green algae—and, specifically, the class Chlorophyceae, to which these taxa (with the exception of *Neochloris* sp.) belong—may be a group in which group I introns in the nuclear 18S rRNA gene are relatively common.

## Material and Methods

### Cultures

*Dunaliella parva* (#1983), and *Characium saccatum* (#111) were obtained from the Culture Collection of Algae at the University of Texas at Austin (UTEX) (Starr and Zeikus 1987). *D. salina* was isolated from Chilean waters and was provided by Dr. Oscar Parra, Universidad de Concepción, Concepción, Chile. It should be noted that we earlier, in abstract form, erroneously described the 18S rRNA gene (and introns) of *D. parva* as representing that of another green alga, *Asteromonas gracilis* (Fuerst et al. 1990, 1991; Wilcox et al. 1990). The complete 18S rRNA gene from *A. gracilis* (UTEX #635) has now been sequenced and found to contain no introns (authors' unpublished observation).

### DNA Isolation

Cells were concentrated by centrifugation and were resuspended in UNSET buffer (Garriga et al. 1984). This was sufficient to lyse the wall-less cells of *Dunaliella.* For *Characium* (vegetative) cells, which possess relatively thick cell walls, liquid nitrogen was used to freeze a small volume of cells in UNSET in the presence of sand. As the material began to soften on warming, it was ground with a mortar and pestle. Once lysed, all material was subjected to a standard phenol/chloroform extraction and ethanol precipitation and was resuspended in TE (Maniatis et al. 1982, p. 458).

### DNA Amplification, Cloning, and Sequencing

As in the approach outlined by Medlin et al. (1988), PCR primers corresponding to the highly conserved regions at each end of the 18S gene included built-in restriction sites which facilitated cloning into M13 mp-18 and mp-19 bacteriophage sequencing vectors. PCR products from two or more PCR reactions were pooled prior to cloning, and multiple M13 clones were pooled for sequencing. Single-stranded templates were sequenced according to the Sequenase version 2.0 protocol (United States Biochemical). Both the "RNA" strand and its complement were sequenced for 77%–80% of each intron sequence, including all regions in which the sequence from one or the other strand had ambiguous positions on sequencing gels.

### RNA Extraction/cDNA Production in *D. parva*

Total RNA was extracted from *D. parva* by using a standard guanidinium isothiocyanate/CsCl ultracentrifugation protocol (Ausubel et al. 1987, sec. 4.2). First-strand cDNA was produced by using the 3′ amplification primer ("SSU2") in a primer extension reaction. Approximately 1–2 μg of RNA and 10 ng of primer were heated to 65°C in 5 × reverse-transcriptase buffer (BRL), allowed to cool slowly, and then placed on ice. Next, a mixture of 2 μg RNAse-free BSA (BRL) and 20 units RNAsin (ProMega) was added, followed by addition of dNTPs (Boehringer Mannheim) to give a final concentration of 0.5 mM of each. Finally, 400 units of Moloney murine leukemia virus (MMLV) reverse transcriptase (BRL) were added, and the reaction was allowed to continue for 1 h at 37°C. One-half of the reaction product was incor-

porated in a PCR reaction, by using SSU2 and a 5′ primer ("1137C"—GAAACTTAAAGGAATTGA>) located just upstream from the 5′ intron. Conditions for PCR were the same as those used to amplify from genomic DNA: 30 cycles, each of 94°C for 45 s, 45°C for 1 min 30 s, and 72°C for 2 min 30 s. To avoid amplification from contaminating DNA, prior to the primer extension the RNA prep was treated with restriction endonucleases known to cut within the gene, and then it was heated briefly to denature the enzymes.

### Sequence Alignment and Analysis

#### *Exons*

To examine phylogenetic relationships of the intron-containing organisms and their relatives, the 18S rRNA gene sequences, exclusive of introns, were manually aligned with the aid of ESEE (Cabot and Beckenbach 1989) for the IBM PC. Regions which either were not clearly alignable for all taxa or correspond to the PCR primers were excluded from the analysis, leaving 1,684 positions, of which 118 are phylogenetically informative. Excluded regions correspond to positions 1–23, 132–134, 490–494, 673–681, 1351–1369, 1677–1705, and 1769–1791 in the *Chlamydomonas reinhardtii* sequence (Gunderson et al. 1987). For distance methods, including neighbor joining, the following programs contained within PHYLIP (Felsenstein 1991) were utilized: a distance matrix was generated using DNADIST with the Kimura two-parameter correction. The matrix was then input into FITCH or NEIGHBOR to generate trees. PAUP (Swofford 1990) was used for parsimony analysis. Parsimony analyses were run in two ways: (1) by weighting all informative positions equally and (2) by down-weighting, by one-half (all taxa with compensatory substitutions) or one-quarter (all but one taxon with compensatory substitutions), those positions which are located in base-paired regions and which show compensatory substitutions. The arbitrary relative values used for weighting in PAUP were 4, 3, and 2. Shortest trees were determined by exhaustive searches, while bootstrap analyses (100 replications) were done by using the branch-and-bound option. Sources for the additional sequences included in the analyses are *C. reinhardtii* (Gunderson et al. 1987); *Volvox carteri* (Rausch et al. 1990); *Characium vacuolatum, Ettlia minuta,* and *Friedmannia israelensis* (Lewis et al. 1992); and *Hydrodictyon reticulatum* (Wilcox et al. 1992).

#### *Introns*

For introns, alignment of conserved regions was done first, followed by alignment of ambiguous variable regions, to give the best match and simultaneously attempt to minimize the number of added gaps. Uncorrected sequence similarities were determined on the basis of the alignments shown in figures 1 and 2. Gaps of one to four nucleotides in length were treated as a series of single-nucleotide gaps. Those gaps which were five or more nucleotides long were treated as five single-nucleotide gaps. As an alternative measure, insertions/deletions were removed, and a corrected distance was calculated, as for exon sequences (see above).

## Results and Discussion

### General Description of Algal 18S Introns

The first indication that introns might be present in the 18S rRNA genes of our study organisms came when PCR yielded products larger than expected. In *Dunaliella parva,* the PCR product corresponding to the whole gene was approximately ~2,600 bp, versus 1,800 bp for typical green-algal 18S genes. In *D. salina* and *Characium*

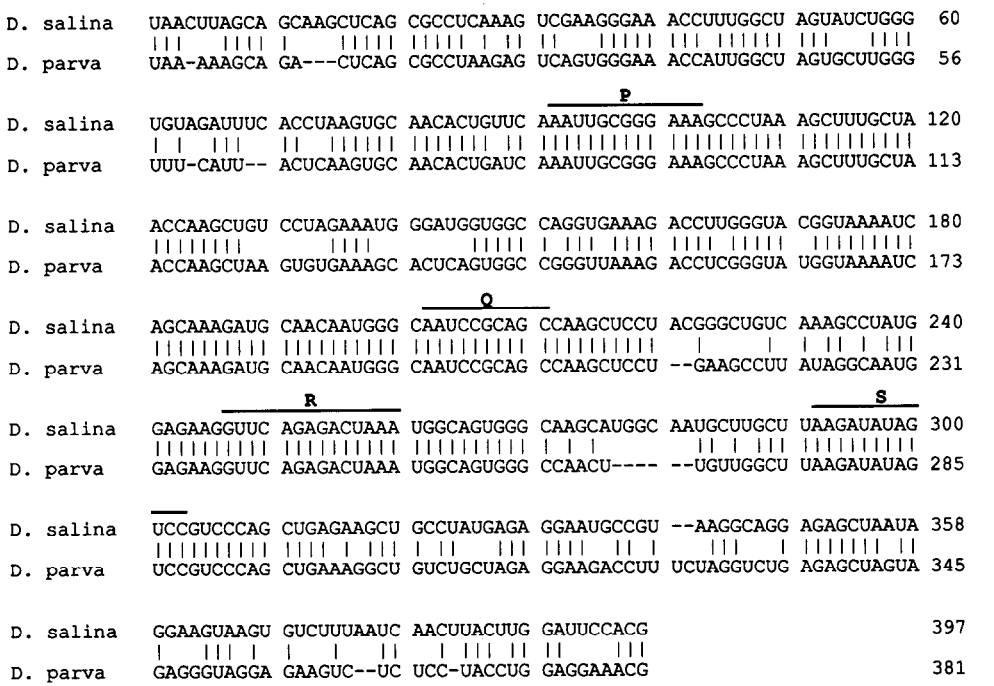



FIG. 1.—Aligned 5′ intron sequences (Dp.SSU,1 and Ds.SSU)

*saccatum,* the size was ~2,200 bp. Subsequent sequence analysis revealed one or, in the case of *D. parva,* two intervening sequences ~400 bp in length (table 1). No open reading frames are present in these relatively small introns. In no case did PCR yield a product of the size corresponding to an intronless form of the gene.

The *D. salina* intron exists in two forms, which differ by a number of nucleotide substitutions and a single base insertion/deletion. Both forms were found to occur within individual cells. This finding will be discussed elsewhere in more detail. The more common of the two forms of the *D. salina* intron is illustrated here.

These algal intervening sequences possess all of the hallmarks of group I introns, including the conserved sequence elements P, Q, R, and S (table 1). Of the various types of group I introns (Michel and Westhof 1990), they most closely resemble those of subgroup IC1.

Putative secondary structures of the introns are depicted in figures 3–6. Each intron possesses a bulged A in P7 and has a G-C pair immediately following this bulged A. Also, in each intron, P6 begins with a G-U pair, followed by a C-G, and P3′ immediately follows sequence element R.

The four introns are located at two different sites within the 18S rRNA gene (see table 1). The *D. parva* 5′ (Dp.SSU,1) and *D. salina* (Ds.SSU) introns share one insertion site, while the *D. parva* 3′ (Dp.SSU,2) and *C. saccatum* (Cs.SSU) introns share the second. The *Ankistrodesmus* (Huss and Sogin 1990), *Pneumocystis* (Sogin and Edman 1989), *Neochloris aquatica,* and *Neochloris* sp. (L. A. Lewis, unpublished observation) introns are each located at a different site within the 18S rRNA gene, none of which is in common with the insertion points of the introns described here.

The cognate pair with the greatest primary sequence similarity (see below)—the

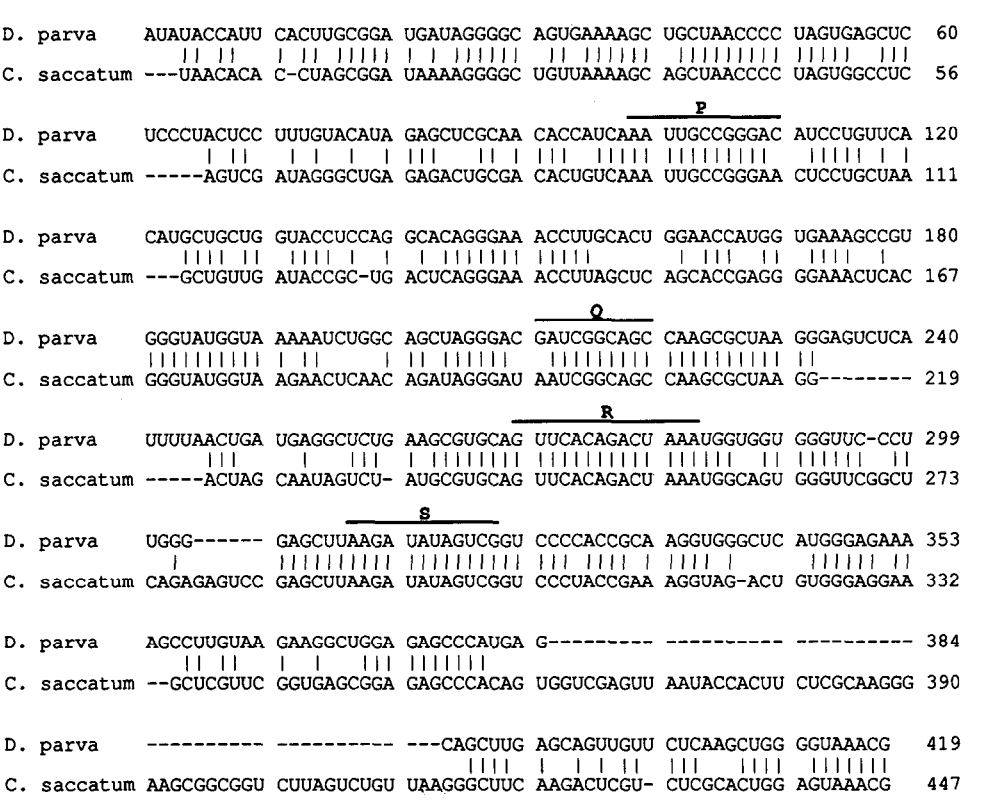



FIG. 2.—Aligned 3′ intron sequences (Dp.SSU,2 and Cs.SSU)

Dp.SSU,1 and Ds.SSU introns—share similar secondary-structural features, including an additional C-G base pair in P6 (figs. 3 and 4), as compared with the Dp.SSU,2 and Cs.SSU intron pair (figs. 5 and 6). The additional length of the Cs.SSU intron compared with the Dp.SSU,2 intron is primarily accounted for by additional sequence near the 3′ end, which results in an extra stem/loop.

## Confirmation of Splicing in *D. parva*

It was determined that neither of the two *D. parva* intervening sequences is present in mature rRNA. RNA was extracted, and first-strand cDNA was produced, which served as a template for PCR using primers bracketing both introns. PCR product was predicted to be 1,467 bp if both of the introns were present and to be 667 bp if both were excised. Figure 7 shows that the PCR product obtained using the cDNA template was of the length expected if both intervening sequences were excised from precursor RNA. This PCR product was cloned and sequenced, confirming that splicing occurred as predicted.

## Distribution of 18S rRNA Introns among the Green Algae

The number of green-algal taxa with published 18S rDNA sequences, together with taxa for which we have either amplified or sequenced the gene, currently totals ~50, of which 6 have been found to possess a total of seven introns (see Introduction). Thus the number of described nuclear-encoded SSU rRNA group I introns presently totals eight, seven of which are found in green algae. As more 18S DNA data for other

**Table 1**
**Size, Location within the Gene, and Conserved Sequence Elements of Algal 18S rRNA Introns**

| Organism (Intron) | Size (bp) | Location[a] | P | Q | R | S |
|---|---|---|---|---|---|---|
| *Dunaliella salina* (Ds.SSU) ..... | 397/8[b] | 1164 | AAUUGCGGGAAA | AAUC-CGCAGC | GUUCAGAGACUAAA | AAGAUAUAGUCC |
| *D. parva* (Dp.SSU,1) ..... | 381 | 1164 | ..... | ....-..... | ..... | ..... |
| *D. parva* (Dp.SSU,2) ..... | 419 | 1766 | .....C..G.C | G...-G..... | .....C..... | .....G |
| *Characium saccatum* (Cs.SSU) ... | 447 | 1766 | .....C..G.. | ....-G..... | .....C..... | .....G |
| Subgroup IC1 consensus[c] ..... | | | AAUUGCGGGRAR[d] | RAYC (-/A) YGCAGC | GUUCAYMGRCYARA | AAGAUAURGUCR |

[a] Relative to published *Chlamydomonas reinhardtii* 18S sequence (Gunderson et al. 1987). Intron is inserted 3′ to position indicated.
[b] See Results and Discussion.
[c] Based on the eight IC1 introns listed by Michel and Westhof (1990). The strict consensus for each site is shown using standard one-letter code.
[d] An adenosine residue follows the first guanine residue in P for one IC1 intron (see Michel and Westhof 1990).

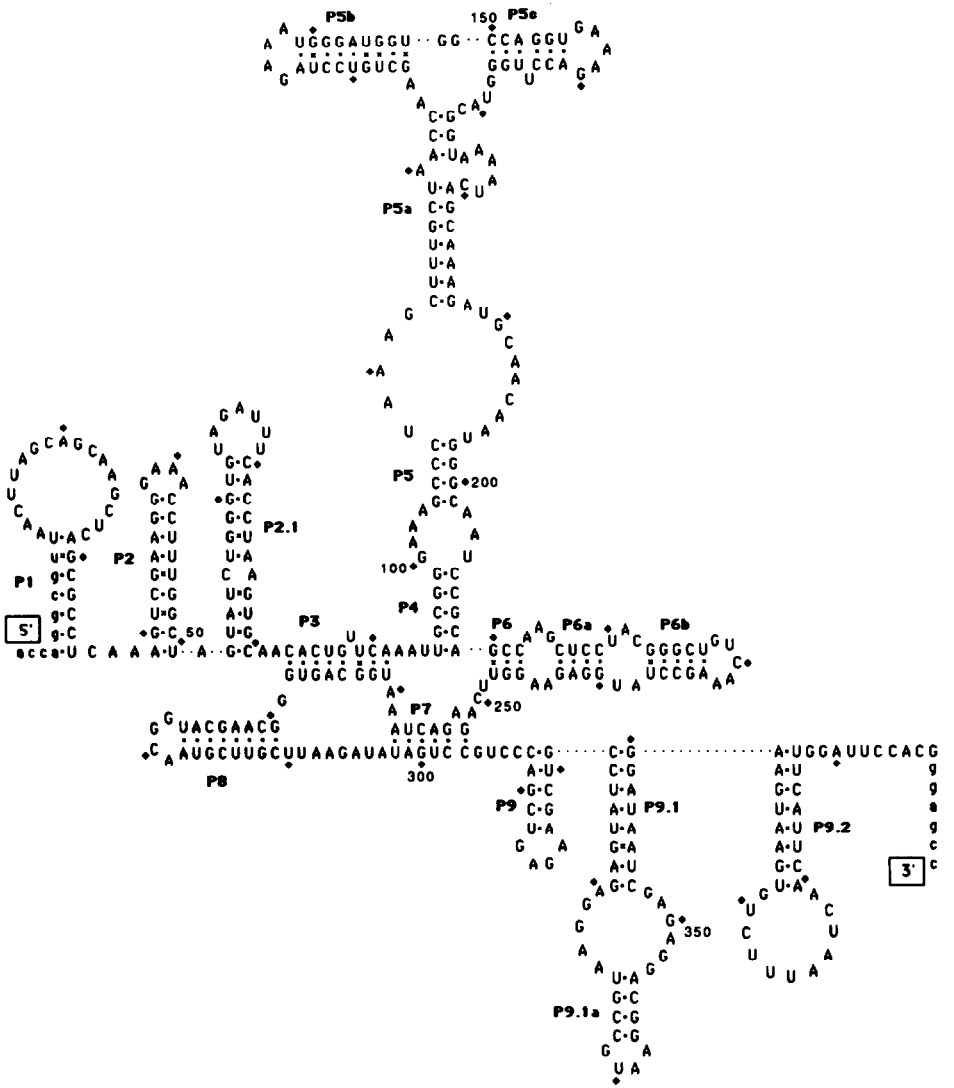



FIG. 3.—Secondary-structural model of 18S rDNA intron in *Dunaliella salina* (Ds.SSU)

eukaryotic lineages become available, however, this ratio may change. Since a considerable amount of the 18S rRNA sequence data gathered both on green algae and on other eukaryotic lineages have been in the form of reverse-transcriptase RNA sequences, a resurvey of such organisms by employing DNA methods is necessary to better assess the distribution of introns in the 18S rRNA gene.

## Phylogenetic Analysis of Intron-containing Chlorophycean Organisms and Their Relatives

The four introns described here are found in three taxa belonging to a group of green algae whose motile cells have basal bodies offset in a clockwise orientation when cross-sections (passing through the basal bodies) of the cell are viewed from above. This basal body arrangement is found in the Volvocales, as well as in some members

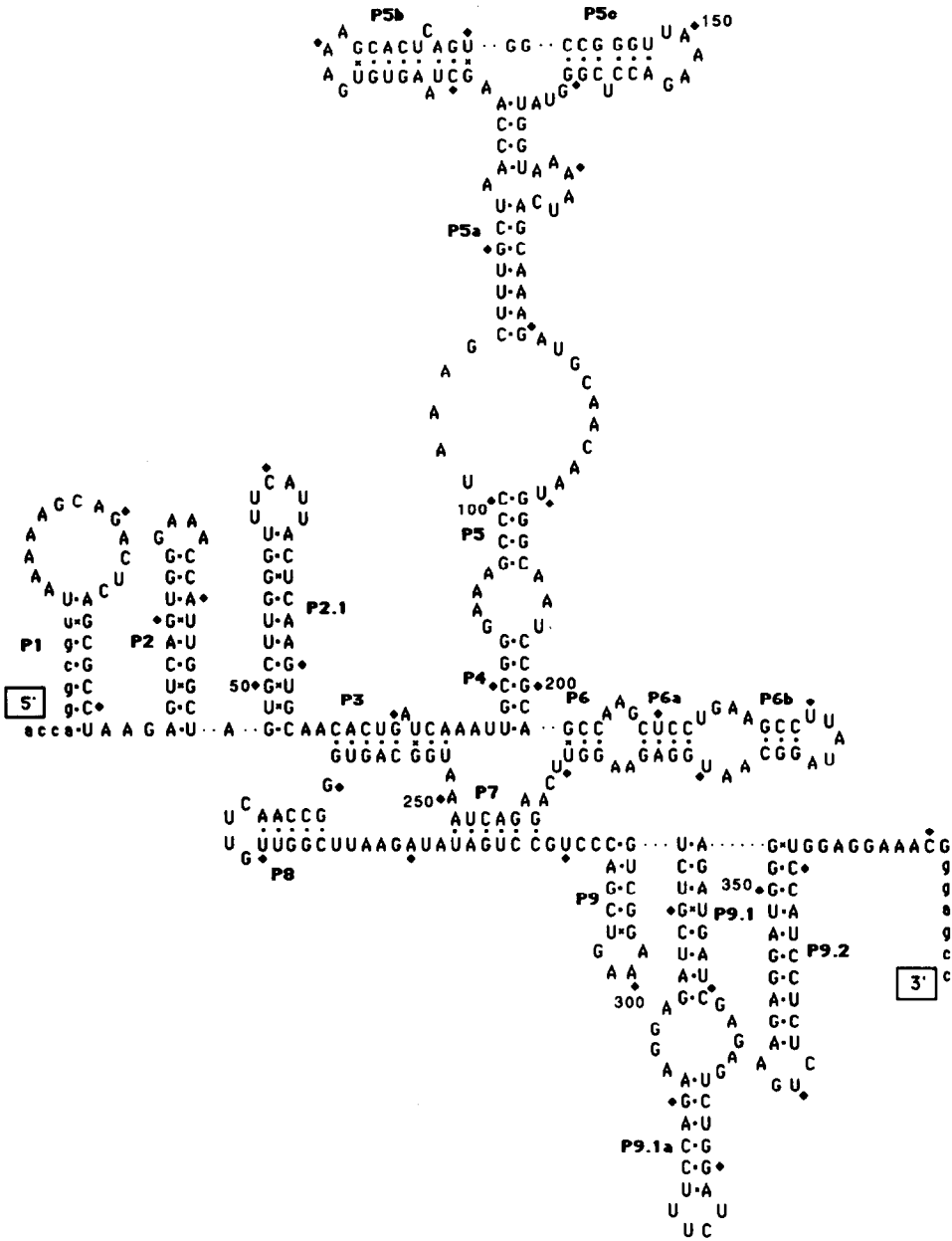



FIG. 4.—Secondary-structural model of 18S rDNA intron in *Dunaliella parva* 5′ (Dp.SSU,1)

of the Chlorococcales (see O'Kelly and Floyd 1984; Watanabe and Floyd 1989; Floyd and Watanabe 1990). The other green-algal taxa known to possess 18S rDNA introns (see above) belong to other green-algal groups. *Neochloris aquatica,* which produces motile cells with directly opposed basal bodies, would appear to belong to a separate lineage of chlorophycean green algae, on the basis of ultrastructural (Watanabe and Floyd 1989) and molecular (Lewis et al. 1992) information. The phylogenetic position of the two other 18S intron-containing green algae is somewhat less clear. *Ankistro-*

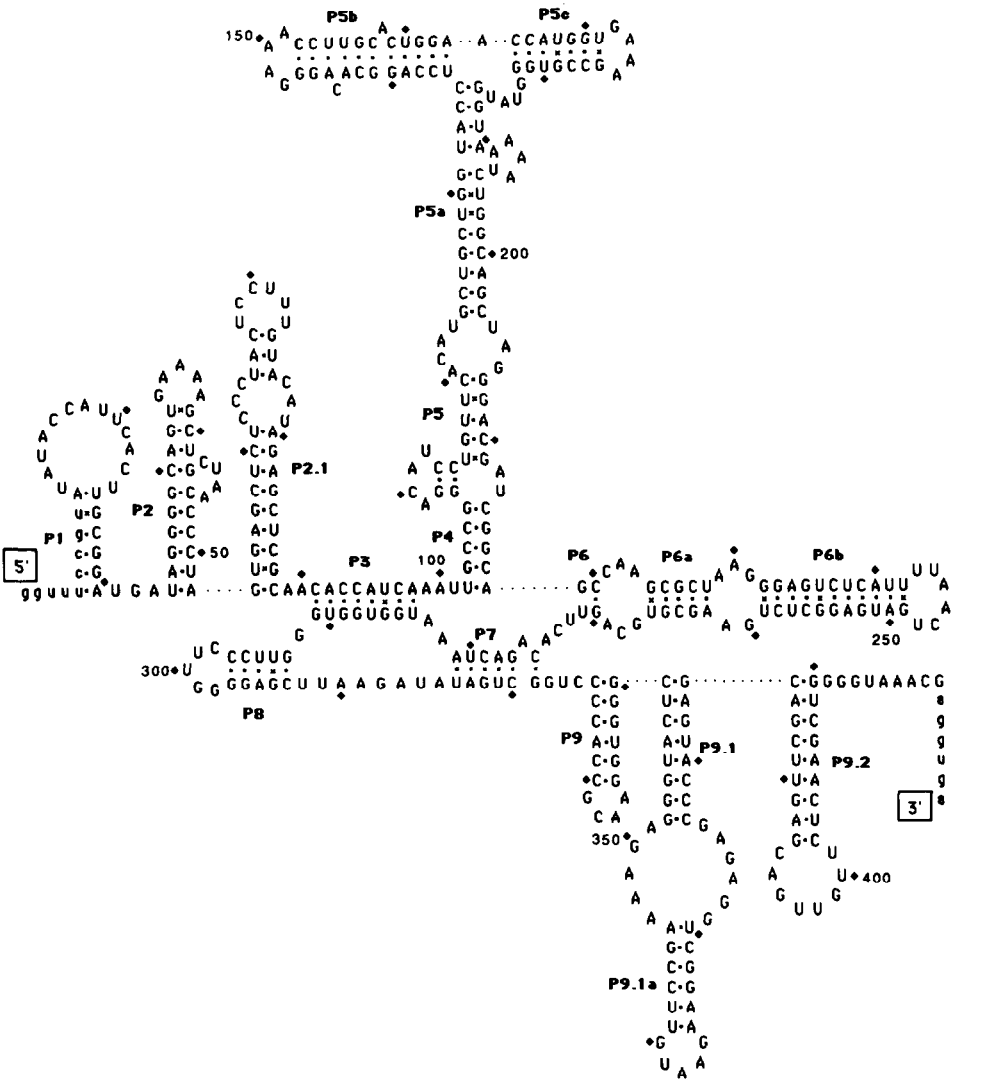



FIG. 5.—Secondary-structural model of 18S rDNA intron in *Dunaliella parva* 3′ (Dp.SSU,2)

*desmus stipitatus,* which reproduces asexually by nonmotile spores, proves to be a difficult organism to place onto trees which include other vegetatively nonmotile, as well as zoospore-producing, green algae. Nonetheless, it appears to have the greatest affinity to chlorophycean taxa (Wilcox et al. 1992). *Neochloris* sp. appears to be a member of a separate green-algal class, the Ulvophyceae (Lewis et al. 1991).

Attempting to account for the distribution of the green-algal 18S rRNA introns described here, we have compared the complete 18S rDNA sequences for the three intron-containing taxa, as well as sequences for several other related organisms having clockwise basal bodies: the vegetatively nonmotile chlorococcalean taxa *Characium vacuolatum* and *Ettlia minuta* and two members of the Volvocales, the unicellular *Chlamydomonas reinhardtii* and colonial *Volvox carteri. Hydrodictyon reticulatum* and *Friedmannia israelensis* were used as outliers. *Hydrodictyon,* like *N. aquatica,*

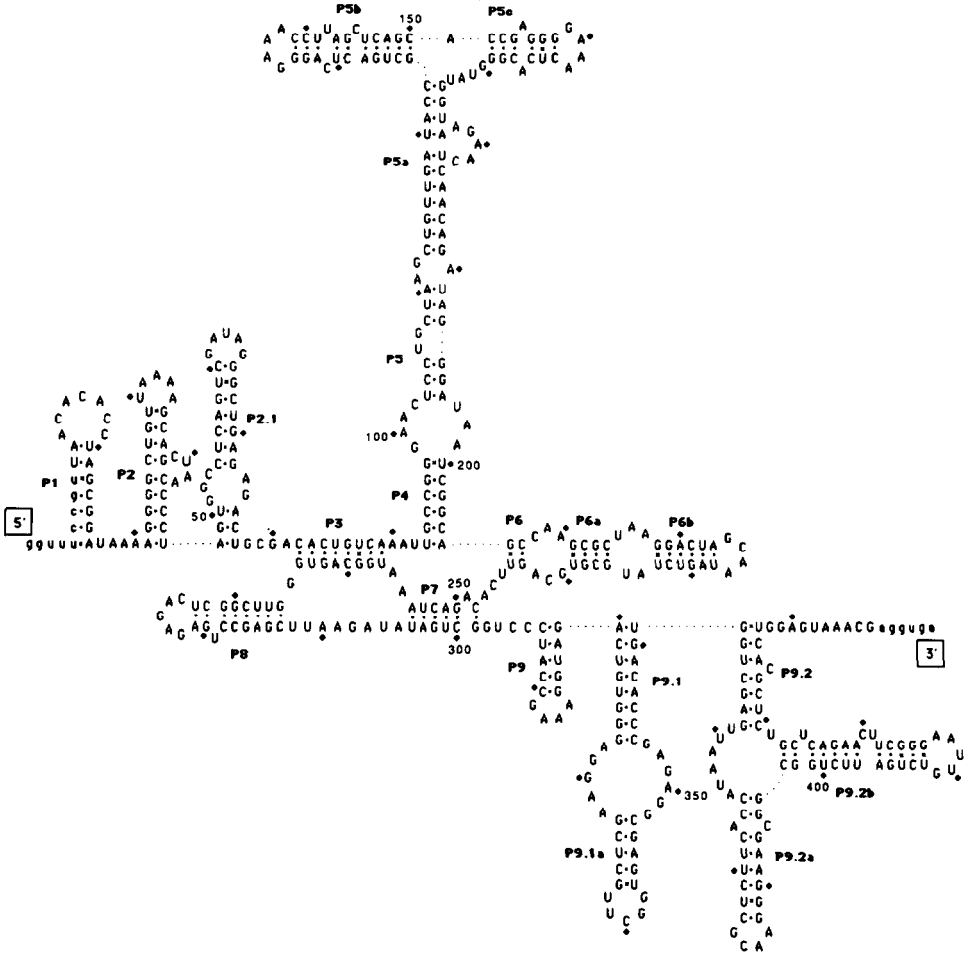
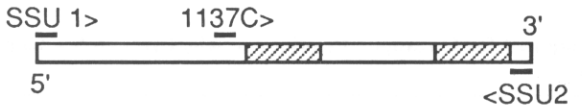



FIG. 6.—Secondary-structural model of 18S rDNA intron in *Characium saccatum* (Cs.SSU)

has motile cells with directly opposed basal bodies, while *Friedmannia,* with counterclockwise basal bodies, is usually placed in another class, the Pleurastrophyceae (sensu Mattox and Stewart 1984). Table 2 shows the nucleotide distances between the various taxa, determined on the basis of the 18S rRNA gene. This matrix was used to construct phylogenetic trees by using the Fitch-Margoliash and neighbor-joining methods. Both distance methods, as well as parsimony analysis, produced the same tree topology (fig. 8). When sequence positions which are involved in base pairing and which exhibit compensatory nucleotide substitutions are down-weighted for parsimony analysis, the topology is unchanged. In parsimony bootstrap analyses, all branches are supported ≥98% of the time (fig. 8).

The tree shows that the two members of the Volvocales—*Chlamydomonas reinhardtii* and *V. carteri*—form a sister group to the remainder of the organisms which possess a clockwise orientation to the flagellar apparatus. Among the latter, the two intron-containing taxa, *D. parva* and *D. salina,* make up a sister group to the group which includes *Ettlia minuta* and the two *Characium* species, the intronless *C. vacuolatum* and the intron-containing *C. saccatum.*



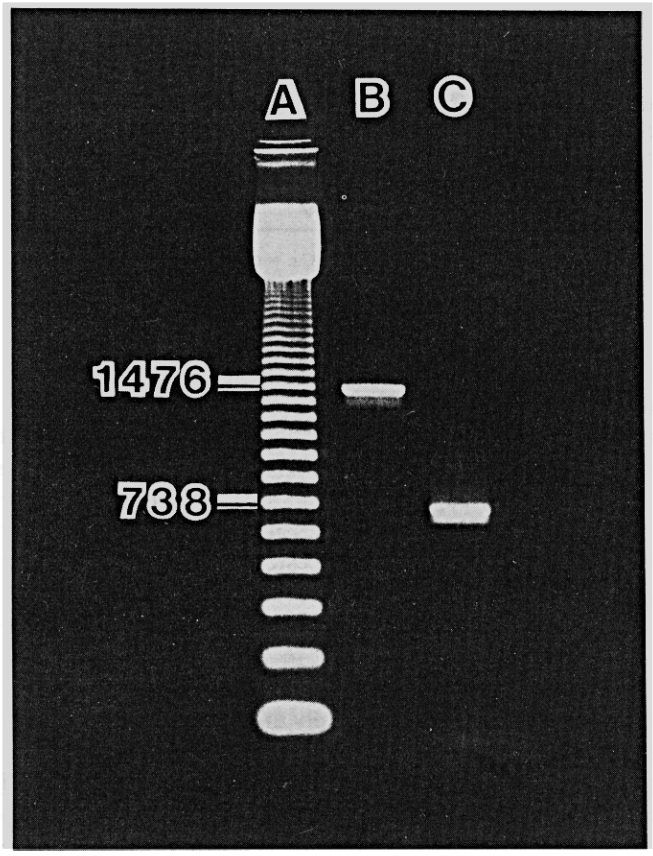




FIG. 7.—EB-stained agarose gel showing PCR product of *Dunaliella parva* 18S gene when primers 1137C and SSU2 are used. Lane A, 123-bp marker (BRL). Numbers correspond to size of indicated bands. Lane B, PCR product when total cellular DNA is used as a template. Lane C, Product of reaction with first-strand cDNA as a template. Note that C is of the size predicted if both introns were excised.

## Transmission of Algal 18S Introns

One of the livelier debates regarding group I introns concerns their mode of transmission—whether it is predominately through inheritance or by some means of horizontal transfer (Doolittle 1990). Often cited as evidence for the latter is the case of *Tetrahymena,* in which nucleotide distances among LSU rDNA intron sequences appear incongruent with a phylogenetic tree based on 18S rDNA sequence data from the same taxa (Sogin et al. 1986). However, phylogenetic analysis of the intron sequences does not appear to preclude the possibility of vertical transmission of the introns (authors' unpublished observation). The inconsistent distribution of introns within the T-even phages is also taken as evidence for a recent transfer of introns (Quirk et al. 1989). Suggested as evidence for ancient, inherited introns maintained

**Table 2**
**Distance Matrix Computed for Ungapped Positions in Algal 18S rRNA Genes (Exclusive of Introns)**

| | C.r. | V.c. | D.s. | D.p. | E.m. | C.s. | C.v. | H.r. |
|---|---|---|---|---|---|---|---|---|
| C.r. .... | | | | | | | | |
| V.c. .... | 1.0 | | | | | | | |
| D.s. .... | 4.9 | 5.1 | | | | | | |
| D.p. ... | 4.7 | 4.8 | 0.7 | | | | | |
| E.m. ... | 6.2 | 6.5 | 5.1 | 4.9 | | | | |
| C.s. .... | 5.9 | 6.1 | 3.6 | 3.5 | 3.5 | | | |
| C.v. .... | 5.8 | 6.1 | 3.5 | 3.2 | 3.3 | 0.7 | | |
| H.r. .... | 5.3 | 5.7 | 3.9 | 3.9 | 6.0 | 5.4 | 5.2 | |
| F.i. .... | 5.9 | 6.1 | 5.1 | 4.9 | 7.1 | 6.5 | 6.5 | 4.3 |

NOTE.—Data are percent of sequence nucleotide distance. C.r. = *Chlamydomonas reinhardtii*; V.c. = *Volvox carteri*; D.s. = *Dunaliella salina*; D.p. = *D. parva*; E.m. = *Ettlia minuta*; C.s. = *Characium saccatum*; C.v. = *Characium vacuolatum*; H.r. = *Hydrodictyon reticulatum*; and F.i. = *Friedmannia israelensis*.

at a particular point of insertion is the discovery of group I introns occurring in tRNA genes of cyanobacteria and plastids (Kuhsel et al. 1990; Xu et al. 1990).

In the case of the four algal introns reported here, if one assumes the introns to have been inherited (in their current positions) along with the 18S gene, the most parsimonious scenario would suggest that the ancestral condition is represented in *Characium,* in which only the 3′ intron is present (see fig. 8). Some time later, the 5′ intron would have been acquired by the ancestor to *Dunaliella,* and while *D. parva* has maintained both introns, *D. salina* would have lost the 3′ intron. Loss of the 3′ intron from the 18S genes of *Characium vacuolatum* and *Ettlia minuta* must be invoked to accommodate the tree topology depicted in figure 8. This scenario also predicts that the cognate introns should show greater similarity within pairs than between pairs (which is the case). We consider the most likely time at which the introns were inserted into the gene to be after the divergence of the two volvocalean taxa, as indicated in figure 8. This placement would obviate invoking the loss of 18S introns from *Chlamydomonas reinhardtii* and *V. carteri.* Sequences of the 18S rRNA gene of other volvocalean taxa, including *V. aureus, Eudorina elegans,* and six *Gonium* species, have shown these organisms to lack introns in this gene (authors' unpublished observation), suggesting that they may never have been present in this group.

The uncorrected genetic distance between the 5′ introns is ~24%, while that between the members of the 3′ pair is 32%. Corrected values (see Material and Methods) are 25% and 35%, respectively. If it is assumed, once again, that the introns were transmitted vertically, one would expect the distance between introns to be correlated with the distance between exons. The *Dunaliella* exons, which share the more similar (5′) introns, are indeed more similar than are those of *D. parva* and *Characium* (table 2). Comparing the distance values between the cognate introns with those for the exon sequences in which they are located, we find that the 5′ introns appear to be diverging at a rate nearly four times that of the 3′ introns (24%–25%/0.7% 5′ vs. 30%–35%/3.5% 3′). This apparent rate difference may be due, at least in part, to a relatively rapid mutational saturation of regions which are under weaker selection and which consequently would be expected to incur more multiple substitutions. Thus a fairly rapid rate would be expected to slow as saturation is approached.

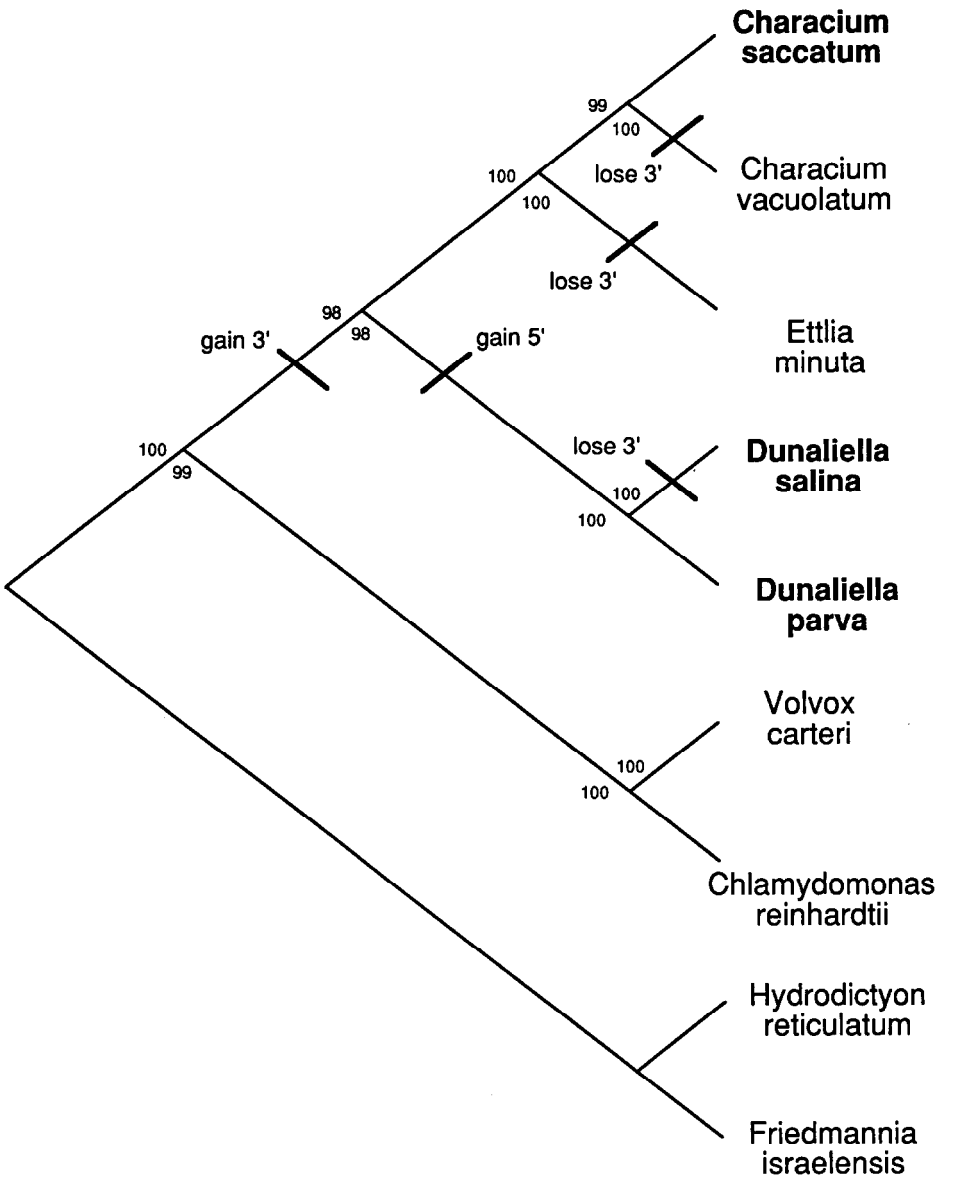



FIG. 8.—Phylogenetic tree inferred from distance (FITCH), neighbor-joining (NEIGHBOR), and parsimony analyses. Numbers refer to parsimony bootstrap values. Those above the lines are from analysis with a data set having all characters weighted equally. Those below come from analysis in which positions exhibiting compensatory substitutions were down-weighted. Lengths of most-parsimonious trees and their consistency index (CI) values were as follows: when all characters were equally weighted, length was 282 and CI was 0.851; and, when sites involved in base pairing and showing compensatory substitution were down-weighted as described in Material and Methods, length was 671 and CI was 0.769.

Pairwise comparisons of a member of either cognate pair of introns with a member of the other pair yield distance values markedly greater than the above figures, generally at least 40% (data not shown). Nonetheless, preliminary analyses of these and other

group I introns by using either various amounts of "core" sequence or only the conserved primary-sequence elements (P, Q, R, and S) suggest that the 5′ and 3′ introns are relatively closely related.

Although not conclusive, all of the available information is consistent with the possibility that the *Dunaliella* and *Characium* introns have been transmitted through descent. To account for the occurrence of the 5′ introns in *D. parva* and *D. salina,* a single step (an intron gain) is required for vertical transmission, while two steps would need to be invoked for horizontal transmission. In the case of the 3′ introns, however, horizontal transmission would require two steps (two gains), compared with four (one gain and three losses) for vertical transmission (see fig. 8). If the 3′ introns were transmitted horizontally, there would seem to be two possible explanations for the degree of sequence divergence which they exhibit: In one explanation, an ancestral form of the intron would have to be acquired independently by the two organisms or would have to be transferred from one to the other shortly after they diverged. In either case, the majority of the sequence divergence would have likely occurred while the introns were in their present locations. In the other explanation, one (or both) of the introns would have to have been acquired fairly recently. In this case, either a recent transfer from one organism to the other or the independent acquisition of an identical intron would not appear to allow enough time to account for the sequence divergence observed. Therefore, for *Characium saccatum* and/or *D. parva* to have acquired its 3′ 18S rDNA intron recently, this/these intron(s) apparently would have had to have been in the form of an "already diverged" intron which had been maintained elsewhere.

The likelihood of horizontal transmission depends on how mobile these introns actually are (i.e., how long they might be expected to remain in a given site and how easily they might reinsert themselves back into the same site within an intronless 18S rRNA gene). The question of whether sequence-specific "homing sites" exist for these introns is a separate issue (although examination of flanking sequences provides no strong indication that such sites do exist). Even if the introns were at some time inserted into homing sites via horizontal transfer, introns which can remain immobile for a relatively long period of time would be expected to be transmitted by evolutionary descent.

We would suggest that the "steps" of intron gain and loss may not be equally probable and that the acquisition of these 18S rRNA group I introns is less frequent than their loss. If such a bias is assumed to exist, a single transposition event within the genome, per intron (i.e., 5′ and 3′), can best account for the distribution of the introns in these algae.

Following the discovery of the *D. parva* introns and then of the *D. salina* intron, we felt that we might eventually discover similar introns in related algae. This was the case in *Characium saccatum.* We have recently determined that three additional *Dunaliella* species possess nuclear SSU rRNA introns, two having only 5′ introns and one having both 5′ and 3′ introns. Further analysis of these additional intron-containing 18S rRNA genes is underway. As data are obtained on these additional *Dunaliella* introns, it is hoped that additional light will be shed on this story.

## Sequence Availability

Complete 18S rDNA sequences, with introns, have been deposited in GenBank. Accession numbers are as follows: *Characium saccatum,* M84319; *Dunaliella parva,* M62998; and *Dunaliella salina,* M84320.

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