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Nuclear DNA Content Estimates in Green Algal Lineages: Chlorophyta and Streptophyta

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• *Background and Aims* Consensus higher-level molecular phylogenies present a compelling case that an ancient divergence separates eukaryotic green algae into two major monophyletic lineages, Chlorophyta and Streptophyta, and a residuum of green algae, which have been referred to prasinophytes or micromonadophytes. Nuclear DNA content estimates have been published for less than 1% of the described green algal members of Chlorophyta, which includes multicellular green marine algae and freshwater flagellates (e.g. *Chlamydomonas* and *Volvox*). The present investigation summarizes the state of our knowledge and adds substantially to our database of C-values, especially for the streptophyte charophycean lineage which is the sister group of the land plants. A recent list of 2C nuclear DNA contents for isolates and species of green algae is expanded by 72 to 157.
• *Methods* The DNA-localizing fluorochrome DAPI (4′,6-diamidino-2-phenylindole) and red blood cell (chicken erythrocytes) standard were used to estimate 2C values with static microspectrophotometry.
• *Key Results* In Chlorophyta, including Chlorophyceae, Prasinophyceae, Trebouxiophyceae and Ulvophyceae, 2C DNA estimates range from 0·01 to 5·8 pg. Nuclear DNA content variation trends are noted and discussed for specific problematic taxon pairs, including Ulotrichales–Ulvales, and Cladophorales–Siphonocladales. For Streptophyta, 2C nuclear DNA contents range from 0·2 to 6·4 pg, excluding the highly polyploid Charales and Desmidiales, which have genome sizes of up to 14·8 and 46·8 pg, respectively. Nuclear DNA content data for Streptophyta superimposed on a contemporary molecular phylogeny indicate that early diverging lineages, including some members of Chlorokybales, Coleochaetales and Klebsormidiales, have genomes as small as 0·1–0·5 pg. It is proposed that the streptophyte ancestral nuclear genome common to both the charophyte and the embryophyte lineages can be characterized as $1C = 0{\cdot}2$ pg and $1n = 6$.
• *Conclusions* These data will help pre-screen candidate species for the on-going construction of bacterial artificial chromosome nuclear genome libraries for land plant ancestors. Data for the prasinophyte *Mesostigma* are of particular interest as this alga reportedly most closely resembles the 'ancestral green flagellate'. Both mechanistic and ecological processes are discussed that could have produced the observed C-value increase of >100-fold in the charophyte green algae whereas the ancestral genome was conserved in the embryophytes.

Key words: 'Ancestral green flagellate' (AGF), C-value enigma, chlorophyta, DNA C-values, nuclear genome size, Streptophyta.

INTRODUCTION

Chlorophyta include the eukaryotic green algae, which possess chlorophyll *a* and *b* and starch stored inside plastids with stacks of 2–6 thylakoids per band (Bold and Wynne, 1985). Approximately 425 genera and 6500 species have been described (Alexopoulos and Bold, 1967). Simplicity and antiquity of green algae (Chlorophyta) have long been accepted as evidence of their apparent ancestry to land plants (McCourt, 1995). Phylogenetic analyses of molecular markers including 18S rRNA and *rbcL* (Bhattacharya *et al*., 1994; McCourt *et al*., 1995, 1996; Huss and Kranz, 1997; Katana *et al*., 2001) and morphological data (Mattox and Stewart, 1984) contradict this view and present a compelling case that an ancient and deep divergence separates green plants into two major monophyletic lineages: Chlorophyta and Streptophyta (Turmel *et al*., 1999; Karol *et al*., 2001) (Fig. 1).

A third polyphyletic green plant lineage, branching at the base of the Chlorophyta–Streptophyta divergence, includes a residuum of related unicellular micromonadophytes (=prasinophytes) (Kantz *et al*., 1990; Steinkötter *et al*., 1994; Karol *et al*., 2001) (Fig. 1). Although the exact relationship of the prasinophytes to land plants remains unclear (Qiu and Palmer, 1999), it seems likely that a prasinophyte-like scaly flagellate was the common ancestor of both green plant divisions (Friedl, 1997). Nuclear genome size and organization remain largely unknown in the prasinophytes. A 2C genome size estimate of about 10 Mbp reported for *Ostreococcus tauri* (Prasinophyceae) is one of the smallest among free-living eukaryotic organisms (Courties *et al*., 1998; Derelle *et al*., 2002). It is assumed that this small genome is evolutionarily derived rather than ancestral (Courties *et al*., 1994) as other members of Mamiellaceae (Prasinophyceae) are reported to represent secondarily reduced forms (Daugbjerg *et al*., 1995). Previously, it was suggested that this apparent miniaturization of prasinophyte nuclear genomes may defeat attempts to use them as a model in reconstruction of an ancstral land plant nucleotype (Cunningham *et al*., 1998; Oakley and Cunningham, 2000). Consequently, the early branching groups in the charophycean lineage (Soltis *et al*., 1999), including Chlorokybales, Klebsormidiales and

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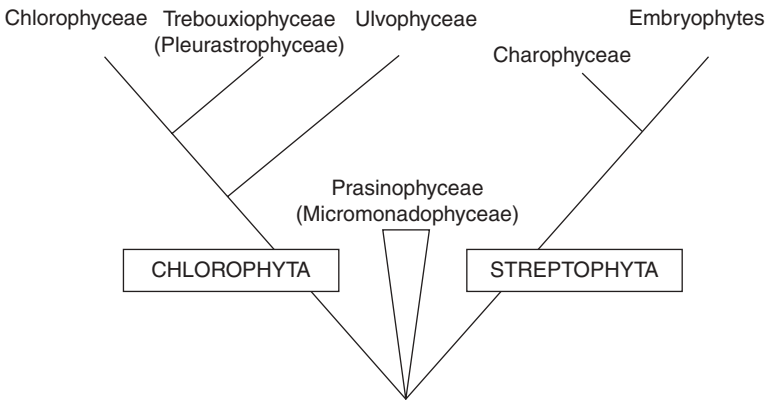

FIG. 1. Summary results of combined analysis using morphological, ultrastructural and large- and small-subunit rRNA gene sequences for chlorophyte and streptophyte algae (McCourt, 1995).

Coleochaetales, may provide the best opportunity for gaining these insights, but there are few published estimates of DNA contents in target members of these orders.

The Second Plant Genome Size workshop and Discussion Meeting [hosted by the Royal Botanic Gardens (RBG), Kew, 8–12 September, 2003] identified major gaps (systematic, regional and plant type) in our knowledge of plant DNA amounts (Bennett and Leitch, 2005*a*). It was noted that no database was available for algae. This major gap was addressed with a compilation of genome size estimates for 247 species of red, green and brown macroscopic algae (Kapraun, 2005). This report included nuclear DNA content estimates for 95 isolates and species of multicellular green marine algae, which are almost exlusively members of Ulvophyceae (Kapraun, 2005). These data are incorporated into a database of plant genome sizes (Kapraun *et al*., 2004) available online on the RBG Kew website (http://www.rbgkew.org.uk/cval/homepage.html). In addition, nuclear DNA content data for green algae, both from our continuing investigations and from the literature, are regularly updated at http://www.uncw.edu/people/kapraund/DNA (see links there to 'Table I. Chlorophyta' and 'Appendix I. Chlorophyta'). The present paper includes nuclear genome size estimates for 72 additional isolates and species of green algae. Of this total, 33 resulted from our ongoing research. Unicellular freshwater microalgae that were underrepresented or excluded previously (Kapraun, 2005) are emphasized here, especially members of Chlorophyceae and Trebouxiophyceae. A significant effort was made to expand our database for streptophyte algae, which are critical in efforts to reconstruct a hypothetical ancestral nuclear genome for the green algal ancestor of the land plants.

Inclusion of published nuclear DNA content data for green algae in the present report was sometimes problematic. The Second Plant Genome Size workshop and Discussion Meeting (Bennett *et al*., 2000; Bennett and Leitch, 2005*b*) identified 'best practice' methodology for nuclear genome size estimation in plant tissues. Virtually none of the published genome size data for algae resulted from investigations adhering to all of the best practice recommendations, primarily because measurement of the relatively small algal nuclear genomes requires standard species different from those specified as appropriate for vascular plants (Kapraun, 2005). Flow cytometry of oceanic picoeukaryotes and phytoplankton has resulted in nuclear DNA content estimates from whole cells for numerous algae including members of Chlorophyceae and Prasinophyceae (Simon *et al*., 1994; Veldhuis *et al*., 1997). In general, only estimates based on isolated nuclei were included in the present study. A comprehensive discussion of standard species and methods is included in the notes related to the Appendix at the end of this paper.

METHODS

Algal material was fixed in Carnoy's solution and stored in 70 % ethanol at 4 °C. Preserved material was rehydrated in water and softened in 5 % w/v EDTA (Goff and Coleman, 1990) for 30 min to 3 h. Specimens were transferred to cover slips treated with subbing solution, air dried and stained with DAPI (0·5 $\mu g\ mL^{-1}$ 4′,6-diamidino-2-phenylindole; Sigma Chemical Co.,' St. Louis, MO, USA) as previously described (Goff and Coleman, 1990; Kapraun and Nguyen, 1994). Detailed procedures for microspectrophotometry with DAPI and requirements for reproducible staining have been specified previously (Kapraun, 1994; Kapraun and Nguyen, 1994) using a protocol modified after Goff and Coleman (1990). Microspectrophotometric data for *Gallus* [chicken erythrocytes or red blood cells (RBC)] with a DNA content of 2·4 pg (Clowes *et al*., 1983) were used to quantify mean fluorescence intensity (I_f) values for algal specimens (Kapraun, 1994). DAPI binds by a non-intercalative mechanism to adenine/thymine-(A/T-) rich regions of DNA (Portugal and Waring, 1988). Consequently, RBC are best used as a standard for estimating amounts of DNA when the A/T contents of both standard and experimental DNA are equivalent (Coleman *et al*., 1981). *Gallus* has a nuclear DNA base composition of 42–43 mol% G+C (Marmur and Doty, 1962). Limited published data indicate similar mean values of 46 mol% for Chlorophyta (Sueoka, 1961; Olsen *et al*., 1987; Freshwater *et al*., 1990; Kooistra *et al*., 1992; Le Gall

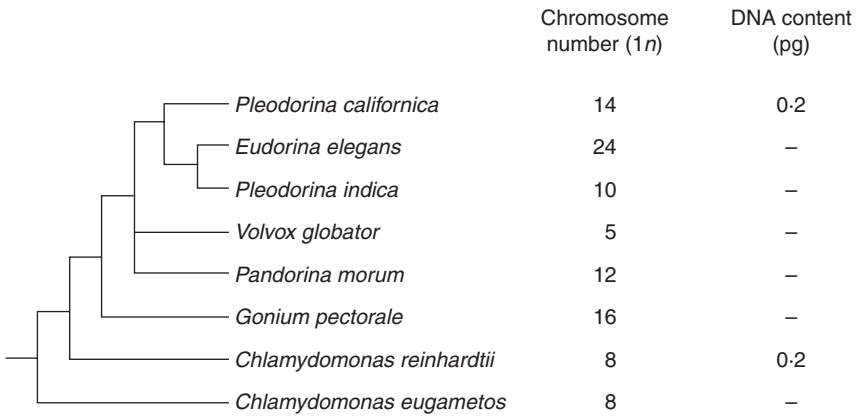

FIG. 2. Estimated 2C nuclear DNA contents and 2*n* chromosome complements (Sarma, 1982) superimposed on a phylogenetic tree for Volvocales based on multiple plastid gene sequences (Nozaki *et al.*, 1999, 2000).

et al., 1993; Simon *et al.*, 1994). Algae investigated in this study are assumed to have a similar range of base pair compositions, and linearity is accepted between DAPI–DNA binding in both RBC and algal samples (Le Gall *et al.*, 1993). Nuclear DNA contents were estimated by comparing the I_f values of the RBC standard and algal sample (Kapraun, 1994). Chlorophyta include taxa with some or all of their cells being multinucleate or endopolyploid (Kapraun and Nguyen, 1994) as well as taxa that exhibit a nuclear 'incremental size decrease associated with a cascading down of DNA contents' (Kapraun, 1994). Specific methodologies were developed for specimens to permit assignment of C level and interpretation of I_f data. Details of materials and methods, as well as information for collection locations, and data for numbers of algal nuclei examined in each sample and estimates of nuclear genome size (pg, ± s.d.) are available at http://www.uncw.edu/people/kapraund/DNA.

RESULTS

The present investigation adds nuclear DNA content estimates for 72 species and isolates of green algae to our database of C-values, emphasizing the streptophyte charophycean lineage that is the sister group of land plants. A previous list of 2C nuclear DNA contents in green algae (Kapraun, 2005) is expanded to 157. DNA content estimates, both from the present research and from published information, are presented as picograms (pg) and as megabase pairs (Mbp) (Appendix).

In Chlorophyta, including Chlorophyceae, Trebouxiophyceae, Ulvophyceae and Prasinophyceae (Figs 1–3), 2C DNA estimates range from 0·01 to 5·8 pg. Significant new data are included in this study (Appendix) for all of the major groups of Ulvophyceae (Fig. 4). In Ulotrichales (Fig. 5), nuclear DNA content data are available for seven species of this large and diverse order (Table 1 online, see Introduction) and suggest that it is characterized by small 2C values of 0·2–0·5 pg (Appendix). Estimates of nuclear DNA contents for species of Ulvales (Fig. 6) range from 2C = 0·14 to 1·1 pg (Appendix). In Trentepohliales, nuclear DNA content estimates of 2C = 1·1–4·1 pg appear to be correlated with a molecular phylogenetic tree derived from small-subunit rRNA gene sequence analysis (Fig. 7). Published 2C nuclear DNA content estimates range from 0·1 to 1·0 pg for most members of Caulerpales. Only species of *Halimeda* and *Codium* have substantially larger genomes of 1·0–6·1pg (Fig. 8). Previously published (Kapraun, 2005) and present nuclear DNA content estimates for members of Dasycladales indicate a range of 0·7–3·8 pg (Fig. 9). In the Cladophorales/Siphonocladales complex, nuclear DNA content estimates indicate that members of clade III have relatively small genomes (2C = 0·2–0·7 pg) whereas members of clade II, including the bulk of Siphonocladales, have much larger genomes of 2C = 2·0–5·7 pg (Fig. 10). Isolates of *Pithophora* spp. have genome sizes of 2C = 1·9 pg (UTEX 1333) and 4·1 pg (UTEX 787), respectively.

In Prasinophyceae (Appendix), nuclear genome size estimates of 0·2 and 0·7 pg were obtained for species of *Pyramimonas* and *Tetraselmis*, respectively (Appendix), suggesting that at least in these prasinophycean algae, nuclear genome sizes more closely approximate those reported in many other green algae (Kapraun, 2005).

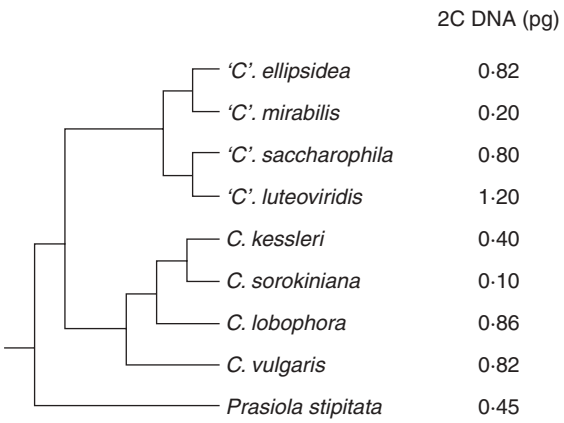

FIG. 3. Estimated 2C nuclear DNA contents superimposed on a phylogenetic tree for Trebouxiophyceae based on 18S rRNA gene sequence analyses (Friedl, 1995; Krienitz *et al.*, 2004).

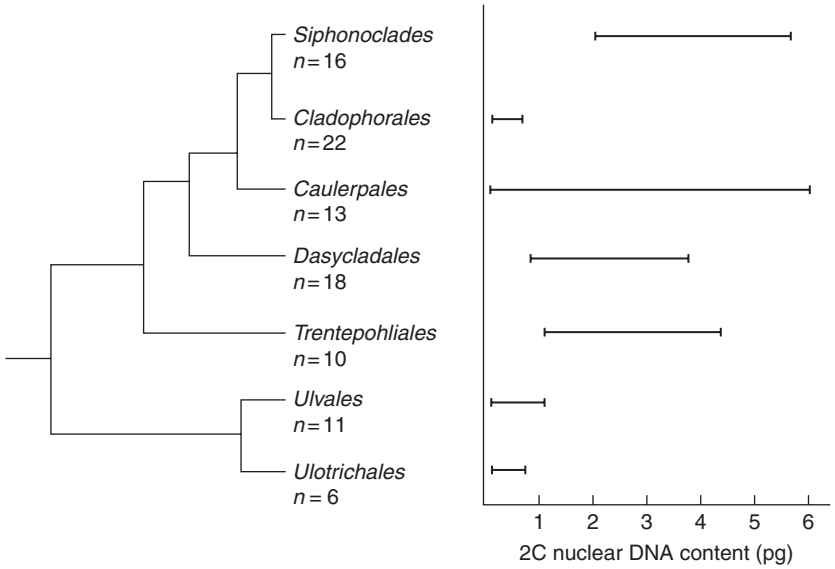

FIG. 4. Estimated 2C nuclear DNA contents superimposed on a cladogram of Ulvophyceae inferred from small-subunit rRNA gene sequence data (Zechman, 1990).

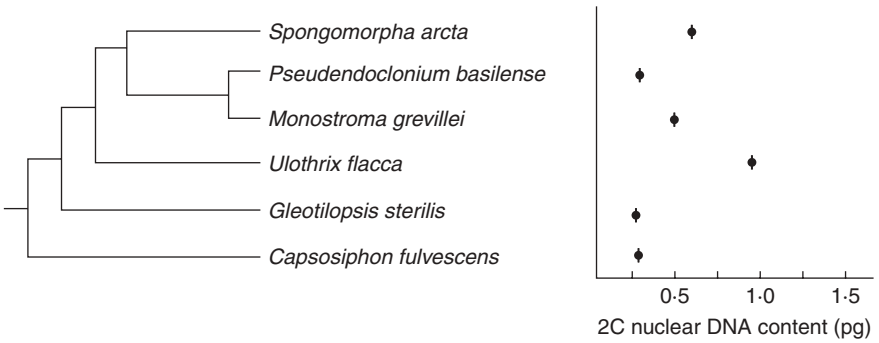

FIG. 5. Phylogenetic tree of Ulotrichales inferred from 18S rRNA and *rbcL* gene sequence analysis (Hayden and Waaland, 2002).

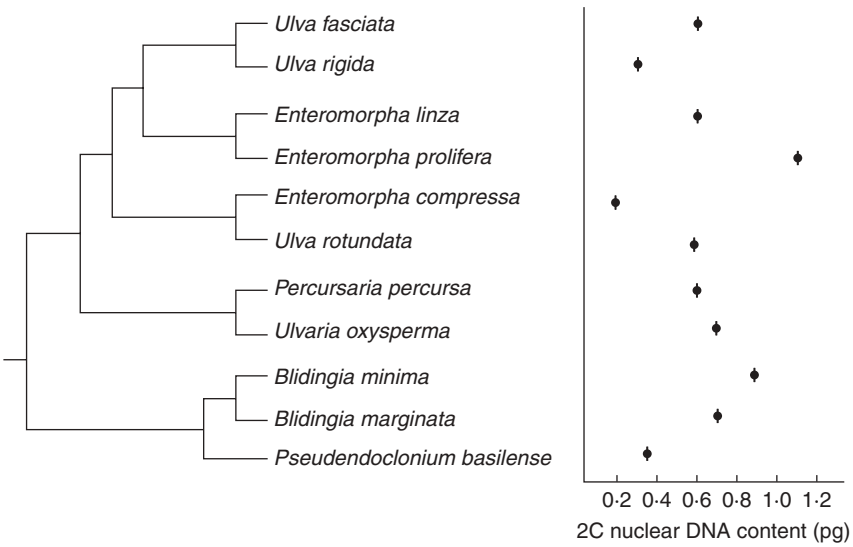

FIG. 6. Phylogenetic tree of the Ulvales inferred from 18S rRNA and *rbc*L gene sequence analysis (Hayden and Waaland, 2002).

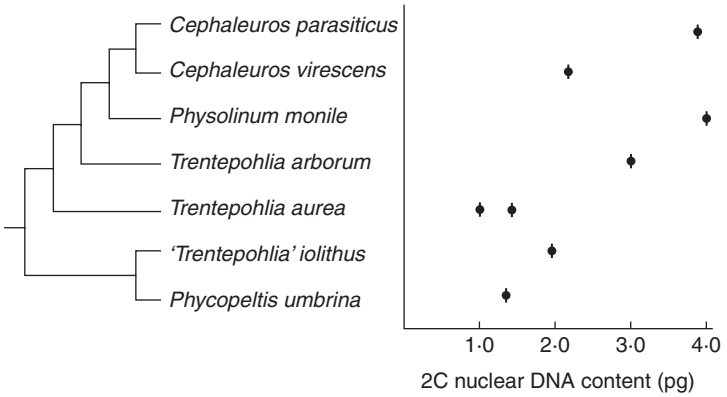

FIG. 7. Estimated 2C nuclear DNA content estimates superimposed on a cladogram of Trentepohliales inferred from small-subunit rRNA genes (López-Bautista and Chapman, 2003, 2005).

In Streptophyta, 2C nuclear DNA contents range from 0·2 to 6·4 pg, excluding the highly polyploid Charales and Desmidiales, which have genome sizes of up to 14·8 and 46·8 pg, respectively. Nuclear DNA content data for Streptophyta superimposed on a contemporary molecular phylogenetic tree indicate that early diverging lineages, including some members of Chlorokybales, Coleochaetales and Klebsormidiales, have genomes as small as 0·1–0·5 pg. In Mesostigmatales, a genome size of 0·74 pg was estimated for an isolate identified as representing *Mesostigama viride* (Appendix). In *Coleochaete* (Coleochaetales) isolates investigated, nuclear DNA content estimates of 1·4, 3·0 and 5·5 pg approximate a doubling sequence, consistent with values that would result from polyploidy. In the present study, a 2C nuclear genome size of 1·2 pg was estimated for *Chaetosphaeridium globosum* (Appendix). In Klebsormidiales, 2C nuclear genome sizes for *Klebsormidium flaccidum* and *Entransia fimbriata* were estimated to be 0·4 and 1·1 pg, respectively (Appendix). An isolate of *Klebsormidium nitens* from Argentina was found to have an estimated nuclear DNA content of 2C = 0·55 pg (Appendix) or 539 Mbp (using 1 pg = 980 Mbp, Bennett *et al*., 2000).

In the present study, nuclear DNA content estimates obtained with DAPI microspectrophotometry for five additional species extend the upward range for presumptive 2C nuclei to 46 pg. In a *Netrium digitus* isolate (UTEX 599), nuclei were too large to be accommodated by the photometer aperture system, but nuclear volume (NV) calculations, as described in the Appendix notes, resulted in a nuclear DNA content estimate of more than 125 pg. This is by far the largest nuclear genome size reported in any green alga (Kapraun, 2005). The present investigation extends the upward range for presumptive 2C nuclei to 7·2 pg in an isolate of *Mougeotia transeaui* (Fig. 11). The 2C nuclear DNA contents in Charales range from 14·0 to 39·2 pg (Fig. 11).

DISCUSSION

Chlorophyta

Chlorophyta contain the classical 'green algae', primarily Chlorophyceae, Trebouxiophyceae (=Pleurastrophyceae) and Ulvophyceae (Mishler *et al*., 1994; Watanabe *et al*., 2001) (Fig. 1). All members of this clade have swimming cells with two or four anterior flagellae arising from basal

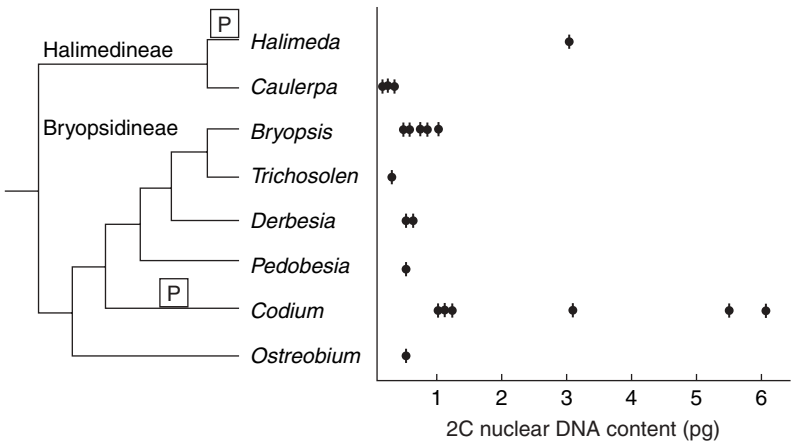

FIG. 8. Estimated 2C nuclear DNA contents superimposed on a phylogenetic tree for Caulerpales based on cladistical analyses (Vroom *et al*., 1998; Woolcott *et al*., 2000). Proposed polyploidy events are indicated by [P].

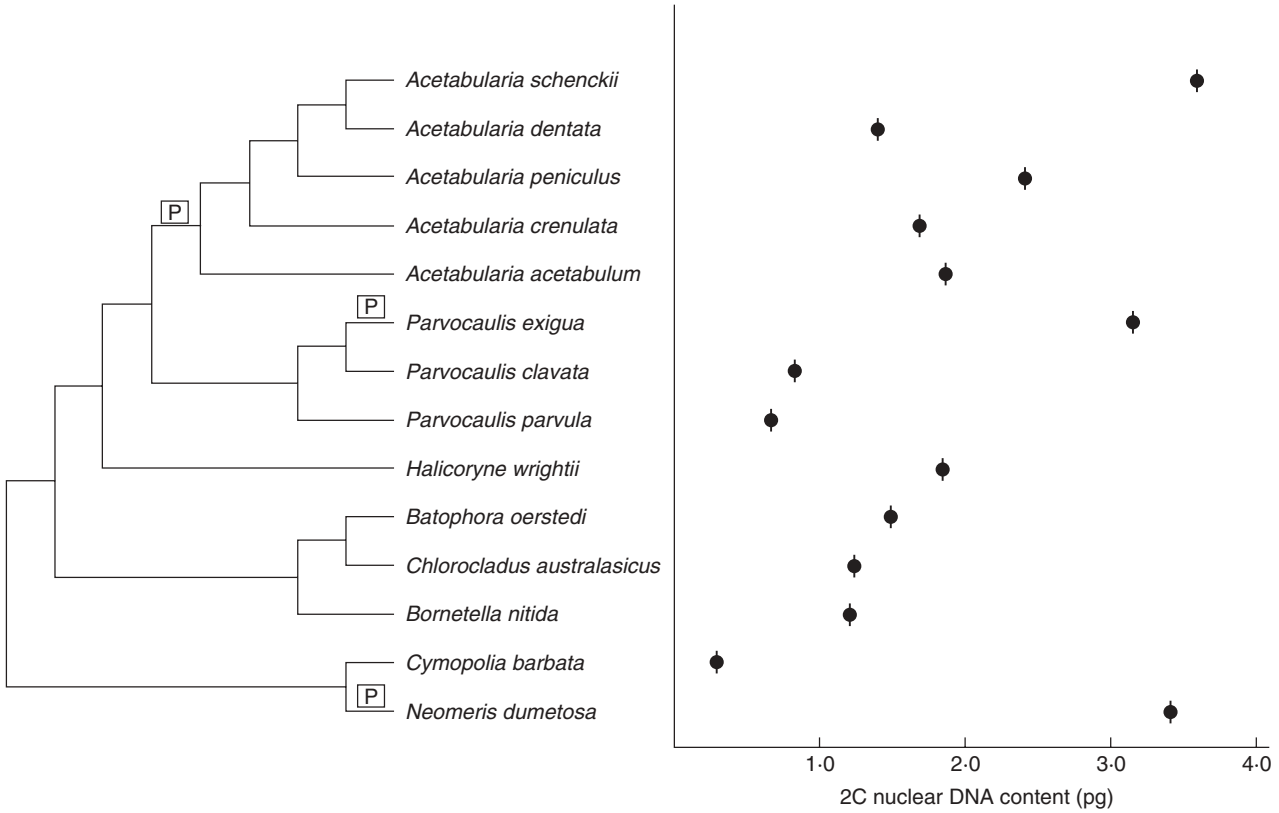

FIG. 9. Estimated 2C nuclear DNA contents superimposed on a phylogenetic tree for Dasycladales based on *rbcL* gene sequence analyses (Zechman, 2003; Berger *et al*., 2003). Proposed polyploidy events are indicated by [P].

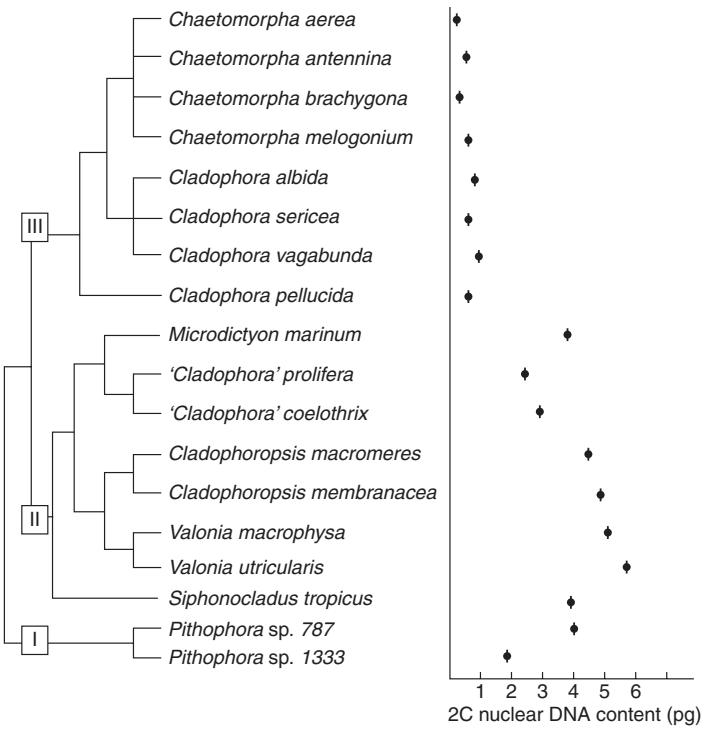

FIG. 10. Estimated 2C nuclear DNA contents superimposed on a phylogenetic tree for the Cladophorales/Siphonocladales complex based on 18S rRNA gene sequence analysis (Hanyuda *et al*., 2002).

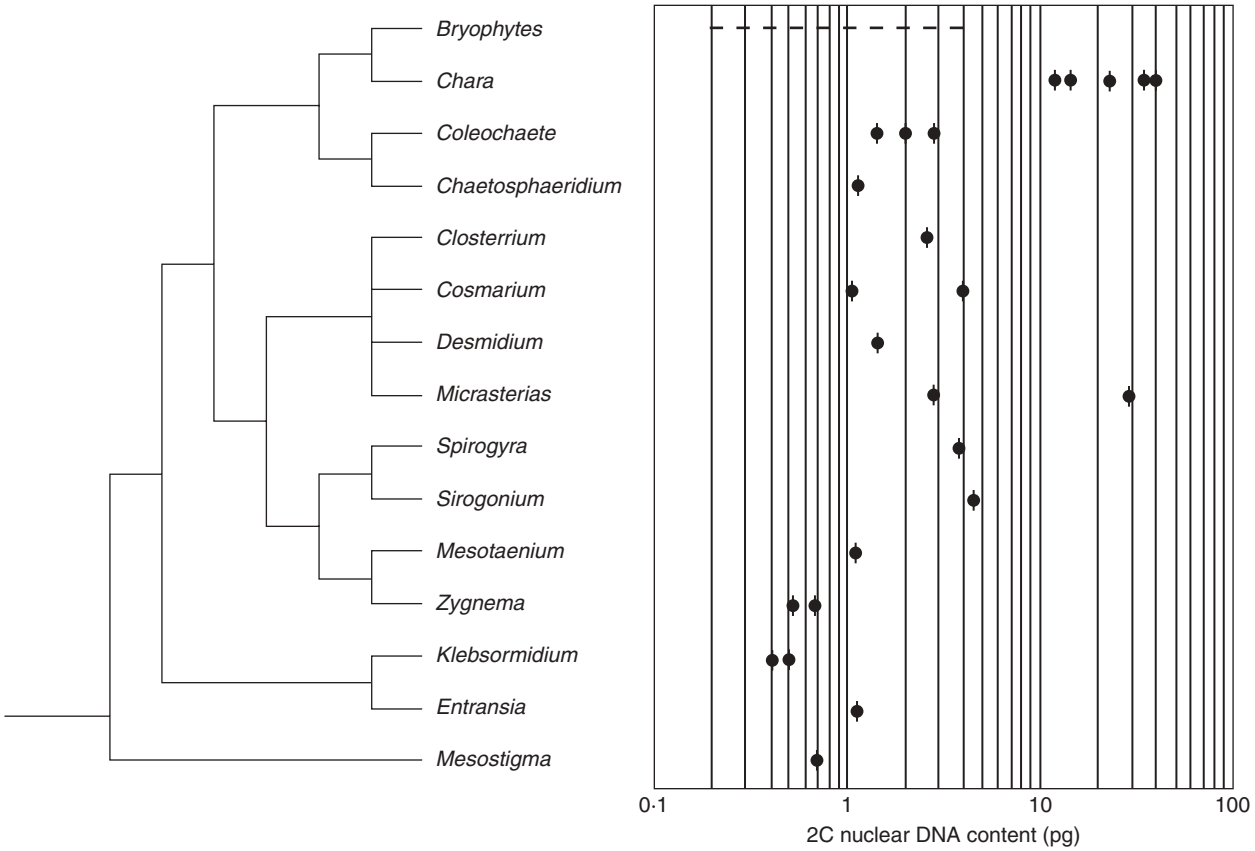

FIG. 11. Ranges of 2C nuclear DNA contents superimposed on a consensus molecular phylogenetic tree for the streptophyte green algae and bryophytes based on sequence analyses (Bhattacharya *et al*., 1994; McCourt *et al*., 2000; Denboh *et al*., 2001; Karol *et al*., 2001; Cimino and Delwiche, 2002; Delwiche *et al*., 2002; Turmel *et al*., 2002*c*; Gontcharov *et al*., 2003). DNA data for streptophyte algae are from Kapraun (2005) and the Appendix, and for the bryophytes from Renzaglia *et al*. (1995) and Voglmayr (2000).

bodies which are arranged cruciately (O'Kelly and Floyd, 1984). Combined analysis of morphology and molecular data strongly supports the monophyly of these three groups (Mishler *et al*., 1994) and the phylogenetic topology followed below (Krienitz *et al*., 2004).

Chlorophycean algae. Chlorophyceae apparently arose during the later stages of green algal evolution and are not an early branching lineage (Watanabe *et al*., 2001). This group includes a predominance of freshwater taxa such as Sphaeropleales (= Chlorococcales; e.g. *Scenedesmus*), and many of the familiar flagellates such as *Volvox* and *Chlamydomonas* (Volvocales). Recently, molecular techniques were used to re-examine phylogenetic relationships in Volvocales (Buchheim *et al*., 1996, 1997, 2002) as previously deduced by morphological and gene sequence data (Nozaki *et al*., 1995; Angeler *et al*., 1999). *Volvox* has been considered to be the limit of the colonial development in the volvocine series, in which *Gonium* and unicellular forms such as *Chlamydomonas* are early branching lineages (Larson *et al*., 1992). Although much of the evolution in the colonial Volvocales superficially appears to constitute a gradual progression in colonial complexity and in types of sexual reproduction, as in the traditional volvocine lineage hypothesis, reverse evolution must be considered for the origin of certain species of *Pleodorina* (Nozaki *et al*., 2000). Apparently, both colonial and unicellular morphotypes have evolved independently in several clades (Nozaki *et al*., 1995, 1999; Nozaki and Krienitz, 2001). Thus, the traditional view of a unidirectional, monophyletic progression from unicellular, through colonial, to multicellular forms, represented by the sequence *Chlamydomonas*, *Gonium*, *Pandorina*, *Eudorina*, *Pleodorina* and *Volvox*, is not supported by gene sequence analyses (Larson *et al*., 1992; Lewis and McCourt, 2004).

In Chlorophyceae, published DNA content estimates for Sphaeropleales (= Chlorococcales), a widely distributed and much investigated group of algae (Buchheim *et al*., 2005; McManus and Lewis, 2005), are limited to an early study which cited *Scenedesmus obliquus* as having a nuclear genome size of 0·4 pg (Charles, 1977). Few nuclear DNA content estimates have been published for Volvocales (Fig. 2). The pioneering investigation of Holm-Hansen (1969) using 'fluorometric measurement' estimated 0·6 pg for *Dunaliella tertiolecta*. Flow cytometry of whole cells gave an estimate of 0·015 pg for this alga (Veldhuis *et al*., 1997). DNA content estimates of 0·01–0·2 pg are given in the Appendix for three additional species of volvocine green algae.

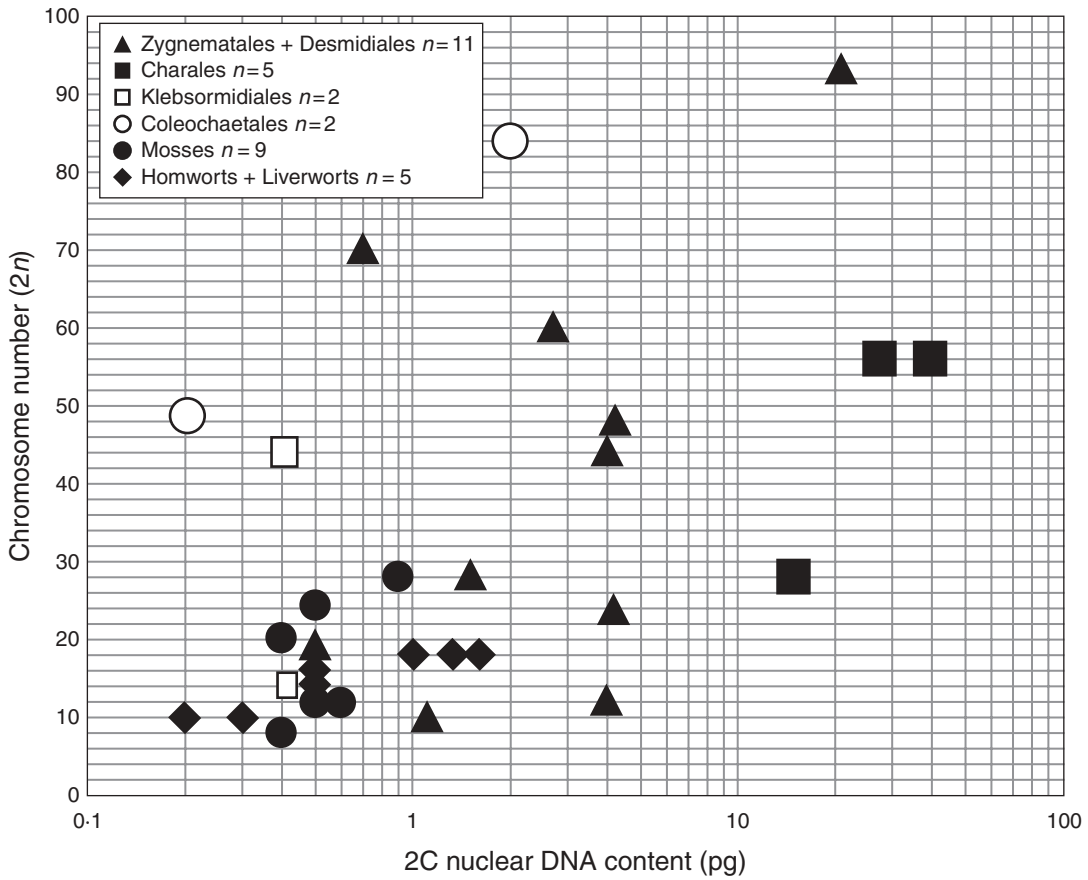

FIG. 12. Comparison of chromosome numbers and nuclear genome sizes in the charophycean and embryophyte lineages. Closed circles: DNA data for streptophyte algae from Kapraun (2005) and the Appendix, and for the bryophytes from Renzaglia *et al*. (1995) and Voglmayr (2000). Open circles: DNA data from Mandoli (2001).

Although relatively few nuclear DNA content estimates have been published for Volvocales (Fig. 2), chromosome numbers have been published for many species. These range from $2n = 8$ to 76, with the vast majority of reported numbers being between 16 and 24 (Sarma, 1982). In general, higher chromosome numbers are associated with colonial forms, and lower chromosome numbers with unicellular forms. Phylogenetic lines giving rise to *Volvox* include both low ($n = 5$) and high ($n = 22$) chromosome complements (Fig. 2). It would be a matter of great interest to augment the small amount of nucleotype data currently available for Volvocales with a more comprehensive investigation comparing nuclear genome size and chromosome numbers with speciation patterns (Schagerl *et al*., 1999; Coleman 2001). Specifically, do recognized volvocine morphotypes correlate with nucleotype parameters including genome size and chromosome number?

Trebouxiophycean algae. Trebouxiophyceae (=Pleurastrophyceae) includes those Chlorophyta with non-flagellate vegetative cells which are typically unicellular, sarcinoid or filamentous (Friedl, 1995; Henley *et al*., 2004; Krienitz *et al*., 2004; Ueno *et al*., 2005). Phylogenetic analysis of 18S rRNA gene sequences has demonstrated a monophyletic origin for these algae, and a sister group relationship to Chlorophyceae (Lewis, 1997; Handa *et al*., 2003; Lokhorst *et al*., 2004) (Fig. 3). Many of the better known members of this group exist in lichen associations, e.g. *Dictyochloropsis* and *Trebouxia* (Lewis and McCourt, 2004).

In Trebouxiophyceae, most nuclear DNA content estimates for Chlorellales (Fig. 3) resulted from an investigation using reassociation kinetics and were published as base pairs (Dörr and Huss, 1990). Pulse field gel electrophoresis (Higashiyama and Yamada, 1991), flow cytometry (Wilhelm *et al*., 1982; Veldhuis *et al*., 1997; Yamamoto *et al*., 2001) and microfluorometric analysis (Cattolico and Gibbs, 1975) have been used as well. The genus *Nannochloris*, and related genera, includes some of the smallest and ultrastructurally simplest phototrophic eukaryotes, with genomes as small as 12·6 Mbp (Arai *et al*., 1998; Yamamoto *et al*., 2001). Using 1 pg = 980 Mbp (after Bennett *et al*., 2000), 2C genome sizes (reported as Mbp) range from 0·02 pg in *Picochlorum atomus* (as *Nannochloris atomus*) (Veldhuis *et al*., 1997) to 1·06 pg in *Chlorella fusca* (Dörr and Huss, 1990).

Chlorellales. Recent molecular studies have resulted in the dispersal of species traditionally referred to *Chlorella* over two classes of chlorophytes, Trebouxiophyceae and Chlorophyceae (Huss *et al*., 1999; Katana *et al*., 2001; Henley *et al*., 2004; Krienitz *et al*., 2004). In the present

investigation, *Chlorella* species included in the Appendix are considered to be trebouxiophycean algae (Huss *et al*., 1999). Phylogenetic analysis of some '*Nannochloris*-like' algae resulted in their transfer to other genera, including *Marvania* and *Picochlorum* (Henley *et al*., 2004).

Prasiolales. Recent molecular data support transfer of *Prasiola*, formerly in the Ulvophyceae, to Trebouxiophyceae (Sherwood *et al*., 2000; Friedl and O'Kelly, 2002; Naw and Hara, 2002). This development is particularly notable as *Prasiola*, and the closely related *Rosenvingiella* (Rindi *et al*., 2004), often develop morphotypes which mimic *Enteromorpha* and *Ulva* (Ulvales). In the present study, the nuclear genome size estimate of 0·9 pg for *Prasiola stipitata* is assumed to represent the 4C value as blades in this species are reported to be diploid (Cole and Akintobi, 1963). Nuclear DNA content estimates range from 2C = 0·1 to 1·2 pg and indicate no apparent correlation between genome size and phylogeny (Fig. 3).

Ulvophycean algae. Ulvophyceae are primarily marine species, most with larger and more complex morphologies than typically found in Chlorophyceae. Molecular data support a model for Ulvophyceae *sensu* Mattox and Stewart (1984) with two separate lineages: a clade including Ulothrichales and Ulvales (Hayden and Waaland, 2002; O'Kelly *et al*., 2004) and a clade with Caulerpales, Cladophorales/Siphonocladales complex, Dasycladales and Trentepohliales (Zechman *et al*., 1990; Hanyuda *et al*., 2002) (Fig. 4). In the present report, presentation of orders reflects a consensus of contemporary phylogenetic treatments (Zechman *et al*., 1990).

Ulotrichales. Ulotrichales as presently delimited (Floyd and O'Kelly, 1990) have been expanded to include Acrosiphoniaciae (*sensu* Kornmann and Sahling, 1977; Sussmann *et al*., 1999). Molecular analyses using 18S rDNA and 18S rRNA gene sequence data have confirmed this placement (Watanabe *et al*., 2001; Lindstrom and Hanic, 2005). Species of *Capsosiphon* and *Monostroma*, included in Ulvales by Bliding (1963, 1968), appear to be more closely related to Ulotrichales (Fig. 5). Nuclear DNA content data, available for seven species of this large and diverse order (table 1 online), suggest that it is characterized by small 2C values of 0·2–0·5 pg (Appendix). No correlation between nuclear genome size and phylogenetic position is apparent (Fig. 5). The relatively small genome sizes reported for species in this order are complemented by relatively small chromosome numbers of $2n = 8-24$ (Kapraun, 1993).

Ulvales. The emended order Ulvales (O'Kelly *et al*. 2004) is monophyletic (Fig. 6), but circumscription of several genera, including the familiar *Ulva* and *Enteromorpha* (Hayden and Waaland, 2002, 2004; Hiraoka *et al*., 2003) and *Blidingia* (Lindstrom and Golden, 2006), remains problematic. In both *Ulva* and *Enteromorpha*, blade and tubular thallus morphotypes apparently arose independently several times throughout the evolutionary diversification of the group (Blomster *et al*., 1998, 1999; Tan *et al*., 1999; Shimada *et al*., 2003). Although results from molecular and culture studies suggest that *Ulva*, *Enteromorpha* and *Chloropelta* should not be recognized as separate genera (Hayden *et al*., 2003; Matsuo *et al*., 2003, 2005), these familiar taxonomic epithets are retained here for convenience (Appendix). The absence of a correlation between nuclear genome size and chromosome number in these species (Kapraun and Bailey, 1992) suggests a significant role of aneuploidy in their evolution (Kapraun, 2005).

Trentepohliales. Molecular investigations place members of this order with the second lineage of Ulvophyceae (Mishler *et al*., 1994; Chapman *et al*., 1995; Lopez-Bautista and Chapman, 2003), which are otherwise almost exclusively marine. Nuclear DNA content estimates of 2C = 1·1–4·1 pg appear to be correlated with a molecular phylogenetic tree derived from small subunit rRNA gene sequence analysis (Fig. 7). Large-scale discontinuous variation in both reported chromosome complements ($n = 4-28$) (Appendix) and nuclear DNA contents are indicative of polyploidy in this order (Lopez-Bautista *et al*., 2000).

Caulerpales. Cladistic analysis of morphological and molecular data supports separation of Caulerpales (Codiales *sensu* Taylor, 1960) into two clades: (1) the homoplastidic Bryopsidineae, which are generally characterized by diplobiontic life histories and non-holocarpic production of gametes (e.g. *Bryopsis* and *Codium*); and (2) the heteroplastidic Halimedineae, which are generally characterized by haplobiontic and diploid life histories and holocarpic production of gametes (e.g. *Caulerpa* and *Halimeda*) (Zechman *et al*., 1990; Vroom *et al*., 1998; Woolcott *et al*., 2000). Published 2C nuclear DNA content estimates range from 0·1 to 1·0 pg for most of these algae. Only species of *Halimeda* and *Codium* have substantially larger genomes (1·0–6·1pg; Fig. 8). As these two genera are placed in separate clades, it appears that large-scale whole genome increase (polyploidy) arose independently at least twice in the evolution of Caulerpales (Fig. 8).

Dasycladales. Recent molecular investigations based on analyses of *rbcL* (Zechman, 2003) and 18S rRNA (Berger *et al*., 2003) gene sequence data revealed three well-supported clades for which morphological synapomorphies exist, but which are not completely in accordance with previous generic concepts. A *Polyphysa* clade is distinguished from *Acetabularia* on the basis of cap morphotype and initiation (Berger and Kaever, 1992; Sawitzky *et al*., 1998). The range of nuclear DNA content estimates for members of Dasycladales (0·7–3·8 pg) (Fig. 9), which suggests large-scale discontinuous variation in all major clades, almost certainly reflects multiple polyploidy events (Kapraun and Buratti, 1998; Kapraun, 2005). It seems noteworthy that similarly large values (approx. 3·2–3·8 pg) were found in the early diverging *Neomeris* clade as well as in the highly derived *Parvocaulis* (=*Polyphysa*) and *Acetabularia* clades. No correlation is apparent between cap morphotypes (Sawitzky *et al*., 1998), cap morphogenesis (Kratz *et al*., 1998) and 'polyploid' nucleotypes in Dasycladales.

The Cladophorales/Siphonocladales complex. Cladophorales and Siphonocladales are a related lineage sharing a gradation of 'architectural' morphological types (Olsen-Stojkovich *et al*., 1986; Van den Hoek *et al*., 1988; Bakker *et al*., 1994). Contemporary molecular studies (Zechman *et al*., 1990; Hanyuda *et al*., 2002) have identified three well-supported clades in this complex: (I) an early diverging assemblage of mostly freshwater species of cladophoracean genera, including *Aegagropila*, *Arnoldiella*, *Pithophora* and *Wittrockiella*; (II) species belonging primarily to Siphonocladales *sensu* Børgesen (1913); and (III) the most derived assemblage including species belonging to the cladophoracean genera *Chaetomorpha*, *Cladophora and Rhizoclonium* (Fig. 10). Confusingly, the characteristic morphologies associated with the genera *Chaetomorpha*, *Cladophora* and *Rhizoclonium* appear to have evolved several times, independently, in all three clades. Thus, these genera as presently circumscribed are clearly polyphyletic.

Karyological studies indicate that species in the most highly derived cladophoracean clade III (*Rhizoclonium*, *Chaetomorpha* and *Cladophora sensu stricto*), without exception, share a unique constellation of karyotype features including: (1) six basic chromosomes, three of which have median and the other three have submedian centromeres; and (2) almost universal polyploidy, resulting in chromosome complements in most species of $x = 12$, 18, 24, 30, 36, etc. (Wik-Sjöstedt, 1970; Kapraun and Gargiuolo, 1987*a*, *b*; Miyaji, 1999). Species in the siphonocladacean clade II have (1) various combinations of both metacentric and acrocentric chromosomes (Kapraun and Breden, 1988; Bodenbender and Schnetter, 1990; Kapraun and Nguyen, 1994), and (2) chromosome complements consistent with an aneuploid origin: $1n = 8$, 12, 14, 16, 18 and 20 (Kapraun, 1993; Kapraun and Nguyen, 1994). Karyological data for basal clade I taxa appear to be limited to species of *Pithophora*, which have chromosome complements of 18, 24, 30 and 36 (Noor, 1968; Verma, 1979; Sarma, 1982) and relatively long chromosomes, up to 3 μm (Godward, 1966). Nuclear DNA content estimates indicate that members of clade III have relatively small genomes (2C = 0·2–0·7 pg) whereas members of clade II, including the bulk of Siphonocladales, have much larger genomes of 2C = 2·0–5·7 pg (Fig. 10). Isolates of *Pithophora* spp., representative of clade I, the earliest diverging among Cladophorales/Siphonocladales investigated, have genome sizes of 2C = 1·9 pg (UTEX 1333) and 4·1 pg (UTEX 787). These values almost certainly represent elevated DNA contents in a polyploid sequence, approximating to 2, 4, 8 and 16 pg (Appendix). As it was beyond the scope of the present investigation to determine chromosome numbers for these isolates, the exact ploidy level remains unknown.

Although the cladophoracean morphotype is reported to have evolved independently in all of the clades, the combination of karyotype pattern ($n = 6$) and nuclear genome size (2C = 0·2–0·7 pg) characteristic of the core clade III of Cladophorales appears to be unique and diagnostic, and may represent synapomorphies (Kapraun, 2005). If it is assumed that small genome size and symmetric karyotype with $1n=6$ is the ancestral condition in the Cladophorales/Siphonocladales complex, then these features have been most faithfully retained in the core cladophoracean algae in clade III. Clade II Siphonocladales, with a genome size increase of approximately an order of magnitude, and specialization of the karyotype by unequal reciprocal translocations (Kapraun and Breden, 1988), appear to be highly derived. Because nuclear volume is strongly correlated with cell size and cell cycle lengths in higher plants (Shuter *et al*., 1983) it is not surprising that these algae with their large, multinucleate cells and relatively long cell generation times have relatively large genomes (Kapraun and Nguyen, 1994). Clade I taxa (e.g. *Pithophora*) appear to have retained the ancestral karyotype, but not the theoretical small genome size. Their substantial increase in nuclear genome size, probably through whole genome duplication or polyploidy, may reflect their reliance on asexual reproduction by multinucleate propagules or akinetes (O'Neal and Lembi, 1983).

Prasinophycean algae

The prasinophytes or micromonads are primarily marine green flagellates. Some of the more commonly recognized taxa include *Halosphaera*, *Pyramimonas* and *Tetraselmis.* They generally have a single plastid, and often possess the accessory pigment prasinoxanthin. The cell membrane of most forms is covered with one or more layers of scales. There are no unique and defining characteristics for the group other than the above constellation of features (Sym and Pienaar, 1993; Graham and Wilcox, 2000). Prasinophytes have been characterized as the form of cell most closely representing the first green alga, or 'ancestral green flagellate' (AGF; Lewis and McCourt, 2004) (Fig. 1). Molecular analyses of 18S rRNA gene sequence data have identified at least seven separate lineages that form a grade at the base of the green algal tree of life (Steinkötter *et al*., 1994; Fawley *et al*., 2000; Zignone *et al*., 2002). Mamiellales include some of the smallest eukaryotes known, e.g. *Crustomastix* (Lewis and McCourt, 2004). There is some evidence that these algae represent secondarily reduced forms (Daugbjerg *et al*., 1995).

Nuclear genome size and organization remain largely unknown in Prasinophyceae (Appendix). Pulse field gel electrophoresis was used to obtain 2C nuclear genome size estimates for *Ostreococcus tauri* of 10·2 Mbp (Courties *et al*., 1998) and 9·7 Mbp (Derelle *et al*., 2002), or about one-quarter of the value reported for *Chlorella* (Higashiyama and Yamada, 1991). Using 1 pg = 980 Mbp (Bennett *et al*., 2000), *Ostreococcus* has a 2C genome size of about 0·01 pg. Flow cytometry analysis of isolated nuclei resulted in genome size estimates for *Micromonas pusilla* of 0·027 pg DNA per cell (Veldhuis *et al*., 1997) and 0·03 pg DNA per cell (Simon *et al*., 1994) and for *Bathycoccus prasinos* of 0·02 pg DNA per cell (Simon *et al*., 1994). These values were published as femtograms (10^{-15}), but are expressed as picograms (10^{-12}) here for consistency. Similar small genome size estimates based on whole cell flow cytometry measurements published for several additional prasinophyte taxa (Simon *et al*., 1994;

Veldhuis *et al*., 1997) are not included in the Appendix as whole cell staining induces some non-specific background fluorescence, resulting in DNA content overestimates, typically by a factor of 3 or less (Veldhuis *et al*., 1997).

Streptophyta

Streptophyta (Bremer *et al*., 1987) include the charophycean lineage along with bryophytes and tracheophytes (Mishler *et al*., 1994; Turmel *et al*., 2002*a*, *b*). Numerous ultrastructural and molecular synapomorphies support this charophyte clade (Lewis and McCourt, 2004). However, the topology of the charophyte tree remains elusive (Friedl, 1997; Delwiche *et al*., 2002). Identification of the charophycean lineage as the sister group of land plants (Manhart and Palmer, 1990) suggests that their common ancestor was a branched, filamentous organism (Cook, 2004) with a haplontic life cycle and oogamous reproduction (Karol *et al*., 2001).

Charophycean algae. The charophycean lineage (Fig. 11) includes Chlorokybales (Qiu and Palmer, 1999), Klebsormidiales (Karol *et al*., 2001), Conjugophyta (Desmidiales and Zygnematales) (Hoshaw *et al*., 1990; McCourt *et al*., 2000; Denboh *et al*., 2001), Coleochaetales (Bhattacharya *et al*., 1994; McCourt, 1995; Cimino and Delwiche, 2002) and Charales (Surek *et al*., 1994; McCourt *et al*., 1996). The precise relationship of Mesostigmatales to the charophycean lineage remains unclear (Delwiche *et al*., 2002). The most important characters that define charophycean algae are the unilateral flagellar root in zooids and open mitosis with a persistent telophase spindle (Mattox and Stewart, 1984). In the present report, presentation of orders reflects a consensus of contemporary phylogenetic treatments (Delwiche *et al*., 2002; Lewis and McCourt, 2004).

Mesostigmatales. For many years the question of the nature of the AGF, the hypothetical ancestor of all land plants, has been controversially discussed (O'Kelly, 1992). Some researchers associate the AGF with a polyphyletic green plant lineage at the base of the split of Chlorophyta and Streptophyta (Fig. 1), which includes the green alga *Mesostigma viride* (Turmel *et al*., 2002*a*). The exact placement of *Mesostigma* remains controversial as some phylogenetic analyses include this species with Prasinophyceae (Lemieux *et al*., 2000; Turmel *et al*., 2002*b*), whereas others consider it to be 'basal' in the charophycean lineage (Palmer *et al*., 2004). Whatever the exact position of *Mesostigma*, there is no doubt that this alga belongs to a deeply diverging lineage given that it represents the first branch in trees inferred from sequences of land plants and all five orders of charophytes (Karol *et al*., 2001). A significant body of research centred around *Mesostigma* is emerging that provides insights into the timing of events that restructured both mitochondrial (mtDNA) and plastid DNA genomes during the evolution of green algae (Turmel *et al*., 2002*b*) and the transition from charophytes to land plants (Turmel *et al*., 2002*a*). *Mesostigma* plastid DNA is highly similar in size (118–360 bp) and gene organization to the plastid DNAs of land plants (Lemieux *et al*., 2000). Apparently plastid gene loss is an ongoing process in streptophytes, with independent losses occurring in multiple lineages. By contrast, *Mesostigma* mtDNA differs greatly at the levels of size (42–424 bp), gene organization and intron content from the bryophytes and land plants sequenced to date. During the evolutionary transition from *Mesostigma* to the liverwort *Marchantia*, mtDNA underwent a four-fold increase in size, was rearranged extensively and gained many introns while maintaining a similar gene content (Turmel *et al*., 2002*b*).

In the present study, a genome size of 0·74 pg was estimated for an isolate identified as *Mesostigama viride* (Appendix). In previous investigations of nuclear genome sizes in green algae, data from microspectrophotometry were corroborated with genome size estimates derived from NV data (Kapraun and Nguyen, 1994). Assuming a plant NV of 15 $\mu m^3 = 1{\cdot}0$ pg (Sparrow and Nauman, 1973; Kapraun *et al*., 1988), the NV of 12·6 μm^3 calculated for *Mesostigma* is equivalent to a nuclear genome size of 0·8 pg, which closely approximates the present estimates.

Chlorokybales. This order is characterized by thalli of sarcinoid packets of cells that grow subaerially (Rogers *et al*., 1980). *Chlorokybus atmosphyticus*, one of the earliest diverging members of Charophyceae (Karol *et al*., 2001; Delwiche *et al*., 2002) (Fig. 11), has a plastid DNA sequence of 149–681 bp that closely resembles the *Mesostigma* plastid DNA in showing a high degree of putatively ancestral features (Turmel *et al*., 2002*b*). Unfortunately, no nuclear DNA content estimates have been published for any member of this important order.

Coleochaetales. Published chromosome numbers for three *Coleochaete* species (Sarma, 1982) include 1*n* complements of 24, 36 and 42, which almost certainly represent a polyploid sequence derived from a basic complement of $x = 12$. It was beyond the scope of the present study to determine chromosome numbers for the *Coleochaete* isolates investigated. However, the nuclear DNA content estimates of 1·4, 3·0 and 5·5 pg, which approximate a doubling sequence, are consistent with values that would be found in a polyploid sequence.

Molecular evidence includes *Chaetosphaeridium globosum* in Coleochaetales (Karol *et al*., 2001). In the present study, a 2C nuclear genome size of 1·2 pg was estimated for this species using microspectrophotometry (Appendix) and 1·23 pg using NV calculations. In *Chaetosphaeridium*, as in *Coleochaete*, the ploidy level of the isolates used in this study was not confirmed with chromosome counts.

Klebsormidiales. This order includes species of *Klebsormidium* and *Entransia*, which have simple, unbranched filaments with parietal laminate or lobed plastids (Lewis and McCourt, 2004). In the present study, 2C nuclear genome sizes for *Klebsormidium flaccidum* and *Entransia fimbriata* were estimated to be 0·4 and 1·1 pg, respectively (Appendix). An isolate of *Klebsormidium nitens* from Argentina, with a reported chromosome complement of $n = 6$ (Sánchez-Puerta and Leonardi, 2001), was investigated in the present study and found to have a

nuclear DNA content of 2C = 0·55 pg (Appendix) or 539 Mbp. Thus, this species may have most nearly retained the proposed small ancestral nucleotype among extant streptophyte algae sampled.

Desmidiales and Zygnematales. Both the true desmids and the filamentous Zygnemataceae have undergone explosive speciation, resulting in thousands of described species (Prescott *et al*., 1972, 1977, 1981; Hoshaw and McCourt, 1988) from every continent. These algae represent the most species-rich group of charophytes with approximately 4000 species described (Gerrath, 2003). Two morphological synapomorphies unite the group: (1) the complete absence of flagellae in any life history stage, and (2) sexual reproduction by conjugation (McCourt *et al*., 2000). Molecular sequence data analyses confirm that the placoderm desmids are monophyletic, and constitute a group separate from the 'false desmids' and filamentous forms (McCourt *et al*., 2000; Gontcharov *et al*., 2003). Apparently, more complex forms evolved from simple filaments, and morphological switching from unicells to filaments occurred several times (Lewis and McCourt, 2004) (Fig. 11). Recent analysis of *rbcL* gene sequence data supports monophyly of *Spirogyra* and *Sirogonium*, two of the largest genera of filamentous Zygnemataceae (Drummond *et al*., 2005). The relationship of Desmidiales and Zygnematales to other streptophyte algae remains uncertain. Some molecular studies imply a close relationship between land plants (bryophytes) and conjugating green algae (Turmel *et al*., 2002*a*, *b*) whereas others suggest a more distant, sister group relationship (McCourt *et al*., 2000; Karol *et al*., 2001; Cimino and Delwiche, 2002; Delwiche *et al*., 2002).

Previous published nuclear DNA content estimates for the true desmids (Desmidiales) range from 2C = 1·1 to 20·7 pg (Hamada *et al*., 1985; Kapraun, 2005). Desmidiales are characterized by extensive polyploidy, with both inter- and intraspecific variation in chromosome complements reported (Hoshaw and McCourt, 1988). For example, published chromosome complements for *Netrium digitus* range from 1*n* = approx. 30 to approx. 592 (Sarma, 1982). Consequently, assignment of a C-value to specific DNA content estimates is arbitrary as chromosome counts were not made in this study for any of the isolates used in DNA quantification. In a previous investigation of Desmidiales (Kapraun, 2005), chromosome complements and nuclear DNA contents were found to be highly correlated ($r^2 = 0{\cdot}7897$), providing circumstantial evidence for the pervasive role of polyploidy in the evolution of this group of charophycean algae.

Previously published 2C nuclear DNA contents in Zygnematales ranged from 0·5 to 4·2 pg (Kapraun, 2005). As in Desmidiales, assignment of a C-value to specific DNA content estimates for isolates of Zygnematales is arbitrary. In general, both chromosome numbers (Sarma, 1982) and nuclear DNA contents (Fig. 11) are smaller in Zygnematales than in Desmidiales. Ploidy level in conjugating green algae may be of taxonomic significance as cell dimensions are considered to be diagnostic (Hoshaw and McCourt, 1988), and cell dimensions are highly correlated with genome size (Wang *et al*., 2005). Although the nucleotype role in gene expression remains poorly understood (Gregory, 2001, 2005*a*, *b*), it seems plausible that some of the described taxa of both desmid and filamentous conjugating green algae represent ploidy races, especially in species-rich genera characterized by a large variation in chromosome complements. It is widely assumed that many desmid genera are probably artificial and often display transitional forms to other genera. A recent molecular investigation of the desmid *Staurastrum* concluded that (1) the taxonomic significance of some morphological characters has been greatly overestimated, and (2) phylogenetic relationships were in conflict with previous formal and informal classification schemes (Gontcharov and Melkonian, 2005).

Charales. The exact relationship of the charophytes to land plants continues to be a subject of investigation. Several recent multigene phylogenies place Charales as the sister taxon to land plants (Karol *et al*., 2001; Cimino and Delwiche, 2002; Delwiche *et al.*, 2002). Charales, commonly known as stoneworts or brittleworts, flourish in fresh and brackish water habitats throughout the world (Bold and Wynne, 1985). Charophytes are prone to calcification and have left an abundant fossil record to the Cretaceous and perhaps beyond (Grambast, 1974; Feist *et al*., 2003). The order is well circumscribed and includes six extant genera (McCourt *et al*., 1996; Sakayama *et al*., 2005), remnants of a once diverse but now largely extinct group (Feist *et al*., 2003). Analysis of 18S rRNA gene sequences indicated a poor correlation between molecular phylogeny and traditional hypotheses based on morphological criteria (Meiers *et al*., 1999). The base chromosome number for *Chara* is *n*=7 and for *Nitella* is *n* = 3. However, many species exhibit polyploidy, with chromosome complements to *n* = 70 reported (Sarma 1982). Published nuclotype data are limited to five species of *Chara* (Maszewski and Kołodziejczyk, 1991; Kunachowicz *et al*., 2001) with 2C nuclear DNA contents ranging from 14·0 to 39·2 pg (Fig. 11). Two of these species, with 2*n* = 28, have 2C DNA contents of about 14 pg. Cytophotometric measurements of DNA content revealed differences in 1C DNA content between female (7·0 pg) and male (7·4 pg) isolates of *Chara tomentosa* (Kunachowicz *et al*., 2001).

SUMMARY AND CONCLUSIONS

DNA C-value remains a key character in biology, biodiversity and molecular investigations as genome size has many important practical implications (Bennett *et al*., 2000). In general, genome size directly influences the cost and difficulty of sequencing projects, and is therefore a primary consideration in choosing future sequencing subjects (Gregory, 2005*a*, *b*). Species with large DNA amounts can make use of standard fingerprinting techniques including AFLP problematic (Fay *et al*., 2005). Low DNA content (genomes approx. 100 Mpb) has been a major criterion in the selection of algae for genomic and genetic analyses (Peters *et al*., 2004; Waaland *et al*.,

2004), including bacterial artificial chromosome (BAC) cloning technology, used for large-scale physical mapping and genomic sequencing (Wang *et al*., 2005). To date, candidate macroalgal (multicellular) species have genomes in the range 127–300 Mpb (Waaland *et al*., 2004). The present study can provide a great service both by expanding the list of target green algal species with appropriately small genome sizes, and by cautioning against use of taxa that may meet many other criteria for genomics investigations (Waaland *et al*., 2004), but have genome sizes too large for available technologies.

Recently, construction and characterization of large-insert BAC libraries has shown promise in the study of how important features in land plants originated and diversified (Liang *et al*., 2004). Unfortunately, the draft genome sequence available for the green alga *Chlamydomonas reinhardtii* (Wang *et al*., 2005) is of limited value in attempts to identify conserved ancestral genes because of the great phylogenetic distance between this chlorophyte and the streptophytes. The present study has indentified several streptophycean algae, including *Klebsormidium* and *Coleochaete*, with genome sizes that make them comparably tractable for genomic investigations.

The availability of a DNA C-values database and a consensus higher-level phylogenetic tree for green algae has opened the way for determining evolutionary trends in DNA amounts. Identification of the chlorophytes, streptophytes and prasinophytes as three of the most important extant green algal lineages provides an opportunity to suggest DNA content transformations which have accompanied their evolution, and to call attention to processes which may be unique or diagnostic for each group. Specific trends observed in this study are summarized below.

Summary for Ulvophyceae

Nuclear DNA content data from previously published investigations (Kapraun, 2005) and present research indicate that clade I of Ulvophyceae (Ulotrichales and Ulvales; O'Kelly *et al*., 2004) is characterized by relatively small nuclear genome sizes, whereas clade II (Caulerpales, Dasycladales, Cladophorales/Siphonocladales and Trentepohliales; Hanyuda *et al*., 2002) is characterized by substantially larger nuclear genome sizes (Fig. 4). It has been suggested that these differences in genome size ranges reflect the results of aneuploidy vs. polyploidy in clades I and II, respectively (Kapraun, 2005).

Summary for the charophycean lineage

The charophycean green algae are a sister group to land plants (Mishler *et al*., 1994; McCourt, 1995; McCourt *et al*., 2000; Lewis and McCourt, 2004). Ultrastructural data are overwhelmingly supported by the analyses of molecular data from nuclear ribosomal repeat units, mainly the small (18S) subunit, but including 5S and large-subunit (25S) rDNA sequences. Plastid genes including *rbcL* and small- and large-subunit rRNA yield similar results (Lewis and McCourt, 2004). Bryophytes are thought to comprise a grade of three monophyletic lineages (mosses, liverworts and hornworts) of uncertain relationship to each other (Shaw and Renzaglia, 2004) and to vascular plants (Palmer *et al*., 2004; Groth-Malonek and Knoop, 2005). Although the topology of the charophyte tree remains elusive (Friedl, 1997; Delwiche *et al*., 2002), comparison of the nucleotypes of charophycean green algae with those of early branching land plants, including bryophytes (Kenrick and Crane, 1997), could provide useful insights into the reconstruction of a common ancestral (generalized) nuclear genome (Pryer *et al*., 2002).

Hornworts, liverworts and mosses, in general, have nucleotypes characterized by small chromosomes (Inoue and Uchino, 1969) and chromosome complements of $2n \geq 30$ and/or 2C nuclear DNA contents >1 pg (Renzaglia *et al*., 1995; Voglmayr, 2000) (Fig. 12). Although greater values for both parameters are known in bryophytes, they appear to be restricted to polyploid species and do not contradict the generalization. For example, more than 80 % of the nuclear DNA C-values in mosses were reported to occur in a narrow peak between 0·25 and 0·6 pg (Voglmayr, 2000). It has been suggested that the small DNA amounts and low C-value variation are linked to the biflagellate nature of bryophyte sperm cells (Renzaglia *et al*., 1995). As nuclear genome size and sperm cell size are tightly correlated (Murray, 2005), and sperm cells are thought to lose motility drastically with increasing size, a strong selection pressure against larger sperm, and therefore also against larger DNA amounts, is hypothesized (Voglmayr, 2000).

These observations gain additional significance in the context of the suggestion that the common ancestor of all angiosperms may have possessed a small genome (Leitch *et al*., 1998, 2005). Small genome size in angiosperms appears to be correlated with phenotypic characteristics such as rapid seedling establishment, short minimum generation times, reduced cost of reproduction and an increased reproductive rate (Bennett, 1987; Midgley and Bond, 1991; Bennett and Leitch, 2005*a*, *b*). Consequently, small genome size may permit greater evolutionary flexibility (Leitch *et al*., 1998), whereas larger size and amplification may lead to 'genomic obesity' (Bennetzen and Kellogg, 1997; Bennetzen, 2002; Gregory, 2005*a*; Knight *et al*., 2005). It is recognized that although nuclear genome size is highly correlated with many cellular and ecological parameters, 'correlation' and 'causation' are far from interchangeable (Gregory, 2005*a*, *b*). The many complex causal factors behind these observations remain obscure.

Published molecular studies have implicated both the conjugating green algae (Turmel *et al*., 2002*a*, *b*) and the charophytes (Karol *et al*., 2001; Cimino and Delwiche, 2002; Delwiche *et al*., 2002) as the closest sister group to land plants. Consequently, it is worth noting that published nucleotype data for these charophycean algae suggest that they share a unique constellation of karyotype features that differ substantially from the most closely related bryophytes. Many taxa in Charales, Desmidiales and Zygnematales are highly polyploid (Sarma, 1982; Hoshaw

and McCourt, 1988; Kunachowicz *et al*., 2001) and can be characterized either by chromosome complements of $2n > 30$ or 2C nuclear DNA contents >1 pg, or both (Fig. 12). In addition, both filamentous and unicellular (desmid) forms of the conjugating green algae have chromosomes with an 'absence of localized centromeres' (Godward, 1966) or 'polycentric chromosomes' (King, 1960; Sarma, 1982; Hoshaw and McCourt, 1988). Karyotype analyses also indicate an extraordinary range in chromosome lengths, from 1 to 20 μm (King, 1960). Both of these green algal groups typically have asymmetric (specialized) karyotypes and large chromosomes up to 12 μm long (Sarma, 1982; Hoshaw and McCourt, 1988; Kunachowicz *et al*., 2001), suggesting a structural distinction with land plant karyotypes.

In contrast to the conjugating green algae and charophytes, some extant members of other groups of streptophytes, including Coleochaetales and Klebsormidiales, have an unspecialized nucleotype with both a small genome size and a small chromosome complement. In the present investigation, nuclear DNA content estimates of <1 pg were found in several isolates of *Coleochaete*, *Entransia* and *Klebsormidium* (Appendix). Unfortunately, published karyotype data are unavailable for many of these taxa. Comparison of haploid genome (1*n* chromosome complement and 2C DNA content) data for these streptophytes suggests that some species may have retained the proposed small ancestral genome size. For example, the chromosome complement of $n = 6$ reported for *Klebsormidium nitens* (Sánchez-Puerta and Leonardi, 2001) with an estimated nuclear DNA content of $2C = 0{\cdot}6$ pg (Appendix) is particularly exciting as it closely approximates both the small genome size and chromosome complement (Fig. 12) reported in the bryophytes (Kapraun, 2005).

Did the relatively large nuclear genomes found in many extant charophycean algae reflect independent, sequential polyploidy events that conferred an advantage associated with increased genome size in the absence of specific upward size constraints? Specifically, large genome size and the correlated large cell size could have been advantageous in buoyant aquatic environments. In addition, in an ancient atmosphere with low levels of oxygen and UV-absorbing ozone, highly polyploid, redundant genomes may have conferred significant survival value. However, as atmospheric conditions changed, their relatively large genomes and large cell sizes, specialized for aquatic habitats, rendered them unsuitable contenders for the colonization of land (Graham, 1993).

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APPENDIX

Notes on chromosome numbers and nuclear DNA content estimates in species of green algae

(a) Taxa are listed alphabetically.

(b) Most chromosome numbers have been published as haploid ($1n$) values for the Chlorophyta (Kapraun, 1993), and $2n$ values given here are extrapolated from $1n$ numbers (and ranges of probable $1n$ numbers).

(c) Most DNA amounts in the literature are given in picograms (pg). Unless otherwise indicated, Mbp values here are derived from estimates for 2C or 4C values using the expression 1 pg = 985 Mbp (Cavalier-Smith, 1985; Bennett *et al*., 2000). These DNA content values should be considered accurate only to 0·1 pg (Kapraun, 2005). Values for some prasinophycean green algae (Simon *et al*., 1994) were published as femtograms (10^{-15}), but are expressed as picograms (10^{-12}) here for consistency. DNA amounts originally published as base pairs (Mbp = megabase pairs or mbp = million base pairs) are indicated with a dagger (†) whereas value published as pg are indicated with an asterisk (*). Most of these base pair values were derived from reassociation kinetics (Bot *et al*., 1989*a*, *b*, 1990, 1991; Kooistra *et al*., 1992; Olsen *et al*., 1987), but LeGall *et al.* (1993) used ethidium bromide with RBC standard and flow cytometry. Additional base pair values were determined from pulsed field gel electrophoresis (e.g. Higashiyama and Yamada, 1991; Courties *et al*., 1998) and flow cytometry (Yamamoto *et al*., 2001).

(d) Algal life histories typically are characterized by an alternation of haploid gametophyte and diploid sporophyte generations (Kapraun, 1993). Thus, DNA content (pg) measurements could be based on either or both 2C replicated haploid ($1n$) nuclei or 4C replicated diploid ($2n$) nuclei. In practice, most published DNA content (pg) values are for 2C diploid nuclei and most 1C and 4C values are extrapolated. Here, the original published DNA content (pg) value for each species is indicated with an asterisk (*). In some samples, ploidy level could not be determined with certainty and assignment of DNA content to specific C-level is speculative.

(e) Previously unpublished data are indicated by an asterisk (*).

(f) Standard species. Species used as a calibration standard for algal research are listed in table 1 (online at http://people.uncw.edu/kapraund/DNA/Chlorophyta.htm).
The vast majority of nuclear DNA estimates for algae have used chicken red blood cells or erythrocytes (RBC) for a DNA standard with 2·4 pg being a generally accepted value for the 4C DNA content of *Gallus gallus* (Clowes *et al*., 1983; Riechmann *et al*., 2000). Mouse (*Mus*) sperm was used as a standard by Hamada *et al*. (1985), the fish *Betta splendens* was used as a standard by Spring *et al*. (1978) and *Allium cepa* was used by Maszewski and Kołodziejczyk (1991). The green alga *Chara tomentosa* was used as a standard by Kunachowicz *et al*. (2001). Initial investigations in our laboratory utilized a standard line based on the fluorescence intensity of an alga with a known DNA content and an angiosperm: *Antirrhinum majus* L. (e.g. Kapraun & Shipley, 1990; Hinson & Kapraun, 1992; Kapraun & Bailey, 1992) or *Impatiens balsamina* L. (e.g. Kapraun & Shipley, 1990). *Saccharomyces cerivisiae* (yeast) was used as a standard in investigations of the Chlorellales by Yamamoto *et al*. (2001) who assumed a DNA content of 13·4 Mbp for *Saccharomyces*. It should be noted that Kumar and Snyder (2001) calculates the nuclear genome of *Saccharomyces* to be 12 Mbp.

(g) Methods. Some of the earliest estimates of nuclear genome size in green algae used microfluorometric analysis (MFA) (Cattolico and Gibbs, 1975). Most contemporary research utilizes flow cytometry (FC) (LeGall *et al*., 1993) and microspectrophotometry (MI) (Kapraun, 1994; Kapraun & Buratti, 1998), which have been shown to be reliable methods for quantification of nuclear DNA contents in green algae. Feulgen microdensitometry (FE) was used by Maszewski and Kołodziejczyk (1991). Reassociation kinetics (RK) has been used successfully as well (Bot *et al*., 1989*a*, *b*, 1990, 1991; Dörr and Huss, 1990; Kooistra *et al*., 1992; Olsen *et al*., 1987). Pulse-field gel electrophoresis (PFGE) or electrophoretic karyotyping has been used successfully with *Chlorella* (Higashiyama and Yamada, 1991).

In the present study, selected nuclear genome size estimates derived from I_f data were corroborated using nuclear volume (NV) estimates, assuming a plant nuclear volume of 15 $\mu m^3 = 1{\cdot}0$ pg (Sparrow and Nauman, 1973). Detailed methodology for estimating nuclear genome size from NV data in green algae using the expression $NV = (\pi d^3)/6$, where d = cell diameter (Kapraun *et al*., 1988) has been published previously (Kapraun and Nguyen, 1994).

Several DNA-localizing fluorochromes have been used in published investigations. DAPI (4′,6-diamidino-2-phenylindole) is certainly the most popular, especially in recent studies (Kapraun, 1994; Kapraun & Buratti, 1998). Hydroethidine (H) (Kapraun & Bailey, 1992), ethidium bromide (EB) (Le Gall *et al*., 1993; Kunachowicz *et al*., 2001) and propidium iodide (PI) (Spring *et al*., 1978) were used in selected green algal investigations.

A key to the references cited for chromosome complements and DNA values in Chlorophyta appears below the table.

APPENDIX

Chromosome number and nuclear DNA content in chlorophycean and charophycean algae

Entry number	Species[a]	2*n*[b]	Original ref. for 2*n*	DNA amount 1C (Mbp)[c]	1C (pg)[d]	2C (pg)[d]	4C (pg)[d]	Original ref. for C-value[e]	Standard species[f]	Method[g]
	Charophycean Green Algae									
	CHARALES									
	Characeae									
1a	*Chara tomentosa* Linnaeus (male)	28	19	7252	7·4*	14·8	29·6	19	*Chara*	MI:EB
1b	*Chara tomentsoa* Linnaeus(female)	28	19	6860	7·0*	14·0	28·0	19	*Chara*	MI:EB
	COLEOCHAETALES									
	Coleochaetaceae									
2	*Chaetosphaeridium globosum* (Nordstedt) Klebahn			588	0·6	1·2*	2·4	*	*Gallus*	MI:DAPI
3	*Coleochaete nitellarum* Jost	84	22	343	0·35	0·7	1·4*	*	*Gallus*	MI:DAPI
4	*Coleochaete orbicularis* Pringsheim	48	22	686	0·7	1·5	3·0*	*	*Gallus*	MI:DAPI
5	*Coleochaete scutata* Brébisson			1287	1·3	2·7	5·5*	*	*Gallus*	MI:DAPI
	DESMIDIALES[1]									
	Desmidiaceae									
6	*Cosmocladium perissum* Roy *et* Bisset			15288	15·6	31·2*	62·4	*	*Gallus*	MI:DAPI
7	*Euastrum pectinatum* (Brébisson) *ex* Brébisson			22932	23·4	46·8*	93·6	*	*Gallus*	MI:DAPI
	Peniaceae									
8	*Gonatozygon monotaenium* de Bary	*c*. 34	18	8624	8·8	17·6*	35·2	*	*Gallus*	MI:DAPI
	KLEBSORMIDIALES									
9	*Entransia fimbriata* Hughes			539	0·55	1·1*	2·2	*	*Gallus*	MI:DAPI
10	*Klebsormidium flaccidum* (Kützing) P.C. Silva, K. Mattox *et* W. Blackwell	44	18	198	0·2	0·4*	0·8	*	*Gallus*	MI:DAPI
11	*Klebsormidium nitens* (Meneghini) Lokhorst	12	21	2695	0·28	0·55*	1·1	*	*Gallus*	MI:DAPI
	MESOSTIGMATALES									
	Mesostigmataceae									
12	*Mesostigma viride* Lauterborn			343	0·35	0·7*	1·4	*	*Gallus*	MI:DAPI

Continued

APPENDIX: *Continued*

Entry number	Species[a]	$2n$[b]	Original ref. for $2n$	DNA amount 1C (Mbp)[c]	1C (pg)[d]	2C (pg)[d]	4C (pg)[d]	Original ref. for C-value[e]	Standard species[f]	Method[g]
	ZYGNEMATALES									
	Mesotaeniaceae									
13	*Mesotaenia kramstae* Lemmermann			539	0·55	1·1*	2·2	*	*Gallus*	MI:DAPI
14	*Roya anglica* G. S. West			784	0·8	1·6*	3·2	*	*Gallus*	MI:DAPI
	Zygnemataceae									
15	*Mougeotia transeaui* Collins			3136	3·2	6·4*	12·8	*	*Gallus*	MI:DAPI
	Chlorophycean Green Algae									
	SPHAEROPLEALES									
	Scenedesmaceae									
17	*Scenedesmus obliquus* (Turpin) Kuetzing	12	22	196	0·2	0·40*	0·8	6		MFA
	VOLVOCALES[2]									
	Chlamydomonaceae									
18	*Brachiomonas sp.*			4·9	0·005	0·01*	0·02	26		FC
19	*Chlamydomonas reinhardtii* P. A. Dangeard	16	3	88	0·09	0·19*	0·38	4		
20	*Dunaliella tertiolecta* Butcher			294	0·3	0·6*	1·2	13		
	Volvocaceae									
21	*Pleodorina californica* Shaw (as *Eudorina californica*)	28	5	78	0·08	0·17*	0·34	25		
	Prasinophycean Green Algae									
	CHLORODENDRALES									
	Chlorodendraceae									
22	*Tetraselmis suecica* (Kylin) Butcher			343	0·35	0·7*	1·4	*	*Gallus*	MI:DAPI
	Halosphaeraceae									
23a	*Micromonas pusilla* (Butcher) Manton *et* Parke			15	0·015	0·03*	0·06	23		FC
23b	*Micromonas pusilla*			13	0·0135	0·027*	0·05	26		FC
	MAMIELLALES									
	Mamiellaceae									
24	*Bathycoccus prasinos* Eikrem *et* Throndsen			10	0·01	0·02	0·04	23		FC
25a	*Ostreococcus tauri* Courties *et* Chretiennot-Dinet	28	10	10·2†	0·005	0·01	0·02	9		PFGE
25b	*Ostreococcus tauri*			9·7†	0·005	0·01	0·02	10		PFGE
	PYRAMIMONADALES									
	Pyramimonadaceae									
26	*Pyramimonas parkeae* Norris *et* Pearson			67	0·07	0·15*	0·3	*	*Gallus*	MI:DAPI
	Trebouxiophycean Green Algae									
	CHLORELLALES[3]									
	Chlorellaceae									
27	**Chlorella ellipsoidea* Gerneck	18	12	400†	0·41	0·82	1·64	12		PFGE
28	*Chlorella fusca var. vacuolata* Shihira *et* Krauss			521†	0·53	1·06	1·12	11		RK
29	*Chlorella homosphaera* Skuja			418†	0·2	0·4	0·8	11		RK

Continued

APPENDIX: *Continued*

Entry number	Species[a]	2*n*[b]	Original ref. for 2*n*	DNA amount 1C (Mbp)[c]	1C (pg)[d]	2C (pg)[d]	4C (pg)[d]	Original ref. for C-value[e]	Standard species[f]	Method[g]
30a	*Chlorella kessleri* Fott *et* Nováková			196†	0·2	0·4	0·8	11		RK
30b	*Chlorella kessleri*			48†	0·05	0·1	0·2	28		FC
31	*Chlorella lobophora* Andreeva			426†	0·43	0·86	1·72	11		RK
32	**Chlorella luteoviridis* Chodat			593†	0·6	1·2	2·4	11		RK
33	**Chlorella minutissima* Fott *et* Novakova			126†	0·13	0·26	0·52	11		RK
34	**Chlorella mirabilis* Andreeva			98†	0·1	0·2*	0·4	11		RK
35	*Chlorella protothecoides* Krüger			195†	0·2	0·4	0·8	11		RK
36	**Chlorella saccharophila* var. *ellipsoidea* (Gerneck) Fott *et* Nováková			808†	0·8	1·6	3·2	11		RK
37	*Chlorella saccharophila var. saccharophila* (Krüger) Migula, Fott *et* Nováková			394†	0·4	0·8	1·6	11		RK
38a	*Chlorella sorokiana* Shihira *et* Krauss			49	0·05	0·11*	0·22	4		MFA
38b	*Chlorella sorokiana*			597†	0·61	1·2	2·4	11		RK
39a	*Chlorella vulgaris* M. Beijerinck	16	12	400†	0·41	0·82	1·64	12		
39b	*Chlorella vulgaris*			140†	0·14	0·28	0·56	11		RK
40	**Chlorella zofingiensis* Dönz			413†	0·4	0·8	1·6	11		RK
41	*Marvania coccoides* (Naumann) Henley *et al.* (= *Nannochloris coccoides* Naumann)			18†	0·02	0·04	0·08	28	yeast	FC
42a	*Nannochloris bacillaris* Naumann	14	2	98	0·1	0·2*	0·4	12		PFGE
42b	*Nannochloris bacillaris*			20†	0·02	0·04	0·08	28	yeast	FC
43	*Picochlorum atomus* (Butcher) Henley *et al.*									
	(as *Nannochloris atomus* Butcher)			9·8	0·01	0·02*	0·04	26		FC
	(as *Nannochloris atomus*)			47†	0·05	0·1	0·2	28	yeast	FC
44	*Picochlorum eukaryotum* (Wilhelm, Eisenbeis, Wild *et* Zahn) Henley *et al.*									
	(*as Nanochlorum eucaryotum* (Wilhem, Eisenbeis, Wild *et* Zahn) Henley *et al.*)			59	0·06*	0·12	0·24	27		FC
	(*as Nanochlorum eucaryotum*)			23†	0·02	0·04	0·08	28	yeast	FC
45	*Picochlorum maculatum* (Butcher) Henley *et al.* (as *Nannochloris maculatus* Butcher)			14†	0·01	0·02	0·04	28	yeast	FC
	PRASIOLALES Prasiolaceae									
46	*Prasiola stipitata* Suhr *in* Jessen	16	8	221	0·23	0·45	0·9*	*	*Gallus*	MI:DAPI
	Ulvophycean Green Algae									
	CAULERPALES Codiaceae									

Continued

APPENDIX: *Continued*

Entry number	Species[a]	$2n$[b]	Original ref. for $2n$	DNA amount 1C (Mbp)[c]	1C (pg)[d]	2C (pg)[d]	4C (pg)[d]	Original ref. for C-value[e]	Standard species[f]	Method[g]
47	*Codium fragile subsp. tomentosoides* (van Goor) P.C. Silva	20	17	284	2·9	5·8	11·6*	*	*Gallus*	MI:DAPI
48	*Codium lucasii* Setchell			882	0·9	1·8*	3·6	*	*Gallus*	MI:DAPI
49	*Codium prostratum* Levring			833	0·8	1·7*	3·4	*	*Gallus*	MI:DAPI
	CLADOPHORALES/SIPHONOCLADALES COMPLEX[4]									
50	*Cladophora coelothrix* Kützing			1421	1·45	2·9	5·8	*	*Gallus*	MI:DAPI
51	*Pithora* sp. (UTEX 787)			2009	2·05	4·1	8·2*	*	*Gallus*	MI:DAPI
52	*Pithophora* sp. (UTEX 1333)			882	0·9	1·9	3·8*	*	*Gallus*	MI:DAPI
	DASYCLADALES[5]									
	Dasycladaceae									
53	*Neomeris dumetosa* Lamouroux			1862	1·9	3·8*	7·6	*	*Gallus*	MI:DAPI
54	*Parvocaulis exigua* (Solms-Laubach) S. Berger *et al.* (=*Polyphysa exigua* (Solms-Laubach) M. J. Wynne)			1568	1·6	3·2*	6·4	*	*Gallus*	MI:DAPI
	TRENTEPOHLIALES									
	Trentepohliaceae									
55	*Cephaleuros parasiticus* Karsten			1911	1·95	3·9*	7·2	20	*Gallus*	MI:DAPI
56	*Cephaleuros virescens* Kunze in Fries	36	14	980	1·0	2·0*	4·0	20	*Gallus*	MI:DAPI
57	*Physolinum monile* (De Wildeman) Printz	22	7	2009	2·05	4·1*	8·2	20	*Gallus*	MI:DAPI
58	*Trentepohlia arborum* (Agardh) Hariot			1470	1·5	3·0*	6·0	20	*Gallus*	MI:DAPI
59a	*Trentepohlia aurea* (Linnaeus) Martius	32,34	1, 24	588	0·6	1·2*	2·4	20	*Gallus*	MI:DAPI
59b	*Trentepohlia aurea*			701	0·71	1·43*	2·86	*	*Gallus*	MI:DAPI
60	*Trentepohlia iolithus* (Linnaeus) Wallroth			980	1·0	2·0*	4·0	*	*Gallus*	MI:DAPI
61	*Trentepohlia odorata* (Wiggers) Wittrock			539	0·55	1·1*	2·2	20	*Gallus*	MI:DAPI
62	*Trentepohlia umbrina* (Kützing) Bornet	24	22	657	0·67	1·34	2·68	*	*Gallus*	MI:DAPI
	ULOTRICHALES[6]									
	Incertae sedis									
63	*Gleotilopsis sterilis* Deason			108	0·11	0·23*	0·46	*	*Gallus*	MI:DAPI
64	*Pseudendoclonium basilense* Vischer			167	0·17	0·34*	0·68	*	*Gallus*	MI:DAPI
	Monostromaceae									
65	*Capsosiphon fulvescens* (C.Agardh) Setchell *et* N.L.Gardner			157	0·16	0·33*	0·6	*	*Gallus*	MI:DAPI
	ULVALES[7]									
	Incertae sedis									
66	*Pseudendoclonium basilense* Vischer			196	0·17	0·34*	0·6	*	*Gallus*	MI:DAPI
	Ulvaceae									
67	*Percursaria percursa* (C.Agardh) Rosenvinge			294	0·3	0·6*	1·2	*	*Gallus*	MI:DAPI

Continued

APPENDIX: *Continued*

Entry number	Species[a]	2*n*[b]	Original ref. for 2*n*	DNA amount 1C (Mbp)[c]	1C (pg)[d]	2C (pg)[d]	4C (pg)[d]	Original ref. for C-value[e]	Standard species[f]	Method[g]
68	*Ulva compressa* Linnaeus (*as Enteromorpha compressa* (Linnaeus) Greville)	20	16	26·8*	0·07	0·14*	0·28	15	*Arabidopsis*	FC
69	*Ulva rotundata* Bliding			294	0·3	0·6*	1·2	*	*Gallus*	MI:DAPI

[1]Traditional taxonomic lists often grouped all conjugating green algae within one order, Zygnematales (Conjugales) (Bold and Wynne, 1985). Results of recent molecular studies support recognition of two orders, Desmidiales and Zygnematales (McCourt *et al*., 2000; Denboh *et al*., 2001).

[2]Molecular data demonstrate that *Chlamydomonas* is not monophyletic (Nozaki *et al*., 2000; Nozaki and Krienitz, 2001) and that revision of the circumscription of these genera will be required (Larson *et al*., 11992). *Dunalliella tertiolecta*, included here in Chlamydomonaceae, is part of a polyphyletic complex that may warrant recognition as a separate order (Nakayama *et al*., 1966).

[3]Recent molecular studies have demonstrated that *Chlorella* taxa are dispersed over two classes: Trebouxiophyceae and Chlorophyceae (Krienitz *et al*., 2004). Chlorellales included here are considered to be trebouxiophycean algae (Huss *et al*., 1999).

[4]Molecular data clearly demonstrate that classifications of the genus *Cladophora* should be revised (Hanyuda *et al*., 2002). Circumscription of families in this complex will require sequence data for additional cladophoralean algae.

[5]Recent molecular investigations indicate that genera of Dasycladaceae are well delineated, but this does not hold true for genera of Polyphysaceae (=Acetabulariaceae). 18S rRNA gene sequence data support transfer of *Acicularia schenckii* and *Polyphysa peniculus* to the genus *Acetabularia* (Berger *et al*., 2003).The familiar binomials are retained here for convenience until a complete taxonomic revision of Dasycladales is available.

[6]Recent phylogenetic investigations have redefined the boundary between Ulotrichales and Ulvales (O'Kelly *et al*., 2004). Species of *Monostroma* appear to be more closely related to Ulotrichales than to the Ulvales (Hayden and Waaland, 2002). No contemporary characterization of families is available for this newly circumscribed order.

[7]Characters used to separate the genera *Ulva* and *Enteromorpha* lack taxonomic significance (Tan *et al*., 1999; Shimada *et al*., 2003). The familiar binomials have been retained here in the absence of formal reassignment of species (Hayden and Waaland, 2003, 2004). Exact placement of *Blidingia* in Ulvales remains uncertain (*Incertae sedis*) as no contemporary characterization of the emended family Monostromaceae is available.

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