DNA content methodology

Algal material was fixed in Carnoy's solution and stored in 70% ethanol at 4 ºC. Preserved material was rehydrated in water and softened in 5% w/v EDTA (Goff and Coleman, 1990) for 30 min – 3 h. Algal specimens were transferred to cover slips treated with subbing solution, air dried and stained with DAPI (0.5ug/mL 4'–6' diamidodino-2-phenylindole)(Sigma Chemical Co., St. Louis, MO 63178) as previously described (Goff and Coleman, 1990; Kapraun and Nguyen, 1990). Detailed procedures for microspectro-photometry with DAPI and requirements for reproducible staining have been specified previously (Kapraun and Nguyen, 1990; Kapraun, 1994) using a protocol modified after Goff and Coleman (1990). Microspectrophotometric data for *Gallus* (chicken erythrocytes or 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 and thymine rich regions of DNA which contain at least four A-T base pairs (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 for algae indicate mean values of 43.5 mol % G + C (n = 9, range = 40-47mol %) for the Phaeophyta (Olsen *et al*., 1987; Stam *et al*., 1988; Le Gall *et al*., 1993), 41.6 mol% G+C (n = 22, range = 28-49 mol%) for the Rhodophyta (Kapraun *et al*., 1993b, 1993c; Le Gall *et al*., 1993), and 46.2 mol% (n = 22, range = 35-56 mol%) for the Chlorophyta (Olsen *et al*., 1987; Freshwater *et al*., 1990; Kooistra *et al*. 1992; Le Gall *et al*., 1993). 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). All three algal groups contain taxa 1) with some or all of their cells being multinucleate and often endopolyploid (Goff *et al*, 1992; Kapraun and Nguyen, 1994; Garbary and Clarke, 2002; Kapraun and Dunwoody, 2002). In addition, both red algae (Goff and Coleman, 1986) and green algae (Kapraun, 1994) have taxa that exhibit a

nuclear "incremental size decrease associated with a cascading down of DNA contents". Consequently, methodologies were developed specific for specimens of each algal group to permit assignment of C level and interpretation of I_f data. Materials and methods, as well as information for collection locations, and data for number of algal nuclei examined in each sample and estimates of nuclear genome size (pg) ± SD are available at http://www.uncw.edu/ people/ kapraund/DNA.

Notes for Tables 1 – 3:Nuclear DNA content estimates in species of red, green and brown algae.

Recently, the Angiosperm Genome Size Workshop (Bennett *et al*., 2000) identified 'best practice' methodology for nuclear genome size estimation in plant tissues (For details and recommendations, see http://www.rbgkew.org.uk/cval/conference.html under Angiosperm Genome Size Discussion Meeting). Virtually none of the published genome size data for algae resulted from investigations adhering to all of the best practice recommendations. Even in cases where the preferred methodology of Feulgen microdensitometry was employed, researchers typically used animal (RBC) rather than plant (*Allium* or *Pisum*) standards. Consequently, all present and previously published data included in these Appendices should be considered accurate only to ± 0.1 pg (Kapraun and Shipley, 1990; Hinson and Kapraun, 1991; Kapraun and Dutcher, 1991; Kapraun and Bailey, 1992).

Most DNA amounts in the literature are given in picograms (pg). Unless otherwise indicated Mbp values in the Appendix 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 (Kapraun2005), resulting in apparent anomalies in derived Mbp estimates as all values are rounded to the next highest number: a 2C value of 0.1 pg is assigned a 1C value of 0.1 pg, not 0.05, and a 2C value of 0.3 is assigned a 1C value of 0.2 pg, not 0.15. In Table 1, for example, both *Chlorokybus atmosphyticus* with 2C = 0.2 pg and *Ostreococcus tauri* with 2C = 0.1 pg are assigned 1C genome size estimates of 98 Mbp. DNA amounts originally published as megabase pairs (Mbp) are indicated with an asterisk (*). Most of these values were derived from reassociation kinetics (Bot *et al*. 1989a, 1989b, 1990, 1991, Kooistra *et al*. 1992, Olsen *et al*. 1987, Stam *et al*. 1988), but LeGall *et al* 1993 used ethidium bromide with RBC standard and flow cytometry. Additional values were published as million base pairs (mbp) determined from pulsed field electrophoresis (*e.g*. Courties *et al*. 1998).

Algal life histories typically are characterized by an alternation of haploid gametophyte and diploid sporophyte generations (Kapraun 1993, Kapraun and Dunwoody 2002). Thus, DNA content (pg) measurements could be based on either or both 2C replicated haploid nuclei or 4C replicated diploid nuclei. In practice, most published DNA content (pg) values are for 2C diploid nuclei and most 1C and 4C values are extrapolated. In the Appendix, 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 [(1)].

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

Standard species

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 a*l. 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. Species used as a standard were not always specified in the preliminary report by Mandoli (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 1009, Hinson & Kapraun 1991, Kapraun & Bailey 1992) or *Impatiens balsamina* L. (e.g. Kapraun & Shipley 1990). Species used as a calibration standard for algal research are listed in Table 1.

Methods:

Both flow cytometry (FC) (LeGall *et al*. 1993) and microspectrophotometry (MI)(Kapraun 1994, Kapraun & Buratti 1998) have been shown to be reliable methods for quantification of nuclear DNA contents in green algae. Feulgen microdensitometry was used by Maszewski and Kołodziejczyk 1991 (Fe). Reassociation kinetics (RK) have been used successfully as well (Bot *et al*. 1989a, 1989b, 1990, 1991, Dörr and Huss, 1990, Kooistra *et al*. 1992, Olsen *et al*. 1987). (PF) Pulse field electrophoresis (electrophoretic karyotyping) techniques have been used successfully with *Chlorella* (Higashiyama and Yamada 1991).

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) and propidium iodide (PI) (Spring *et al*. 1978) were used in selected green algal investigations.