Theor Appl Genet (1995) 90:11–16


M. A. Lodhi · B. I. Reisch

# Nuclear DNA content of *Vitis* species, cultivars, and other genera of the Vitaceae

Received: 10 June 1994 / Accepted: 28 July 1994

**Abstract** The nuclear DNA content was analyzed in *Vitis* species, hybrid cultivars, and genera of the Vitaceae using flow cytometry. Significant variation was found among *Vitis* species, hybrids, and other genera of the Vitaceae (*Ampelopsis* and *Parthenocissus*). DNA content was estimated to range from 0.98 to 1.05 pg/2C within *V. labrusca* (ns) and 0.86 to 1.00 pg/2C within *V. vinifera* (ns). Genotypes from *Vitis* and *Parthenocissus* were similar in nuclear DNA content (approximately 1.00 pg/2C) whereas they differed significantly from *Ampelopsis* (1.39 pg/2C). No correlation between DNA content and the center of origin of genotypes of the Vitaceae was noted. Based on the present study, the *Vitis* genome size is 475 Mbp, 96% of which is non-coding. Knowledge of DNA content is useful in order to understand the complexity of the *Vitis* genome and to establish a relationship between the genetic and physical map for map-based cloning.

**Key words** *Ampelopsis* · Flow cytometry · Grapevines · *Parthenocissus*

## Introduction

Grape (*Vitis* species) is one of the most important fruit crops of the world (Anonymous 1993). Our understanding of the grape genome has been increasing rapidly (Hain et al. 1993; Hébert et al. 1993; Lodhi et al. 1993, 1994; Tesniére et al. 1993; Thomas et al. 1993) because of the worldwide interest in this crop. Grapes belong to the family Vitaceae and the genus *Vitis* has been grouped in two sections, 11 series, and more than 60 species (Galet 1988). Many of these species are valuable sources of genes for resistance to diseases, to insects and to abiotic stress (Alleweldt and Possingham 1988). To our knowledge no complete study has been conducted on DNA content in the Vitaceae. Fluorometric studies have been used to differentiate ploidy level among grapes (Saurer and Antcliff 1969; Rajasekaran and Mullins 1983) and to quantify the nuclear DNA of *V. vinifera* cv Cabernet Sauvignon (Thomas et al. 1993). A knowledge of DNA content is thought to be important in establishing evolutionary relationships (Price 1976; Srivastava and Lavania 1991; Dickson et al. 1992; Costich et al. 1993), in genome mapping (Arumuganathan and Earle 1991a; Flavell et al. 1993a), in studies of growth rate and habit, and generation time (Bennett 1985, 1987), and in studies of ecological or environmental adaptation (Grime and Mowforth 1982; Sins and Price 1985; Grime 1990; Ceccarelli et al. 1992).

Enormous variation has been found in the DNA content of plant genomes (Bennett 1987; Price 1988). This variation is significant from an evolutionary and phylogenetic point of view (Costich et al. 1993). The variation results from the accumulation of repeated DNA (Flavell 1985, 1986; Cullis 1990; Lapitan 1992) and unique DNA sequences (Furuta and Nishikawa 1991), as well as by increased ploidy (Flavell 1986; Hammatt et al. 1991; Costich et al. 1993). An accumulation of DNA over time results in population divergence and contributes to the formation of new species (Narayan and Rees 1976, 1977; Bachmann and Price 1977; Stein et al. 1979; Flavell 1985). Moreover, variation in nuclear DNA content has also been reported within several plant species: e.g., *Microseris* (Price et al. 1983), *Zea* (Rayburn et al. 1985, 1993), and *Scilla* (Grueilhuber and Septa 1985). Studies of closely related species indicate that both evolutionary increases and decreases in DNA content are common (Price 1976; Rao and Sharma 1987). Though in some plant genera, such as *Gibasis*, an evolutionary decrease in DNA content has been reported (Kenton 1984), an increase in DNA content is

Communicated by G. Wenzel

M. A. Lodhi[1] · B. I. Reisch
Departmet of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456, USA

*Present address:*
[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA

generally observed, as for example in *Secale* (Bennett et al. 1977), *Crepis* (Jones and Brown 1976) and *Lolium* (Hutchinson et al. 1979). Some authors have suggested that additional DNA does not signify any particular function and represents an evolutionary by-product (Poggio and Hunzikar 1986).

In view of the importance of nuclear DNA content determinations, an attempt has been made in this report to quantify nuclear DNA from the members of three genera of the Vitaceae (*Vitis*, *Ampelopsis* and *Parthenocissus*), including 22 *Vitis* species, as well as complex interspecific hybrids and commercial cultivars, by laser flow cytometry. Our goal was to compare the grape genome with other plant genomes and thus deduce the complexity of the *Vitis* genome. We also wished to estimate the relationship between the genetic and physical genome map.

## Materials and methods

The plant material used in this study (see Table 1) was collected from the USDA-ARS, Plant Genetic Resources Unit, Geneva, New York and the Grape Breeding and Genetics Program, New York State Agricultural Experiment Station, Geneva, New York. Young leaves which were not fully expanded were collected from growing shoots and kept on ice until use. Nuclei were prepared for flow cytometric analysis using the protocol of Arumuganathan and Earle (1991b), except that the concentration of propidium iodide was raised to 0.20 mM (Calbiochem No. 537059).

Nuclear DNA content was analyzed on an Epics Profile Flow Cytometer (Coulter Electronics, Hialeah, Fla., USA) with an air-cooled argon-ion laser to all visible lines. The amount of DNA was estimated by the fluorescence of the nuclei with propidium iodide relative to that of chicken red blood cells (CRBC). The results were obtained in the form of four histograms: logarithmic (LFL) and linear-light scatter (FL) and forward-angle (FS) and side-light scatter (SS). In the present study only FL (256 channels) was used to calculate the DNA content and compared with LFL where necessary. The DNA amount of CRBC (2C = 2.33 pg, Galbraith et al. 1983) was used to convert the DNA estimates for *Vitis* into absolute amounts, using the following equation:

$$\text{DNA content of the sample (pg/2C)} = \frac{\text{mean position of plant nuclear peak}}{\text{mean position of CRBC nuclear peak}} \times 2.33$$

Each reading was based on propidium iodide fluorescence from 400–4 000 plant nuclei. Data were collected independently in 1992 and 1993 and an average DNA content was determined based on all observations. Total base pairs were calculated from the average DNA content in pg/2C based on the approximation that 1 pg of nuclear DNA contains 965 Mbp (Bennett and Smith 1976). A General Linear Model was used to perform an analysis of variance (SAS Institute 1985) and means were separated with a Student-Newman-Keul's test.

## Results and discussion

Forward-angle light scatter (FS) indicates the particle size and is used to locate the data from nuclei for subsequent analysis. In each case two particle sizes were selected, one representing CRBC nuclei and the other the sample nuclei (Fig. 1). In the bottom left corner of each FS panel are the particles of plant debris that were excluded from the analysis at run time by using a bitmap gate. The other type of histogram represents the relative linear fluorescence intensity (FL) stained with propidium iodide. In our FL analysis only two fluorescence peaks were detected, one for the sample DNA and the other for the CRBC standard. Only one sample peak for $G_1$ phase cells depicting the 2C DNA value was obtained. No $G_2$ + M phase peaks (for a 4C DNA value) were observed. 4C peaks would have been expected between 2C and CRBC peaks. A similar result was reported in rice and wheat (Arumuganathan and Earle 1991a) and in cacao (Figueiro et al. 1992) where it was attributed to the developmental stage of the leaves. For our analyses we collected leaf tissues between 0700 and 0800 h and the cells were most likely in the presyn- thesis gap stage ($G_1$). Chromosomes in this phase are in a diploid state so only the 2C DNA value would be detected. The obscurity of the 4C peaks could also be attributed to the high fluorescent background of the plant debris.

Differences in nuclear DNA content were found among *Vitis* species (Table 1) but were not statistically significant. Values from 0.85 pg/2C (*V. doaniana*) to 1.12 pg/2C (*V. aestivalis lincecumii* and *V. rupest. .s*) were observed. The *Vitis* species (2n = 38) included in the present study represent 8 of the 11 series of Asian and North American origin. The nuclear DNA content of *Parthenocissus* was calculated to be 1.07 pg/2C and that of *Ampelopsis* as 1.38 pg/2C (for both 2n = 40). No difference in the nuclear DNA amount was noted between *Vitis* and *Parthenocissus*, but a significant difference was present between *Ampelopsis* and all the others. The difference with *Ampelopsis* could be due to chromosome size as well as to the addition of two chromosomes. To our knowledge no cytological work has been reported on most of the *Vitis* species or for the *Ampelopsis* included in our analysis. These results are not in agreement with Shetty (1959) who found the chromosome of *Parthenocissus* to be larger than those of *Vitis*. Nuclear DNA variation detected in the present study among *Vitis* species, *Ampelopsis*, and *Parthenocissus* could be of taxonomic significance and might bear upon phylogenetic relationships.

Non-significant variation for DNA content (8–14%) was observed among cultivars of *V. labrusca*, *V. vinifera* and diploid *Vitis* hybrids (Table 1). Our results are consistent with the observations of Rayburn et al. (1989) who found about 14% DNA-content variation in four different corn lines. Furuta et al. (1984) was able to detect DNA-content variation in individual chromosomes in different strains, as well as a significant difference between homologous chromosomes in different strains, of common wheat. Among interspecific *Vitis* hybrids, DNA content ranged from 0.88 pg/2C in *Vitis* hyb. 'Lady Patricia' to 1.03 pg/2C in *Vitis* hyb. 'Chancellor' (ns). An interspecific tetraploid form of 'Himrod', which arose as a sport from diploid 'Himrod', was also included to test DNA variation in grape polyploids. The

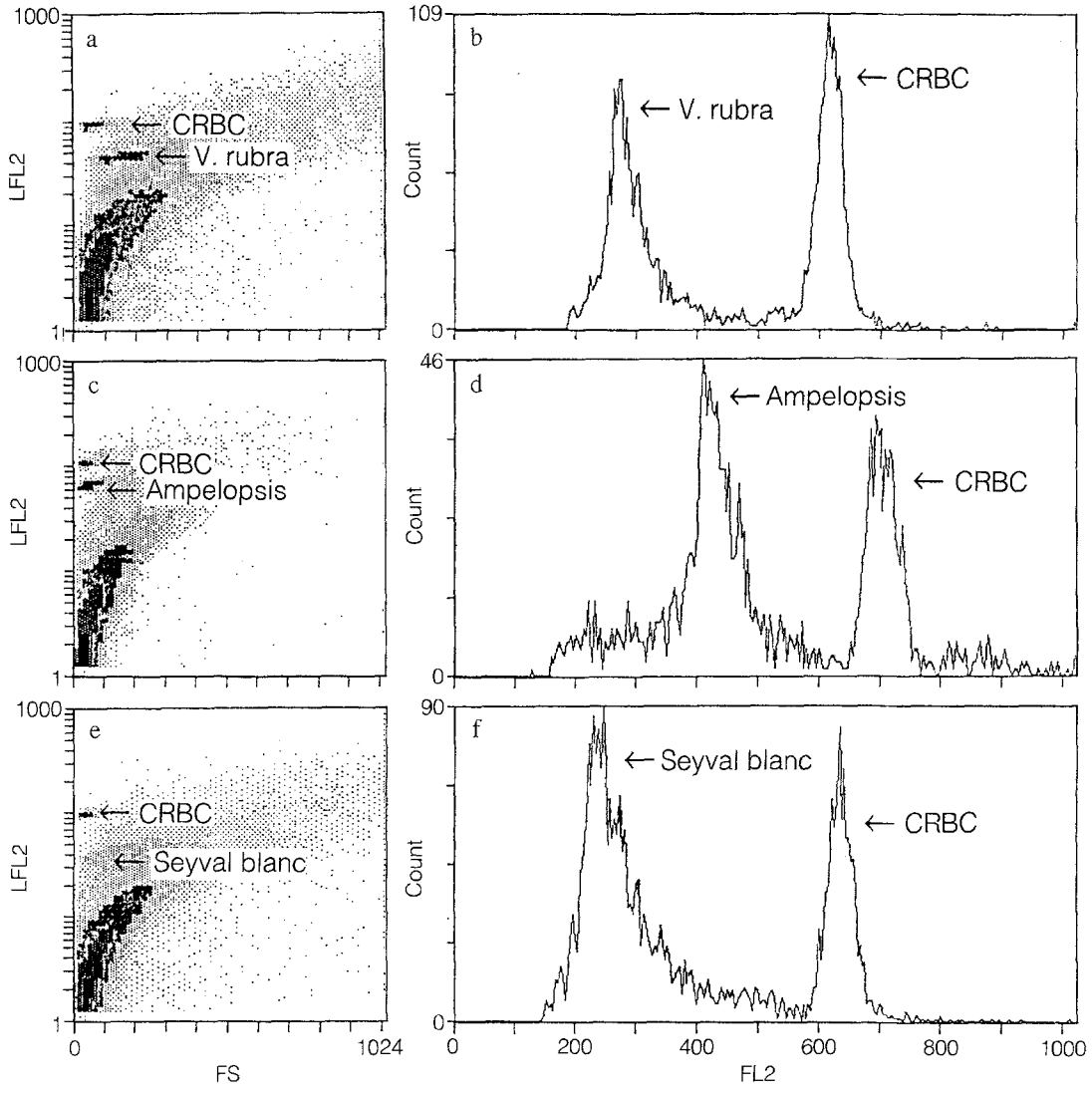


**Fig. 1 a–f** Output of flow cytometric analysis of three genotypes re-analyzed with EPICS Elite software. Panels **a**, **c** and **e** represent the log of fluorescence versus forward-angle light scatter (*FS*) indicating the particle size of 2n genotypes. Panels **b**, **d** and **f** show relative fluorescence intensity (*FL*) versus nuclei count. These panels display the number of nuclei per channel (count) as a function of relative fluorescence intensity resulting from the flow cytometric analysis of nuclei stained with propidium iodide. In all FL histograms, two peaks are observed, the one on the right is the fluorescence intensity detected for chicken red blood cells (*CRBC*) and the one on the left is for the sample genotype. Fluorescence intensity from a number of nuclei for the calculation of DNA content were obtained by taking a particular area under each distinctive peak in FL histograms

amount of DNA in tetraploid 'Himrod' (1.33 pg/2C) was less than twice the amount in the diploid. This phenomenon has been observed in other polyploid species (Parida et al. 1990; Furuta and Nishikawa 1991 and references therein) and might be the result of nuclear diminution accompanied by chromosome shortening as observed in wheat (Bhaskaran and Swaminathan 1960). Because of its recent origin, nuclear diminution might not adequately explain the unexpected result with tetraploid 'Himrod'. Experimental error may also be a causal factor.

Also included in Table 1 are three progeny plants of an interspecific cross 'Cayuga White' × 'Aurore', each designated by a number. These plants were chosen based on the vegetative vigor of the vine. 'Cayuga White' × 'Aurore' #89 was the least vigorous; a smaller and weaker vine, with a shorter internodal length, small leaves and few thinner shoots. Progeny #99 was the most vigorous with very long and thick shoots and #135 was intermediate in vigor between the other two. No significant difference was observed in the DNA content of the parents and the progeny. Also no correlation was noted between DNA content and vine growth as was reported in *Acacia* (Mukherjee and Sharma 1990). Our results do not follow the nucleotype concept (Bennett 1972) which emphasizes the influence of bulk DNA content on the phenotype of an organism. The DNA content in all three progeny plants is close to that of both parents, i.e., 1.00 pg/2C. In $F_1$ hybrids of maize, DNA content was close to the parental means in some crosses whereas variation from the parental means was observed in others (Rayburn et al. 1993). In the maize study inbred lines were used as parents while in our case the parents were interspecific hybrids that could result in

**Table 1** DNA content and origin of *Vitis* species, cultivars and other genera of the Vitaceae

| Genotype | No. of observations | DNA[a] (pg/2C) | Mbp/C |
|---|---|---|---|
| *Vitis* species | | | |
| a. Asia | | | |
| *V. amurensis* | 3 | 1.01b | 487 |
| *V. betulifolia* | 3 | 0.98b | 473 |
| *V. coignetiae* | 2 | 1.00b | 483 |
| *V. flexuosa* | 3 | 0.91b | 439 |
| *V. thunbergii* | 2 | 1.09b | 526 |
| *V. yenshanensis* | 3 | 1.08b | 522 |
| b. North America | | | |
| *V. acerifolia* Selection I | 3 | 0.97b | 469 |
| *V. acerifolia* Selection II | 2 | 0.96b | 464 |
| *V. aestivalis* | 3 | 0.95b | 459 |
| *V. aestivalis argentifolia* | 2 | 0.95b | 459 |
| *V. aestivalis lincecumii* | 1 | 1.12b | 540 |
| *V. berlandieri* | 3 | 1.01b | 488 |
| *V. champini* | 3 | 0.92b | 444 |
| *V. cinerea* | 2 | 0.98b | 473 |
| *V. doaniana* | 3 | 0.85b | 411 |
| *V. labrusca* | 3 | 1.05b | 507 |
| *V. monticola* | 3 | 1.09b | 526 |
| *V. riparia* | 3 | 0.97b | 469 |
| *V. rubra* | 2 | 0.98b | 473 |
| *V. rupestris* | 3 | 1.12b | 541 |
| *V. vulpina* | 3 | 0.88b | 425 |
| Cultivars of *V. vinifera* | | | |
| 'Cabernet Sauvignon' | 1 | 1.00b | 483 |
| 'Chardonnay' | 3 | 0.86b | 415 |
| 'Pinot noir' | 3 | 0.86b | 415 |
| 'Thompson Seedless' | 1 | 0.92b | 444 |
| Cultivars of *V. labrusca* | | | |
| 'Catawba' | 3 | 1.00b | 483 |
| 'Concord' | 2 | 0.99b | 478 |
| 'Concord Seedless' | 2 | 1.06b | 511 |
| 'Niagara' | 3 | 0.98b | 478 |
| Interspecific hybrid cultivars | | | |
| 'Chancellor' | 2 | 1.03b | 497 |
| 'Einset Seedless' | 2 | 1.02b | 493 |
| 'Himrod' | 3 | 0.95b | 459 |
| 'Himrod' (tetraploid) | 3 | 1.33a | 321 |
| 'Lady Patricia' | 2 | 0.88b | 425 |
| Seibel 5455 | 2 | 1.00b | 483 |
| 'Seyval blanc' | 4 | 0.88b | 425 |
| Seyve-Villard 12-375 | 2 | 0.97b | 469 |
| Parents and Progeny | | | |
| 'Cayuga White' | 2 | 1.00b | 483 |
| 'Aurore' | 3 | 0.98b | 473 |
| C. White × Aurore #89 | 3 | 1.06b | 511 |
| C. White × Aurore #99 | 2 | 0.99b | 478 |
| C. White × Aurore #135 | 3 | 0.95b | 459 |
| Genera of Vitaceae | | | |
| *Ampelopsis brevipedunculata* | 3 | 1.38a | 666 |
| *Parthenocissus tricuspidata* | 3 | 1.07b | 516 |

[a] Average DNA content from 1992 and 1993 observations. Means followed by the same letters a or b are not significantly different according to the Student-Newman-Keul's Test at $P \leq 0.05$

macromutations without significant addition or deletion of DNA. Such changes can affect the growth and vigor of plants without changing the DNA content. Poggio and Hunzikar (1986) were also unable to establish any correlation between DNA content and morphological, biochemical and ecological characteristics in *Bulnesia*.

According to the present study, the grape genome is 475 Mbp (average of *Vitis* species, *V. vinifera* and *V. labrusca* cultivars, Table 1) which is about three times the size of *Arabidopsis thaliana* (145 Mbp), one-fifth the size of maize, *Zea mays* (2292–2716 Mbp), two-thirds the size of apple, *Malus* × *domestica* (743–796 Mbp), half the size of tomato, *Lycopersicon esculentum* (907–1 000 Mbp), and the same size as rice, *Oryza sativa* (419–463 Mbp), as reported by Arumuganathan and Earle (1991a). It is slightly smaller than the genome of diploid blueberries, *Vaccinium* species (608 Mbp; Costich et al. 1993), and fractionally larger than that of *Theobroma cacao* (415 Mbp; Figueira et al. 1992). Figueira et al. (1992) attributed small nuclear content in cacao to small chromosome size (it varies from 1.25 to 2.85 μm; Martison 1975). The average chromosome size in *Vitis* species (0.85–1.07 μm; Shetty 1959) is smaller than in cacao. Nuclear DNA content is related to the cumulative size of the chromosome of a species (Bennett et al. 1982; Lukaszewski et al. 1982). Though grape has smaller chromosomes, the diploid chromosome number is almost twice that of cacao (2n = 20).

The total amount of nuclear DNA varies between $5 \times 10^7$ to over $8 \times 10^{10}$ bp in somatic cells of flowering plants (Bennett and Smith 1976). The DNA which specifies all the properties of plants has been estimated to occupy about $10^7$–$10^8$ bp (Flavell 1980). An average mRNA molecule is about 1 200 bases long. Assuming that on an average there are about 15 000 genes/haploid genome and that each mRNA is a product of a single gene, $1.8 \times 10^7$ bp of DNA will be involved in transcription (Flavell 1980).

The genome size in grape is approximately $4.75 \times 10^8$ bp/C; therefore, based on the above calculations, the coding region ($1.8 \times 10^7$ bp) is only 3.8% of the genome. The rest of the genome ($4.57 \times 10^8$ bp) is most likely repetitive or non-coding in nature. Similar figures for the coding fraction of total plant nuclear DNA have already been reported; (5–10%, Flavell 1980; 3–5%, Narayan 1991). The amount of repetitive DNA is very important in genome mapping and map-based cloning. Large non-coding regions of DNA may indicate a relatively large distance between genes. Non-coding DNA is likely to contribute to major distortions between the genetic and physical maps within and between species and may lead to large differences in the distances between genes within and between species (Flavell et al. 1993b). It has been suggested that the physical relationship to genetic maps can vary between 80 and 1 000 kbp/cM in different chromosomal regions (Tanksley et al. 1992; Flavell et al. 1993a). Genetic maps developed in 'Cayuga White' and 'Aurore' grapes cover 1 232 to 1 481 cM, respectively (Lodhi et al., unpublished). Therefore, one cM approximates to 300 kbp in *Vitis*, a suitable distance for 'genome walking' and gene cloning (Flavell et al. 1993a). Such a map-based cloning strategy was adopted to clone a bacterial

(*Psudomonas syringae* pv *tomato*) speck resistance gene in tomato (Martin et al. 1993). Tomato has a larger genome size compared to *Vitis* and one cM equals approximately 750 kbp (Tanksley et al. 1992), more than twice the amount of DNA per cM compared with grape.

Highly significant positive correlations have been found between the nuclear DNA content and total chromosome length in *Lycoris* (Nishikawa et al. 1979) and barley (Bennett et al. 1982). A higher DNA content was found to be a characteristic of temperate-zone genotypes and grasses (Grime and Mowforth 1982), whereas no such correlation was found in several *Helianthus* species (Sims and Price 1985). All genotypes in our study were of Asian or North American origin. A non-significant variation in DNA content is seen in *Vitis* species as well as in interspecific hybrid cultivars. No variation was seen between *Vitis* and *Parthenocissus*, but *Ampelopsis* had a greater DNA content. We did not find any relationship between the DNA content and the origin of *Vitis* species or genera. From the present study, it is not possible to establish a phylogenetic relationship between *Vitis* species and genera. Very little intraspecific DNA variation was seen in cultivars of *V. vinifera* and *V. labrusca*. These cultivars developed independently as chance seedlings or due to genomic mutations. It has been suggested that a 5–10% variation in nuclear DNA can cause significant change in an organism (Price 1988; Cavallini et al. 1993) which may result in physiological and environmental adaptations (Mukherjee and Sharma 1993).

Flow cytometry was found to be a reliable and efficient technique for the measurement of DNA content in *Vitis* species and related genera. The information collected on DNA content in *Vitis* was used to investigate the complexity of the *Vitis* genome. Because of its relatively low complexity, 'genome walking' and map-based cloning are suitable for use in *Vitis*.

**Acknowledgments** The authors thank Philip Forsline, USDA-ARS, Plant Genetic Resources Unit, Geneva, New York for providing the plant material used in this study. The help of Elizabeth Earle and Lou Batts, Plant Breeding and Biometry, Cornell University, for the use of laboratory facilities and flow cytometric analysis is acknowledged. Grateful thanks are due to Carol Bayles and James Slattery, Flow Cytometry and Imaging Facility, New York State Center for Advanced Technology in Biotechnology (NYSCATB), Cornell University, Ithaca, New York, for help with the analysis. The authors also thank James Slattery, NYSCATB, Norman Weeden, Horticultural Sciences, New York State Agricultural Experiment Station, Geneva, New York, and Bernard Baum, Centre for Land and Biological Resources Research, Ontario, Canada for helpful suggestions on the manuscript. The scholarship of the Rotary Foundation for graduate studies of M. A. Lodhi is duly acknowledged. This research was supported by Grant No. 1888-90 from BARD, the United States-Israel Binational Research and Development Fund, and by the New York Wine and Grape Foundation.

## References

Alleweldt G, Possingham JV (1988) Progress in grapeving breeding. Theor Appl Genet 75:669–673

Anonymous (1993) FAO Production Year Book, vol 46. Food and Agricultural Organization of the United Nations, Rome

Arumuganathan K, Earle ED (1991a) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–219

Arumuganathan K, Earle ED (1991b) Estimation of nuclear DNA content by flow cytometry. Plant Mol Biol Rep 9:229–241

Bachmann K, Price HJ (1977) Repetitive DNA in Cichorieae (Compositae). Chromosoma 61:267–275

Bennett MD (1972) Nuclear DNA content and minimum generation time in herbaceous plants. Proc R Soc Lond B181:109–135

Bennett MD (1985) Intraspecific variation in DNA amount and the nucleotypic dimension in plant genetics. In: Freeling M (ed) Plant genetics. A. R. Liss, New York, pp 283–302

Bennett MD (1987) Variation in genomic form in plants and its ecological implications. New Phytol 106:177–200

Bennett MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Phil Trans R Soc Lond B274:227–274

Bennett MD, Gustafson JP, Smith JB (1977) Variation in nuclear DNA in the genus *Secale*. Chromosoma 61:149–176

Bennett MD, Smith JB, Ward JP, Finch RA (1982) The relationship between chromosome volume and DNA content in unsquashed metaphase cells of barley, *Hordeum vulgare* cv Tullen 346. J Cell Sci 56:101–111

Bhaskaran S, Swaminathan MS (1960) Metaphase chromosome length and DNA content in relation to polyploidy in *Triticum* species. Exp Cell Res 20:598–599

Cavallini A, Natali L, Cionini G, Gennai D (1993) Nuclear DNA variability within *Pisum sativum* (Leguminosae): nucleotypic effects on plant growth. Heredity 70:561–565

Ceccarelli M, Falistocco E, Cionini PG (1992) Variation in genome size and organization within hexaploid *Festuca arundinaceae*. Theor Appl Genet 83:271–278

Costich DE, Ortiz R, Meagher TR, Bruederle LP, Vorsa N (1993) Determination of ploidy level and nuclear DNA content in blueberry by flow cytometry. Theor Appl Genet 86:1001–1006

Cullis CA (1990) DNA rearrangements in response to environmental stress. Adv Genet 28:73–97

Dickson EE, Arumuganathan K, Kresovich S, Doyle JJ (1992) Nuclear DNA content variation within the Rosaceae. Am J Bot 79:1081–1086

Figueira A, Janick J, Goldsbrough P (1992) Genome size and DNA polymorphism in *Theobroma cacao*. J Am Soc Hort Sci 117:673–677

Flavell RB (1980) The molecular characterization and organization of plant chromosomal DNA sequences. Annu Rev Pl Physiol 31:569–596

Flavell RB (1985) Repeated sequences and genome change. In: Hohn B, Dennis ES (eds) Genetic flux in plants. Springer-Verlag, Berlin Heidelberg, New York, pp 139–156

Flavell RB (1986) Repetitive DNA and chromosome evolution in plants. Phil Trans R Soc Lond B312:227–242

Flavell RB, Dean C, Moore G (1993a) Exploiting plant genomes, small and large. In: Heslop-Harrison JS, Flavell RB (eds) The chromosome. BIOS Scientific Publishers, UK, pp 249–264

Flavell RB, Gale MD, O'Dell M, Lucas H, Murphy G, Moore G (1993b) Molecular organization of genes and repeats in the large cereal genomes and implications for isolation of genes by chromosome walking. In: Chandley A, Sumner A (eds) Chromosomes today, Vol 11. Chapman and Hall, London, pp 199–213

Furuta Y, Nishikawa K (1991) Variation in nuclear and individual chromosomal DNA content and its role in evolution of plants. In: Gupta PK, Tsuchiya T (eds) Chromosome engineering in plants: genetics, breeding and evolution, Part A. Elsevier, New York, pp 71–86

Furuta Y, Nishikawa K, Makino T, Sawai Y (1984) Variation in DNA content of 21 individual chromosomes among six subspecies in common wheat. Jpn J Genet 59:83–90

Galbraith DW, Harkins KR, Maddox JM, Ayres NM, Sharma DP, Firoozabady E (1983) Rapid flow cytometric analysis of cell cycle in intact plant tissues. Science 220:1049–1051

Galet P (1988) Cépages et vignobles de France, Tome 1. Les vignes Américaines, 2nd edn. Imprimerie Charles Déhan, Montpellier, France

Grime JP (1990) Ecological effects of climate change on plant populations and vegetation composition with particular reference to the British flora. In: Jackson M, Ford-Lloyd BV, Parry ML (eds) Climatic changes and plant genetic resources. Belhaven Press, London, pp 40–60

Grime JP, Mowforth MA (1982) Variation in genome size—an ecological interpretation. Nature 299:151–153

Grueilhuber J, Septa F (1985) Geographical variation of genome size at low taxonomic levels in *Scilla bifolia alliance* (Hyacinthaceae). Flora 176:431–438

Hain R, Reif HJ, Krause E, Langbartels R, Kindle H, Vornam B, Wiese W, Schmelzer E, Schreier PH, Stöcker RH, Stenzel K (1993) Disease resistance results from foreign phytoalexin expression in a novel plant. Nature 361:153–156

Hammatt N, Blackhall NW, Davey MR (1991) Variation in the DNA content of *Glycine* spp. J Exp Bot 42:659–666

Hébert D, Kikkert JR, Smith FD, Reisch BI (1993) Optimization of biolistic transformation of embryogenic grape-cell suspensions. Plant Cell Rep 12:585–589

Hutchinson J, Rees H, Seal AG (1979) An assay of the activity of supplementary DNA in *Lolium*. Heredity 43:507–509

Jones RN, Brown LM (1976) Chromosomal evolution and DNA variation in *Crepis*. Heredity 36:613–641

Kenton A (1984) Chromosomal evolution in the *Gibasis linearis* group (Commelinaceae). III. DNA variation, chromosomal evolution and speciation in *G. venustula* and *G. heterophylla*. Chromosoma 90:303–310

Lapitan NLV (1992) Organization and evolution of higher plant nuclear genomes. Genome 35:171–181

Lodhi MA, Reisch BI, Weeden NF (1993) Molecular genetic mapping of the *Vitis* genome (abstract). Am J Enol Vitic 44:469

Lodhi MA, Reisch BI, Weeden NF (1994) Genetic linkage maps of *Vitis* and QTL detection by interval mapping (abstract). Plant Genome II Conference, 24-27 January 1994 San Diego, California

Lukaszewski AJ, Wozna J, Apolinarska B, Naganowska B (1982) The DNA content of the individual chromosomes of rye. Theor Appl Genet 62:145–148

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

Martison VA (1975) Cytological studies of diploid and tetraploid *Theobroma cacao*. Genetica 45:341–348

Mukherjee S, Sharma AK (1990) Mitotic cell duration and its relationship with nuclear DNA content, chromosome number and size and rate of growth in species of *Acacia* growing under stress. Ind J Exp Biol 28:508–510

Mukherhee S, Sharma AK (1993) In situ nuclear DNA content in perennial fast and slow growing Acacias from arid zones. Cytobios 75:33–36

Narayan RKJ (1991) Molecular organisation of the plant genome: its relation to structure, recombination and the evolution of chromosomes. J Genet 70:43–61

Narayan RKJ, Rees H (1976) Nuclear DNA variation in *Lathyrus*. Chromosoma 54:141–154

Narayan RKJ, Rees H (1977) Nuclear DNA divergence among *Lathyrus* species. Chromosoma 63:101–107

Nishikawa K, Futura Y, Endo H (1979) Consideration of chromosome evolution on the basis of nuclear DNA content and total chromosome length in *Lycoris*. Jpn J Genet 54:387–396

Parida A, Raina SN, Narayan RJK (1990) Quantitative DNA variation between and within chromosome complements of *Vigna* spp. (Fabaceae). Genetica 82:125–134

Poggio L, Hunzikar JH (1986) Nuclear DNA content variation in *Bulnesia*. J Hered 77:43–48

Price HJ (1976) Evolution of DNA content in higher plants. Bot Rev 42:27–52

Price HJ (1988) DNA variation among higher plants. Ann Mis Bot Gard 75:1248–1257

Price HJ, Chambers KL, Bachmann K, Riggs J (1983) Inheritance of nuclear 2C DNA content variation in intraspecific and interspecific hybrids of *Microseris* (Asteraceae). Am J Bot 70: 1133–1138

Rajasekaran K, Mullins MG (1983) The origin of embryos and plantlets from cultured anthers of hybrid grapevines. Am J Enol Vitic 34:108–113

Rao VLK, Sharma AK (1987) Amount of DNA and genotype differences in *Hordeum*. Cytologia 52:593–598

Rayburn AL, Price HJ, Smith JD, Gold JR (1985) C-band heterochromatin and DNA content in *Zea mays*. Am J Bot 72:1610–1617

Rayburn AL, Auger JA, Benzinger EA, Hepburn AG (1989) Detection of intraspecific DNA content variation in *Zea mays L.* by flow cytometry. J Exp Bot 40:1179–1183

Rayburn AL, Biradar DP, Bullock DG, McMurphy LM (1993) Nuclear DNA content $F_1$ hybrids of maize. Heredity 70:294–300

SAS Institute (1985) SAS/STAT Guide for personal computers. SAS Institute Inc., Cary, North Carolina, USA

Saurer W, Antcliff AJ (1969) Polyploid mutants in grapes. HortScience 4:226–227

Shetty BV (1959) Cytotaxonomical studies in Vitaceae. Biblio Genet XVIII:167–272.

Sims LE, Price HJ (1985) Nuclear DNA content variation in *Helianthus* (Asteraceae). Am J Bot 72:1213–1219

Srivastava S, Lavania UC (1991) Evolutionary DNA variation in *Papaver*. Genome 34:763–768

Stein DB, Thompson WF, Belford HS (1979) Studies on DNA sequences in Osmundaceae. J Mol Evol 13:215–232

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Röder MS, Wing RA, Wu W, Young ND (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Tesniére CM, Romieu C, Vayda ME (1993) Changes in gene expression of grapes in response to hypoxia. Am J Enol Vitic 44:445–451

Thomas MR, Matsumoto S, Cain P, Scott NS (1993) Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification. Theor Appl Genet 86:173–180