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The Relationship between Satellite Deoxyribonucleic Acid, Ribosomal Ribonucleic Acid Gene Redundancy, and Genome Size in Plants

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ABSTRACT

The buoyant density of ribosomal DNA is similar in species with or without satellite DNA, and in all species examined was distinguishable from that of the satellite DNA. In melon tissues (*Cucumus melo*) the percentage satellite DNA is not correlated with the percentage hybridization to ribosomal RNA. Satellite DNA sequences do not appear to be dispersed between those coding for ribosomal RNA. There is no correlation between the presence of satellite DNA and high ribosomal RNA gene redundancy, but there is a correlation between satellite DNA and small genome size, which results in a correlation between satellite DNA and a high percentage hybridization to ribosomal RNA. Satellite DNAs are defined as minor components after CsCl centrifugation.

The initial observations on satellite DNAs in plants (defined here as minor components after CsCl centrifugation) suggested a relationship between satellite DNA and the sequences coding for rRNA, the rDNA (11). Three plants containing a satellite component had a higher percentage of rDNA than two plants without, and when the satellite of pumpkin was separated from the mainband by CsCl gradient fractionation, rRNA hybridized mainly to the satellite fraction, accounting for 3.5% of the satellite DNA. An additional satellite, with a much higher bouyant density of 1.722 g cm^{-3}, has also been reported to appear in various plant tissues when maintained under conditions of physiological stress (6, 15). This stress satellite was interpreted as a massive amplification of rDNA, but subsequent studies have shown that the additional satellite produced in response to at least one of the stress conditions was of bacterial rather than plant origin, having nothing to do with rDNA (13).

Although it is clear that some satellite DNAs, such as the mouse satellite, have no relationship to the rDNA, rDNA is resolved as a satellite component in certain species. The rDNA satellite accounts for 0.3% of the DNA in *Xenopus laevis* somatic tissues (3), but is as much as 30% of the DNA prepared for germinal vesicles (4), where it is present in several thousand extrachromosomal nucleoli. In both cases approximately 50% of the satellite hybridizes with rRNA. The Dytiscid water beetles also contain a conspicuous mass of extrachromosomal DNA (Giardina's body) in their oogonia and oocytes. In *Colymbetes fuscus* the ovariole tips contained 23% satellite compared with 3.4% in somatic cells. Hybridization to RNA was also 7-fold greater in the ovariole tissue, accounting for approximately 3% of the satellite DNA. In *Dytiscus marginalis* the ovariole tips contained twice as much satellite as somatic cells, 35% *versus* 18%, and rDNA accounted for 0.12% of the satellite component (5). Only a small percentage of the satellite component in plants and in the water beetles is therefore complementary to rRNA, compared with 50% in *Xenopus*.

Because there is little cytological evidence for extrachromosomal DNA replication during plant development and no evidence for massive rDNA amplification (1), the observed correlation between satellite DNA and rDNA in plants requires further examination. The similarity in buoyant density between satellite and rDNA may be fortuitous, for rDNA has a similar density in species containing no satellite DNA (9, 16). Furthermore, a high percentage hybridization with rRNA does not necessarily imply a high redundancy of the rRNA gene, inasmuch as determination of this latter value depends on the size of the genome. In fact, high numbers of rRNA genes are found in species with low percentage hybridization but with large genomes (10, 18). The relationship between satellite DNA, defined as a minor component on neutral CsCl centrifugation, and rDNA has therefore been examined in terms of their respective buoyant densities in a larger range of plant species, and considered in the light of both percentage hybridization and the level of rRNA gene redundancy.

MATERIALS AND METHODS

DNA was prepared from total tissue by homogenization in a detergent mix followed by chloroform deproteinization. Further purification involved digestion with RNase and pronase, recovery by high speed centrifugation, and purification on a CsCl equilibrium density gradient (16). All DNA samples were monitored by Model E CsCl analytical centrifugation (19).

Explants of artichoke (*Helianthus tuberosus*) were cultured in medium containing ^{32}P orthophosphate or ^{3}H uridine, and seedings of swisschard (*Beta vulgaris* var. cicla), pea (*Pisum sativum*), onion (*Allium cepa*), wheat (*Triticum aestivum*), flax (*Linum usitatissimum*), maize (*Zea mays*) and Norway spruce (*Picea abies*) were grown in water culture in the presence of ^{32}P orthophosphate for 5 to 10 days. Total nucleic acid was prepared and then fractionated by gel electrophoresis, and the cytoplasmic rRNAs, 1.3×10^6 and 0.7×10^6 daltons, were eluted and recovered by high speed centrifugation (16).

Hybridization was carried out with the denatured DNA fixed to Millipore filters in 6 × SSC (SSC: 0.15 M NaCl, 15 mM sodium citrate, pH 7.2) at 70 C with 2 or 5 $\mu g\ ml^{-1}$ of rRNA for 4 or 2 hr, respectively (16). For the determination of the buoyant density of the rDNA, 50 to 100 μg of DNA was fractionated by CsCl equilibrium gradient centrifugation, each

fraction was fixed to a Millipore filter, and the filters were hybridized with rRNA (16).

The nuclear DNA content of the species was determined by comparative Feulgen photometry (12) of 10 telophase (2 c), or the modal DNA value from 50 interphase nuclei, from 2 replicates of root tips or shoot apices. Measurements were made relative to *Allium cepa*, containing 33.5 × 10^{-12} g DNA per telophase nucleus, respectively (12). With certain gymnosperms the Feulgen staining of root tips was rather variable and determinations were made on isolated nuclei (14).

RESULTS AND DISCUSSION

Buoyant Densities of Satellite and rDNA. The buoyant densities of the majority of plant satellite DNAs are within the range 1.703 to 1.712 g cm^{-3} (8), which also encompasses the density of rDNAs (16). However, when examined in detail, the peak hybridization of rDNA in melon seed DNA was at 1.711 g cm^{-3}, clearly different from the satellite at 1.707 g cm^{-3} (Fig. 1A). Similarly, with cucumber gherkin fruit DNA, the peak hybridization of rDNA at 1.708 g cm^{-3} was denser than

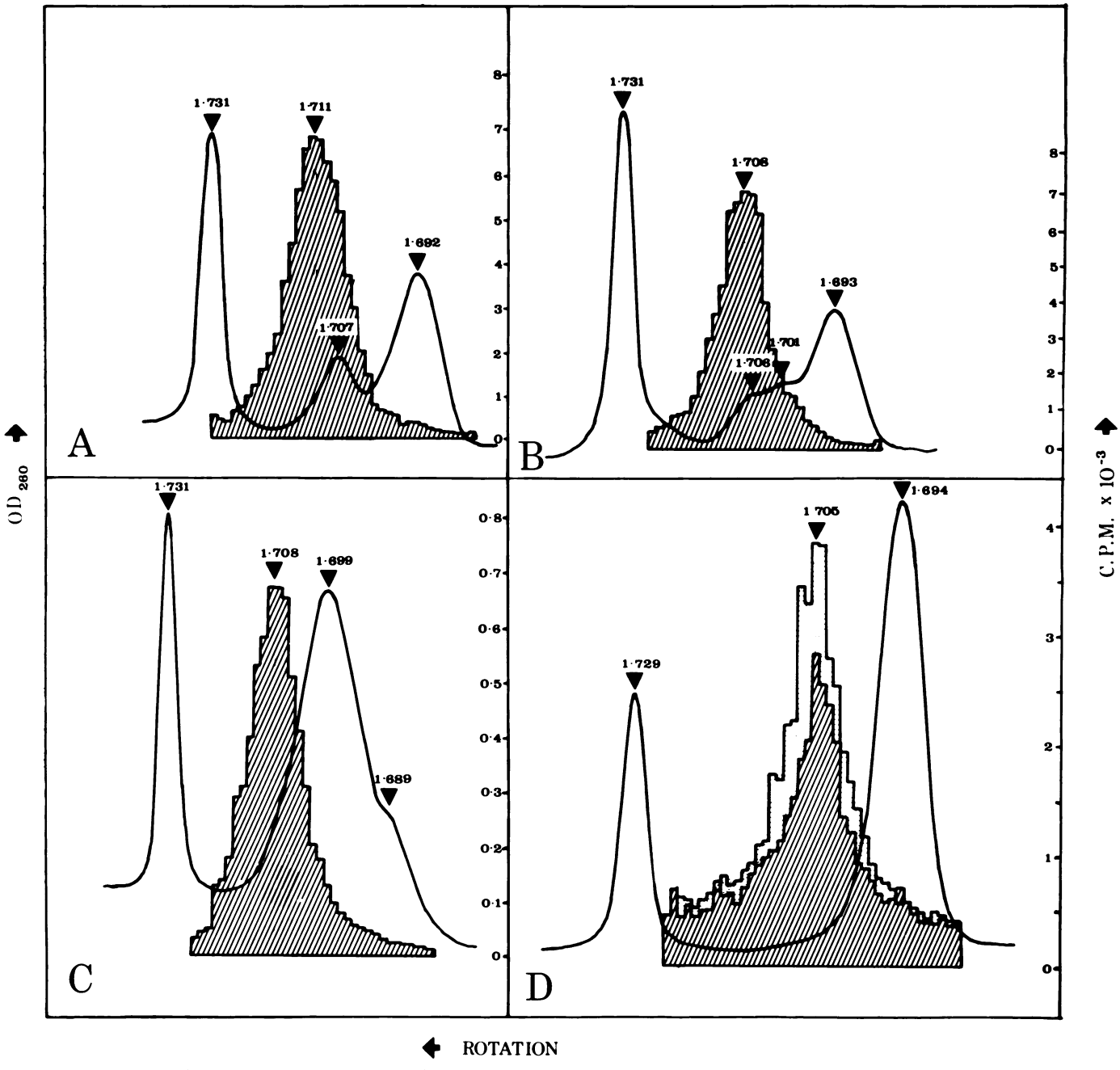

FIG. 1. Buoyant densities of satellite and rDNAs. Fifty to 100 μg of total DNA from melon seed (A), cucumber gherkin fruit (B), flax shoot, (C), and *Tulbaghia* shoot (D), plus *Micrococcus lysodeikticus* DNA (1.731 g cm^{-3}) were fractionated by preparative CsCl-equilibrium centrifugation for 66 hr at 35,000 rpm at 25 C in an MSE 50 rotor (continuous scan). The DNA in each fraction was denatured, fixed to a Millipore filter, and the filters hybridized with ^{32}P-labeled 1.3 × 10^6 dalton rRNA (3 μg/ml, 2 hr, 70 C, 6 × SSC) prepared from artichoke explants (histogram). The buoyant density of the hybridization peak was calculated from the densities of the mainband, satellite, and *M. lysodeikticus* DNA, determined from Model E analysis, assuming a linear gradient between the mainband and marker. In D, the absorbance profiles of the 2× and 4× *Tulbaghia* DNAs are superimposed, the hybridization of 2× DNA being indicated by the shaded histogram, and the 4× by the open histogram.

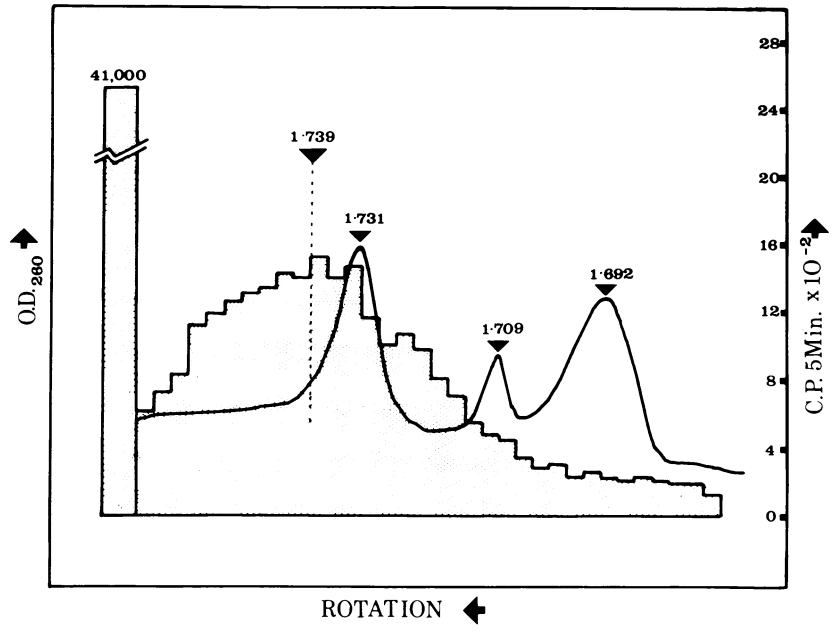

FIG. 2. Buoyant density of melon rRNA/DNA hybrid and satellite DNA. Ten μg of purified melon satellite DNA, containing 0.1 μg of the 1.3×10^6 mol wt rRNA gene, were denatured at 100 C for 5 min in 0.1 × SSC. The DNA was rapidly cooled, made up to 2 × SSC, and hybridized with 5 μg of 1.3×10^6 mol wt ^{32}P-labeled rRNA (50-fold excess) at 70 C for 1 hr. The mixture was then diluted to 1 × SSC, incubated for a further 3 hr at 60 C, cooled, and together with marker DNAs (melon mainband, 1.692 g cm^{-3}, *M. lysodeikticus* 1.731 g cm^{-3}) was fractionated by preparative CsCl-equilibrium centrifugation for 66 hr at 35,000 rpm at 25 C in the MSE 50 rotor (continuous scan). Fractions were diluted with 2 ml of 0.1 × SSC, and the radioactivity was determined by Cerenkov counting (histogram). The pellet, containing the free rRNA, was dissolved in 2 ml and similarly counted.

either of the satellites at 1.701 and 1.706 g cm^{-3} (Fig. 2B). With certain species, such as flax, satellite DNA is less dense than the mainband, 1.689 and 1.699 g cm^{-3}, while the rDNA was denser at 1.708 g cm^{-3} (Fig. 1C). In *Tulbaghia,* which contains no satellite, the rDNA had a density of 1.705 g cm^{-3} (Fig. 1D). With the range of plant species analyzed, the density of the peak of hybridization—the rDNA—was often similar but always distinguishable from the density of the satellite component, and was within the same range of r-DNA densities observed in species without satellite DNA (Table I).

The varying percentage of satellite present in different tissues of melon (14) offered another approach to consider the relationship between satellite DNA and rRNA hybridization. The percentage hybridization of seed DNA, which contained 25% satellite, to rRNA was less than that of the fruit DNA, containing only 18% satellite (Table II). In fact, the levels of hybridization correlated well with the percentages of the mainband, 75% in seed and 82% in the fruit.

This lack of correlation between the buoyant densities of the satellite and rDNA, and between percentage hybridization and percentage satellite in melon, suggests that the general relationship between satellite DNA and rDNA may be completely fortuitous. In certain species both components have similar base compositions and therefore fractionate at similar positions in a CsCl gradient.

The possibility that rDNA is integrated within the satellite DNA may be tested by CsCl analysis after aqueous hybridization of rRNA with satellite DNA. With large DNA the satellite would become much denser as the result of attached sequences of hybridized rRNA. Satellite and rRNA–DNA hybrid would only be resolved when the size of the DNA analyzed was reduced to approximately half that of the sequence under consideration, *i.e.* 1 to 1.5 × 10^6 daltons for the rRNA transcription unit, or 2 to 3 × 10^6 daltons of double stranded DNA. The hybrid and satellite would of course be resolved in any size of DNA if the rDNA was not an integral part of the satellite. CsCl gradient analysis of a 1.3 × 10^6 rRNA/melon satellite DNA (double stranded size of 7.20 × 10^6 daltons) hybridization mixture resolved a broad band of radioactivity at 1.739 g cm^{-3}, corresponding to the rRNA-DNA hybrid (16), and pelleted the bulk of the rRNA at the bottom of the tube (Fig. 2). The satellite DNA was at its normal renatured density of 1.709 g cm^{-3} (8). This resolution of hybrid and satellite DNA indicates that for this size of fragment, 7.2 × 10^6 daltons, the rDNA sequences are not an integral part of the satellite. This suggests that the non rDNA component of the satellite DNA is not dispersed between the rRNA genes, and is not analogous to spacer DNA in the *Xenopus* rDNA satellite. These results are perhaps consistent with the observation that the rDNA accounts for less than 5% of the satellite in plants, and in water beetles, compared with 50% in Xenopus.

Satellite DNA, rRNA Gene Redundancy and Genome Size. The species analyzed are arranged in Table III in order of increasing rRNA gene redundancy. It is clear that there is no correlation between high rRNA gene redundancy and the presence of satellite DNA. Several examples of different ploidy levels are included in the table. In euploids of *Hyacinthus orientalis,* the level of gene redundancy is proportional to the level of ploidy (18). This relationship also holds for *Tradescantia paludosa* and *T. virginiana,* diploid and tetraploid species, respectively. As previously reported (17), diploid and tetraploid species of *Nicotiana* do not show this relationship. The two tetraploid species examined, *N. rustica* and *N. tabacum* contain a similar number of rRNA genes, but this number is lower than that present in the diploid species, *N. glutinosa* and *N. sylvestris,* which themselves differ by a factor of two. These results are consistent with the conclusion of Siegel *et al.* (17) that a loss of rRNA genes is associated with tetraploidy in the genus *Nicotiana* but suggests a much greater range of redundancy within the genus. A similar reduction in redun-

Table I. *Buoyant Densities of Satellite and rRNAs*

Buoyant densities of mainband and satellite components were determined from Model E analysis relative to *M. lysodeikticus* DNA (1.731 g cm^{-3}). The density of the rDNA was determined from the hybridization peak from CsCl-fractionated DNA (Fig. 1). All the species were hybridized with artichoke rRNA (200–500 $\times 10^3$ cpm μg^{-1}).

Species	Buoyant Density		
	rDNA	Satellite	Mainband
		g cm^{-3}	
Cucumis melo (melon)	1.709–1.711	1.706	1.692
Cucumis sativus (cucumber)	1.709–1.711	1.702, 1.706	1.694
Cucumis sativus (cucumber gherkin)	1.708	1.701, 1.706	1.693
Phaseolus coccineus (runner bean)	1.701	1.702	1.693
Brassica rapa (turnip)	1.705	1.704	1.696
Aquilegia alpina	1.710	1.708	1.696
Citrus sinensis (orange)	1.711	1.712	1.694
Linum usitatissimum (flax)[1]	1.707–1.710	1.689	1.699
Oenothera fruticosa	1.705	1.699	1.704
Pisum sativum (pea)[1, 2]	1.703–1.705		1.695
Hyacinthus orientalis (hyacinth)[2]	1.712		1.700
Helianthus tuberosus (artichoke)[1, 2]	1.705		1.695
Zea mays (maize)[1, 2]	1.710–1.711		1.701
Passiflora antioquiensis (passion flower)[2]	1.712		1.700
Thalictrum aquilegiifolium	1.711		1.695
Beta vulgaris (swisschard)[1, 2]	1.704–1.714		1.694
Allium cepa (onion)[1, 2]	1.704–1.705		1.691
Triticum aestivum (wheat)[1, 2]	1.708–1.709		1.702
Juniperus chinensis pyramidalis[3]	1.707		1.695
Pseudotsuga douglasii (Douglas Fir)[3]	1.707		1.694
Tulbaghia violaceae[3]	1.705		1.694

[1] Also hybridized with homologous rRNA ($>30 \times 10^3$ cpm μg^{-1}).

[2] Data taken from Ref. 16.

[3] Also hybridized with Norway spruce rRNA (17×10^3 cpm μg^{-1}).

Table II. *Percentage of Satellite and Percentage Hybridization in Melon Seed and Fruit DNA*

The percentage of satellite DNA in melon seed and fruit DNAs was determined by Model E analytical equilibrium centrifugation. The percentage hybridization was determined with ^{32}P-labeled 1.3×10^6 and a mixture of 1.3×10^6 plus 0.7×10^6 mol wt rRNAs from artichoke (400×10^3 cpm μg^{-1}).

	Seed	Fruit
	%	
Satellite	25	18
Hybridization		
1.3×10^6 rRNA (2 μg/ml, 3 hr)	0.19	0.22
$1.3 \times 10^6 + 0.7 \times 10^6$ rRNA (2 + 1 μg/ml, 3 hr)	0.29	0.32
1.3×10^6 rRNA (5 μg/ml, 1 hr)	0.16	0.20

dancy is indicated with *Helianthus*, where the diploid *H. annuus* contains 6700 copies compared with only 1580 in the hexaploid *H. tuberosus*. A tetraploid of *Tulbaghia violaceae*, initially derived by colchicine treatment of the diploid, appeared as a normal tetraploid on detailed cytological examination and genome size, but contained almost 4 times, rather than twice, the redundancy of the diploid. Hybridization to

Table III. *Ribosomal RNA Genes, Genome Size, and Satellite*

The percentage of hybridization was determined with 1.3×10^6 or 1.3 plus 0.7×10^6 (indicated with asterisk) artichoke rRNA ($200–500 \times 10^3$ cpm μg^{-1}). The values in brackets indicate the intraspecies autopolyploidy level or the chromosome number for interspecies comparison.

Species	Genes per Telophase Nucleus	DNA Hybridized	Presence of Satellite	DNA $\times 10^{12}$ g per Telophase Nucleus
		%		
Citrus sinensis (orange)	1250	0.15	Sat.[1]	1.8
Thallictrum aquilegiifolium	1400	0.12		2.6
Helianthus tuberosus (artichoke) [102]	1580	0.022*		24
Passiflora antioquiensis (passion flower)	1800	0.13		3.0
Vicia benghalensis	1900	0.079		5.2
Linum usitatissimum (flax)	1980	0.33	Sat.	1.3
Cucumis melo (melon)	2000	0.23	Sat.	1.9
Lagenaria vulgaris	2100	0.33	Sat.	1.4
Nicotiana tabacum [48]	2200	0.049		9.6
Nicotiana rustica [48]	2200	0.049		9.6
Beta vulgaris (swisschard)	2300	0.20		2.5
Taxus baccata (yew)	2500	0.024		22.3
Nicotiana glutinosa [24]	3200	0.13		5.3
Oenothera fructicosa	3400	0.25	Sat.	2.9
Luffa cylindrica	3600	0.26	Sat.	3.0
Phaseolus coccineus (runner bean)	4000	0.46	Sat.	1.9
Juniperus chinensis pyramidalis	4100	0.046*		30
Aquilegia alpina	4600	0.91	Sat.	1.1
Tulbaghia violaceae [2×]	4650	0.024		42
Tradescantia paludosa [12]	4800	0.029		36
Nicotiana sylvestris [24]	4900	0.24		4.4
Momordica charantia	5500	0.29		4.1
Secale cereale (rye)	5700	0.065		19
Zea mays (maize)	6200	0.18		7.5
Trillium grandiflorum	6300	0.015		92
Helianthus annuus (sunflower) [34]	6700	0.16		8.8
Pseudotsuga douglasii (Douglas fir)	7200	0.064		25
Cucumis sativus (cucumber gherkin)	7700	0.62	Sat.	2.7
Pisum sativum (pea)	7800	0.17		10
Brassica rapa (turnip)	8600	1.17	Sat.	1.6
Tradescantia virginiana [24]	8600	0.030		62
Cucumis sativus (cucumber)	8800	0.96	Sat.	2.0
Cucurbita pepo (pumpkin)	9800	0.82	Sat.	2.6
Cucurbita pepo (marrow)	10500	0.99	Sat.	2.3
Pinus sylvestris (Scots Pine)	10700	0.096*		37
Triticum aestivum (wheat)	12700	0.092		30
Allium cepa (onion)	13900	0.090		33.5
Picea albertiana conica	13900	0.11*		36
Tulbaghia violaceae [4×]	16800	0.043		85
Hyacinthus orientalis (hyacinth) [2×]	16800	0.074		49
Picea abies (Norway spruce)	19300	0.15*		38
Hyacinthus orientalis [3×]	22600	0.069		71
Picea sitchensis (Sitka spruce)	24700	0.14		38
Larix decidua (larch)	26800	0.26*		30
Hyacinthus orientalis [4×]	31900	0.070		99

[1] Sat.: satellite.

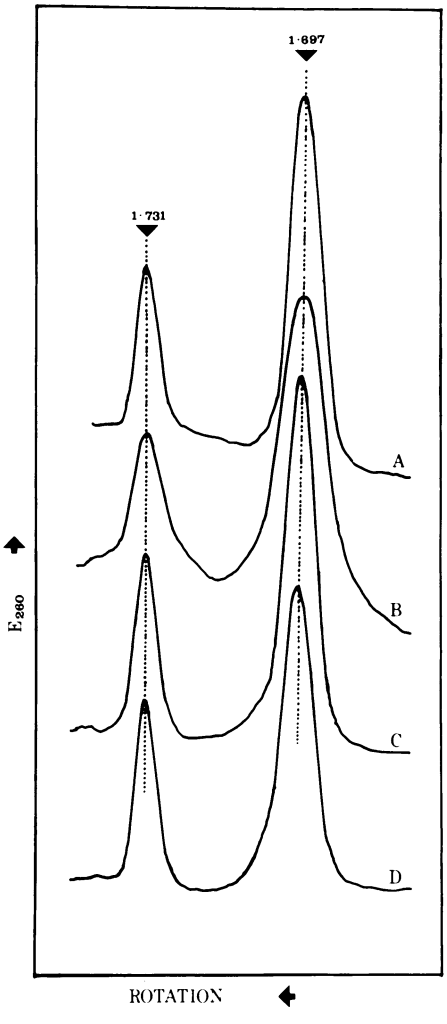

FIG. 3. Model E analysis *Nicotiana* sp. DNAs. Total DNA was prepared from root tips of *Nicotiana tabacum* (A), *N. rustica* (B), *N. glutinosa* (C), and *N. sylvestris* (D), and was analyzed by Model E equilibrium centrifugation at 44,000 rpm, 25 C for 24 hr. The buoyant density was calculated relative to *M. lysodeikticus* marker DNA at 1.731 g cm^{-3}.

CsCl-fractionated DNA indicated that the Tulbaghia 2× and 4× DNAs differed only in the amount of hybridization and not in the buoyant density of the rDNA (Fig. 1D). The results also indicate that gymnosperms in general do not have the very high gene redundancy suggested from the 4 species previously analyzed (7), but cover a range similar to that found with the angiosperms. The hybridization values shown in Table III are with artichoke rRNA, but very similar results were obtained with Norway spruce rRNA, indicating that the very high degree of conservation of the rRNA sequence previously noted for angiosperms (9, 11) extends to the gymnosperms.

Although there is no correlation between high rRNA redundancy and the presence of satellite DNA, the latter does appear to be associated with a higher percentage hybridization to rRNA, although there is some overlap. The lower percentage hybridizations of the satellite-containing species, *e.g.* orange and melon, are similar to the higher percentage hybridizations of nonsatellite containing DNAs, such as swiss-chard, *Momordica*, maize, and *N. sylvestris*. In this study there was no indication of significant satellite components specific to the diploid *Nicotiana* species (Fig. 3), contrasting with previous reports (17). This relationship between satellite and high percentage hybridization, however, really reflects a strong correlation between the presence of satellite DNA and a small genome. All those species with satellite have less than 3×10^{-12} g. It is interesting to note that the only member of the Cucurbitaceae that has no satellite, *Momardica*, has the largest genome size (4.1×10^{-12} g.) of these Cucurbitaceae. Conversely, *Aquilegia*, the only member of the Ranunculaceae containing satellite DNA (see Table I in ref. 8) has the smallest genome—1.1×10^{-12} g—of the Ranuculaceae species analyzed.

It was previously reported that none of 11 monocots examined contained satellite DNA (8). An additional 15 species have now been examined (*Convallaria majalis*, 1.698; *Crocus chrysanthus*, 1.699; *C. vernus*, 1.698; *Galanthus nivalis*, 1.694; *Iris danfordiae*, 1.698; *I. reticulata*, 1.699; *Leucojium aestivum*, 1.695; *Luzula purpurea*, 1.693; *Narcissus pseudonarcissus*, 1.697; *Phleum pratense*, 1.702; *Poa annua*, 1.698; *Scilla sibirica*, 1.700; *Tradescantia paludosa*, 1.696; *Trillium grandiflorum*, 1.698; and *Tulbaghia violaceae*, 1.693 g cm^{-3}), still with no evidence for any satellite component. The correlation between genome size and the presence of satellite DNA offers an explanation for this observation, since absence of satellite would be predicted from the monocot genome sizes, all of which are considerably greater than 3×10^{-12} g, the threshold value for satellite within the dicots. From published data of DNA content of monocots (2), none contained less than 3×10^{-12} g per telophase nucleus, and the smallest monocot genome examined in this study, *Phleum pratense*, with 3.7×10^{-12} g DNA (2), contained no satellite. The definition of satellite DNAs, minor components on CsCl analyses, must be stressed in this context. Simple repetitious sequences comparable in renaturation rate to satellite DNA are probably present in all plant genomes (1), and certainly Ag^+/Cs_2SO_4 satellites are resolved from many monocot genomes (unpublished data). Such sequences, however, appear to be resolved only as discrete CsCl satellite components in very small genomes.

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