Pl. Syst. Evol. 153, 119—132 (1986)

Plant Systematics and Evolution

Genome Size in Gymnosperms

By

D. Ohri and **T. N. Khoshoo**

(Received May 31, 1985; in definite form September 20, 1985)

Key Words: Gymnosperms. – Nuclear DNA content, evolution, polyploidy, woody habit.

Abstract: The DNA 2C and per chromosome values of 57 species belonging to 22 genera of gymnosperms have been analysed. The overall range is 12-fold with a modal value of about 30.0 pg. *Cycadales* exhibit a 2-fold difference. Among *Coniferales* with a 4-fold variation, the *Pinaceae* have higher mean DNA contents as well as a greater range and diversity than other families. Remarkable interspecific differences are found in *Cycas, Picea, Larix, Pinus, Callitris, Cupressus*, and *Chamaecyparis*. Despite this, there is a constancy of basikaryotypes within these genera. *Gnetum* shows a distinctly low DNA value.

The phylogenetic implications of the variation in DNA content in a major category like gymnosperms offer much interest. Even more they are the most ancient seed plants. The group, however, is considered to be an assemblage of heterogenous taxa, the common character of naked seed having evolved in many parallel lineages of Paleozoic plants (TAYLOR 1976, TAYLOR & DELEVORYAS 1982). Moreover, the extant genera are the end points in their respective evolutionary lines without direct relationships. After having dominated the vegetation during the later Paleozoic and Mesozoic eras the group now comprises only some 70 genera and roughly 730 species. Therefore, our study is only of limited value for explaining the phylogenetic relationships between the various gymnosperm groups.

The DNA contents of 236 gymnosperm species have been analysed by PRICE & al. (1973a). The discrepancies in their techniques and the low reliability of their results have been discussed (HESEMANN 1980). Furthermore, roughly 500 species belonging to 60 genera have been studied cytologically. However, a comparison of absolute chromosome sizes among different taxa is not feasible as various authors have used different tissues and techniques. Besides, different species or even genera

Table 1. Chromosome number (CN), 2C and per chromosome (Ch) DNA content in 22 genera (57 species) of gymnosperms. * = Chromosome numbers analysed in the present study. $\bar{x}$ = Mean of four replications. S.E. = Standard Error

Species	Source	CN (2n=)	DNA in pg	
			2C	Ch
			$\bar{x}$ ± S.E.	
I. *Cycadales*				
1. *Cycadaceae*				
Cycas circinalis L.	NBRI, Lucknow, India.	22*	29.51 ± 1.36	1.34
C. revoluta THUNB.	-do-	22*	25.54 ± 1.28	1.16
Encephalartos villosus LEM.	-do-	18*	42.17 ± 1.41	2.34
Zamia angustifolia JACQUIN	-do-	16	24.12 ± 1.45	1.50
II. *Ginkgoales*				
2. *Ginkgoaceae*				
Ginkgo biloba L.	NBRI, Lucknow, India.	24	19.86 ± 1.42	0.82
III. *Coniferales*				
3. *Araucariaceae*				
Araucaria cookii R. BROWN ex LIND.	NBRI, Lucknow, India.	26*	19.14 ± 1.51	0.73
A. cunninghamii D. DON	-do-	26*	21.77 ± 1.67	0.83
Agathis robusta (C. MOORE) F. M. BAILEY	-do-	26*	21.64 ± 1.31	0.83
4. *Podocarpaceae*				
Podocarpus gracilior PILGER	-do-	24	22.75 ± 1.32	0.94
5. *Pinaceae*				
Abies sibirica LEDEB.	Agricultural Canada Research St. Mordon, Manitoba, Canada.	24*	31.63 ± 1.41	1.31
Larix sibirica LEDEB.	-do-	24*	24.59 ± 1.11	1.02
L. × *eurolepis* A. HENRY	Hortus Botanicus Univ. van Amsterdam Plantage Middenlaan Amsterdam, Netherlands.	24	30.77 ± 1.35	1.28
L. gmelini (RUPR.) KUZ.	Hortus Botanicus Tallinensis, Academiae Scientiarum Estonie Tallin-19, Kloorstrimet-sa Tee 44, USSR.	24*	27.98 ± 1.33	1.16

Table 1 (continued)

Species	Source	CN (2n=)	DNA in pg	
			2C	Ch
			$\bar{x} \pm$ S.E.	
Picea glauca (MOENCH) VOSS	Hortus Botanicus Principalis Academie Scientiarum Moscow, USSR.	24*	40.41 ± 1.48	1.68
P. mariana (MILL.) BRITT.	-do-	24*	31.61 ± 1.41	1.31
P. pungens ENGELM.	-do-	24*	40.03 ± 1.39	1.66
P. orientalis (L.) LINK	Hortus Botanicus Univ. van Amsterdam, Plantage Middenlaan, Netherlands.	24*	37.16 ± 1.19	1.54
Pinus caribaea MORELET	U.S. National arboretum Washington DC., U.S.A. (Guatemala).	24*	39.09 ± 1.28	1.62
P. elliottii ENGELM.	-do- (Alabama).	24*	35.27 ± 1.36	1.46
P. taeda L.	-do- (Louisiana).	24*	37.62 ± 1.51	1.56
P. taeda	-do- (Georgia).	24	37.79 ± 1.16	1.57
P. taeda	-do- (Texas).	24	38.14 ± 1.14	1.58
P. taeda	-do- (Mississippi).	24	37.68 ± 1.35	1.56
P. virginiana MILL.	-do- (North-Carolina).	24	33.21 ± 1.28	1.38
P. mugo TURRA	Inverni Della Beffa, Milanoa Italy.	24*	40.12 ± 1.33	1.67
P. mugo	Hortus Botanicus Tallinesis, Academiae Scientiarum, Estonie Tallin-19 Kloorstrimet-sa Tee 44, USSR.	24	40.29 ± 1.39	1.67
P. banksiana LAMB.	-do-	24*	34.35 ± 1.37	1.43
P. divaricata (AIT.) DUM.-COURS.	Jardin Botanique de Montreal Quebec, Canada.	24	34.53 ± 1.18	1.43
P. rigida MILL.	-do-	24*	41.59 ± 1.21	1.73
P. ponderosa DOUGL.	Agricultural Canada Research St. Mordon, Manitoba, Canada.	24*	39.69 ± 1.41	1.65

Table 1 (continued)

Species	Source	CN (2n=)	DNA in pg	
			2C	Ch
			$\bar{x} \pm$ S.E.	
P. resinosa AIT.	Glenden Hall Research Lab., Univ. of Toronto, Ontario, Canada.	24*	46.72 ± 1.51	1.94
P. contorta var. *latifolia* S. WATSON	Botanical Garden Univ. of British Columbia, Vancouver, Canada.	24*	35.64 ± 1.38	1.48
P. pinaster AIT.	Jardin Botanic Blanes, Prov. Gerona, Spain.	24*	48.71 ± 1.49	2.02
P. excelsa HOOK.	Jardin, Botanique de la ville de Limoges, France.	24	48.15 ± 1.21	2.00
P. maritima LAMB.	-do-	24*	48.08 ± 1.34	2.00
P. gerardiana WALL.	Kalpa, (H.P.), India.	24*	57.35 ± 1.41	2.38
P. patula SCHLECHT. & CHAM.	Kalika, (U.P.), India.	24*	36.91 ± 1.04	1.53
P. wallichiana A. B. JACKS	Arizol, (Kashmir), India.	24*	49.22 ± 1.23	2.05
P. wallichiana	Bhutan.	24	49.15 ± 1.11	2.04
P. roxburghii SARG.	Solan, (H.P.), India.	24*	38.81 ± 1.24	1.61
P. thunbergii PARL.	Botanical Garden Tohoku Univ., Japan	24	43.97 ± 1.51	1.83
P. densiflora SIEB. & ZUCC.	-do-	24*	42.96 ± 1.46	1.79
6. *Taxodiaceae*				
Cunninghamia lanceolata (LAMB.) HOOK. f.	FRI Dehra Dun, India.	22*	27.12 ± 1.45	1.23
Taxodium mucronatum	FRI Dehra Dun, India.	22*	17.48 ± 1.22	0.79
Metasequoia glyptostroboides HU & CHENG	NBRI, Lucknow, India.	22	12.95 ± 1.14	0.58
7. *Cupressaceae*				
Callitris glauca R. BR.	FRI Dehra Dun, India.	22*	16.53 ± 1.12	0.75

Table 1 (continued)

Species	Source	CN (2 n =)	DNA in pg	
			2C	Ch
			$\bar{x} \pm$ S.E.	
C. rhomboidea R. Br. ex L. C. Rich.	Royal Botanic Gardens, South Yarra, Victoria, Australia.	22*	22.33 ± 1.02	1.01
C. verrucosa A. Cunn. ex Endl.) Müller	-do-	22*	20.84 ± 1.42	0.94
Tetraclinis articulata (Vahl) Mast.	Botanischer Garten der Univ. Erlangen, W. Germany.	22*	23.59 ± 1.31	1.07
Cupressus arizonica Greene	Jardin, Botanic Blanes, Prov. Gerona, Spain.	22*	23.59 ± 1.23	1.08
C. sempervirens L.	-do-	22	22.74 ± 1.31	1.03
C. sempervirens	Univ. Campus Srinagar, Kashmir, India.	22	22.61 ± 1.24	1.02
C. sempervirens	FRI Dehra Dun, India.	22	23.02 ± 1.34	1.04
C. sempervirens var. *stricta* Aiton	Botanischer Garten der Univ. Bonn, W. Germany	22	23.09 ± 1.42	1.04
C. macrocarpa Hartw.	Royal Botanic Gardens, South Yarra, Victoria, Australia.	22*	28.36 ± 1.24	1.28
C. guadalupensis Sarg.	Jardin Botanique de Montreal Quebec, Canada.	22	23.67 ± 1.26	1.07
C. glabra var. *conica* Sudw.	Jardin Botanique de la Ville et de l'Univ. de Caen, France.	22	22.97 ± 1.33	1.04
Chamaecyparis lawsoniana (A. Murr.) Parl.	Hortus Botanicus Univ. van Amsterdam, Netherlands.	22*	30.10 ± 1.50	1.36
C. pisifera (Sieb. & Zucc.) Endl.	Botanischer Garten der Univ. Bonn, W. Germany.	22	22.11 ± 1.41	1.00
C. obtusa (Sieb. & Zucc.) Endl.	Botanical Garden Tohoku Univ., Japan.	22*	27.44 ± 1.12	1.24

Table 1 (continued)

Species	Source	CN (2n=)	DNA in pg	
			2C	Ch
			$\bar{x} \pm$ S.E.	
Thuja plicata D. DON	Jardin Botanique de la ville et de l'Univ. de Caen, France.	22	24.59 ± 1.11	1.11
T. occidentalis L.	Botanical Garden, Univ. of Turku, Finland.	22*	23.27 ± 1.34	1.05
Biota orientalis ENDL.	Univ. Campus Srinagar, Kashmir, India.	22*	22.79 ± 1.25	1.03
IV. *Ephedrales*				
8. *Ephedraceae*				
Ephedra tweediana C. A. MEY	NBRI, Lucknow, India.	14*	17.75 ± 1.27	1.26
9. *Gnetaceae*				
Gnetum ula BRONGN	-do-	22	4.54 ± 0.51	0.20

are characterized by similar basikaryotypes. In the present study, therefore, 57 species belonging to 22 genera have been studied in respect to their 2C DNA values, using a uniform procedure. This, we hope, will add a new dimension to the discussion of cytogenetical processes of evolution in gymnosperms.

Material and Methods

Seeds were obtained from different sources listed in Table 1, and were germinated in saw dust. Root tips (leaf tips in case of *Cycas, Encephalartos, Zamia, Ginkgo*, and *Metasequoia*) were excised when they were approximately 3 cm long. After washing they were fixed in 4% formaldehyde in neutral phosphate buffer for 2 hours, followed by thorough washing in distilled water for 24 hours. Subsequently, root/leaf tips were hydrolysed in 5N HCl at room temperature (20 °C) for 1 hour; then given a wash in distilled water for 1 minute and transferred to Feulgen solution adjusted to pH 2.2 with 1N NaOH. After 1 hour in Feulgen they were given three washes of 10 minutes each in SO_2 water to bleach any stain left in cytoplasm. The root/leaf tips were then transferred to distilled water and squashed under the coverslip in glycerol. Four slides of each material were prepared and 60 2C nuclei at late telophase were read on a Vickers microdensitometer at 565 nm. In each case the mean of arbitrary units was converted to picograms of DNA using means of identical number of readings from *Allium cepa* root tips, processed

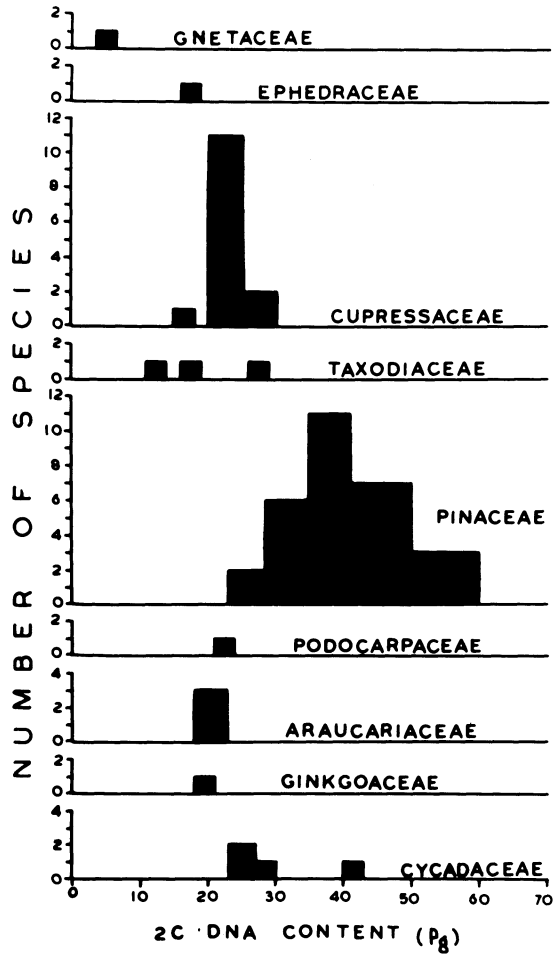


Fig. 1. Frequency diagrams representing the quantitative DNA variation pattern in various families. Abscissa: DNA amount subdivided into frequency classes of 10 picograms; ordinate: number of species in each frequency class

simultaneously in the same tubes as the gymnosperm material. The 2C DNA value of *Allium cepa* was taken as 33.55 pg (VANT HOF 1965). DNA content per chromosome was obtained by dividing 2C values by chromosome number (2 n).

Results

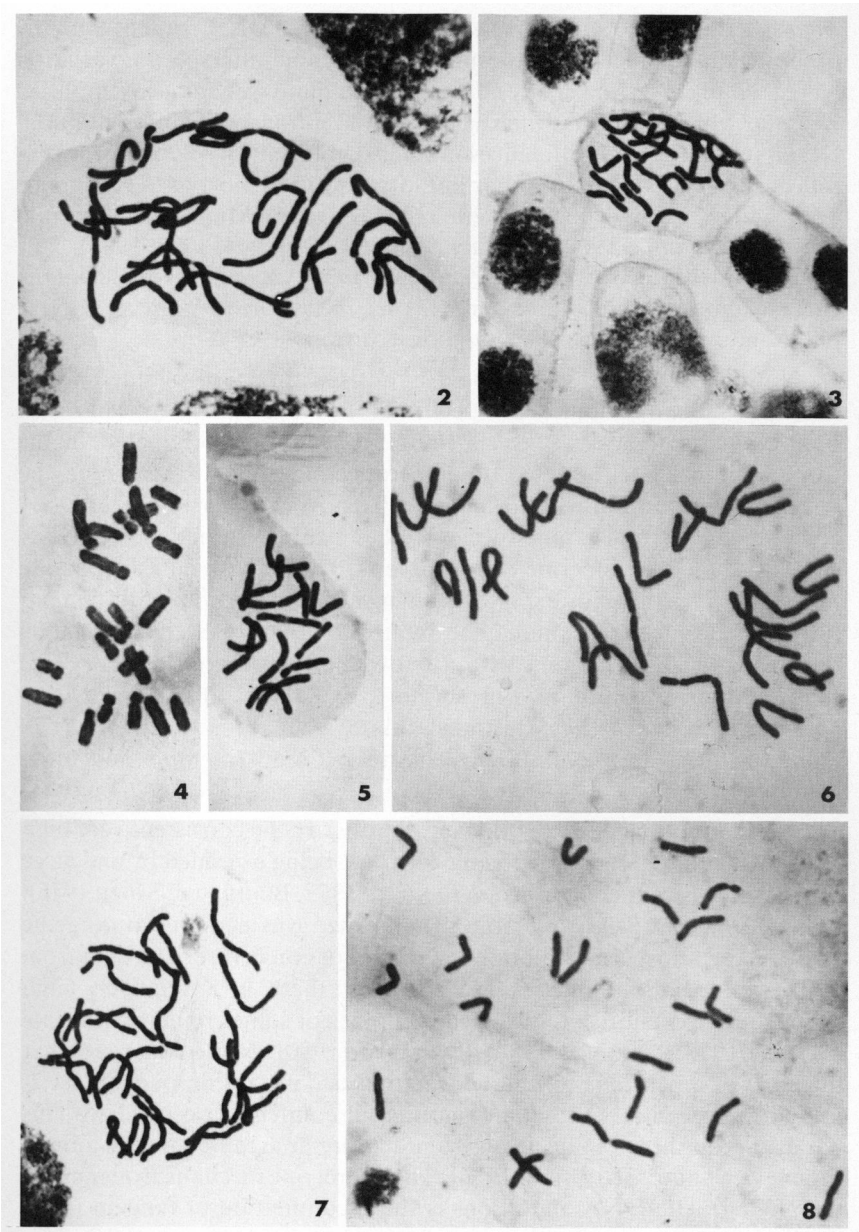
The respective chromosome numbers, DNA 2C and per chromosome values of 57 species belonging to 22 genera are listed in Table 1. The range between a minimum (*Gnetum ula*: 4.54 pg) and a maximum (*Pinus*

gerardiana: 57.35 pg) is 12.63-fold, with a modal value of about 30 pg (Fig. 1). A notable feature is that this variation has occurred primarily at the diploid level (Figs. 2–8). Among *Cycadales* studied the maximum DNA content is exhibited by *Encephalartos villosus* (42.17 pg). *Ginkgo biloba*, the only extant species of *Ginkgoales*, has 19.86 pg. Among *Coniferales*, the genera belonging to *Pinaceae* show higher mean DNA contents as well as a greater range and diversity than other families (Fig. 1). Within the order DNA contents exhibit a 4.42-fold difference. Because of extremely scanty data in *Araucariaceae, Podocarpaceae* and *Taxodiaceae*, no indication about the range of DNA content variation can be obtained in these families. *Pinaceae* and *Cupressaceae*, however, exhibit a variation of 2.33- and 1.82-fold, respectively. Furthermore, the genus *Pinus* shows a higher mean DNA content than other genera of *Pinaceae*. Very remarkable interspecific differences are found in *Cycas* (25.54–29.51 pg), *Picea* (31.6–40.4 pg), *Larix* (24.5–30.7 pg), *Pinus* (33.2–57.3 pg), *Callitris* (16.5–22.3 pg), *Cupressus* (22.6–28.3 pg) and *Chamaecyparis* (22.1–30.1 pg) (Table 1). *Ephedrales* with *Ephedra tweediana* (17.7 pg) exhibit a lower DNA value than the members of *Coniferales*. *Gnetum ula* is quite distinct, having the lowest DNA value (4.54 pg) among all gymnosperms. Furthermore, the DNA contents are not correlated with chromosome numbers (Table 1). A notable feature is that no intraspecific differences were detected in different seed sources of *Pinus mugo, P. taeda, P. wallichiana* and *Cupressus sempervirens* (Table 1).

Discussion

As compared with angiosperms and their 600-fold DNA-variation (BENNETT & al. 1982), the genome size of gymnosperms with just 12.63-fold variation can be described as fairly homogeneous. If the value of *Gnetum* is excluded, this range is reduced to only 4.42-fold. This is in keeping with the general conservatism of gymnosperms with respect to chromosomal diversity, breeding system, life form, habitats, etc. (KHOSHOO 1962, LEVIN & WILSON 1976, PRAGER & al. 1976, EHRENDORFER 1976).

It is quite impossible to demonstrate any general phylogenetic trends considering the antiquity and great divergence of living gymnosperm families. The only definite conclusion which can be drawn is that both phylogenetic increase and decrease in DNA content must have taken place many times during evolution. Among *Coniferales* a general trend towards decrease with phylogenetic advancement may be indicated by the fact that both *Cupressaceae* and *Taxodiaceae* ($x = 11$) have markedly less per chromosome DNA values than *Pinaceae* ($x = 12$) (Table 1). Even among



Figs. 2–8. Somatic chromosomes of Gymnosperms. × 700. – Fig. 2. *Pinus roxburghii*, 2 n = 24; – Fig. 3. *Taxodium mucronatum*, 2 n = 22; – Fig. 4. *Cycas circinalis*, 2 n = 22; – Fig. 5. *Ephedra tweediana*, 2 n = 14; – Fig. 6. *Pinus pinaster*, 2 n = 24; – Fig. 7. *Pinus ponderosa*, 2 n = 24; – Fig. 8. *Callitris glauca*, 2 n = 22

Pinaceae the genus *Pinus* possesses higher average DNA content than the other genera (Table 1). Furthermore, remarkable interspecific variation has taken place during species divergence in many genera as exemplified by *Pinus* which shows as much as a 1.72-fold intrageneric differentiation. Despite such marked differences, a remarkable karyotypic uniformity is reflected by the constancy of basikaryotypes within most of the genera or even within closely allied genera (Figs. 2, 6, 7) MEHRA & KHOSHOO 1956a, b; KHOSHOO 1962; PEDERICK 1970; SAYLOR 1964, 1972, 1983). This demonstrates some sort of constraint upon DNA increments in each of the chromosomes of the complements, as has been shown in many diverse plant and animal species (see OHRI & KHOSHOO 1986).

Nothing, however, can be said about the direction of interspecific alterations. In an ecological perspective of the reproductive cycle in *Pinus*, FRANCINI-CORTI (1962) suggests that the genus has originated in the tropics and has later adapted to temperate conditions. If this is true, the direction of evolution within *Pinus* has been from lower to higher DNA content, as the tropical pine species studies exhibit distinctly lower DNA contents than the temperate ones (Table 1). The highest value is found in *P. gerardiana*, a temperate species growing in highly xeric habitats. Similar phylogenetic considerations can be applied to other genera by correlating the trends in DNA content with a knowledge of their past history and ecogeographic distribution. Furthermore, the DNA contents of *Ephedra* and particularly *Gnetum* do substantiate what EHRENDORFER (1976) remarked "the deep evolutionary hiatus between *Gnetatae* and other gymnosperms ...".

Another notable feature of gymnosperms are the consistent very high DNA values as compared with angiosperms, being exceeded by and large only in some perennial monocots (BENNETT 1972, BENNETT & SMITH 1976). Exactly how and why this large genome size was acquired during the course of gymnosperm evolution is a matter of conjecture. The data from DNA reassociation kinetics indicate that there is a relatively high proportion of repetitive DNA in the genomes of some conifers (MIKSCHE & HOTTA 1973, RAKE & al. 1980). RAKE & al. (1980) also demonstrates that the ratio of repetitious to unique fractions is constant despite almost 2-fold difference in DNA content among four conifer species studied. This has also been shown to be a consistent feature in many plant and animal genera (see OHRI & KHOSHOO 1986). Therefore, one mechanism leading to the phylogenetic DNA alterations is the accumulation of tandem DNA duplications (OHNO 1970, SPARROW & NAUMAN 1976, GRANT 1976). This has apparently been possible because the function of water conduction in conifers is performed by tracheids in contrast to woody angiosperms which have vessels. As there is a general proportionality between nuclear

and cell size (PRICE & al. 1973b) the check in woody dicots is excercised by the cambial cells which form wood fibres (see DARLINGTON 1937, STEBBINS 1950, KHOSHOO 1962). While in conifers the need for efficient water conduction has resulted in strong selection for bigger cell size as depicted by giant cambial cells in *Pinus strobus* (WILSON 1964, 1966) and consequently increased nuclear volume and DNA content (CAVALIER-SMITH 1978). In this context it is significant to note the association of very high DNA value (as in *Pinus gerardiana*: 57.35 pg) with temperate and highly xeric habitat (Table 1). It would be worth studying tracheid dimensions vis-à-vis DNA content in conifer species in relation to ecological conditions. Here it is interesting to note a general correlation of seed size with DNA content among pine species studied. Some of the smallest (*P. virginiana*) and largest (*P. gerardiana*) seed sizes are associated with minimum (33.21 pg) and maximum (57.35 pg) DNA values respectively. This selection for higher DNA contents associated with slow rates of development may be responsible for the absence of annual habit (CAVALIER-SMITH 1978). Curiously, gymnosperm shrubs and lianas have comparatively lower DNA values, the distinctly lowest being those of *Gnetum* which also has vessels.

Furthermore, the greater DNA redundancy has in all probability put a constraint on further divergent evolution at polyploid levels as the treshold value for genome size was already reached and further increases by polyploidy could not be tolerated (see OHNO 1970, MIKSCHE & HOTTA 1973, EHRENDORFER 1976, GRANT 1976). It is pertinent to remark here that endopolyploidy which is so characteristic of angiosperms has so far not been found in gymnosperms (NAGL 1978); this could mean that increases in nuclear and cell size during ontogeny, also, are not tolerated. Besides, all the cases of generative polyploidy among *Coniferales* are found in *Cupressaceae* and *Taxodiaceae*, families which incidentally have comparatively smaller chromosomes and lower DNA values than *Pinaceae* (KHOSHOO 1959, EHRENDORFER 1976).

Lastly, a point worth discussing is the intraspecific DNA variation reported for many North American conifer species. Their DNA amount have been shown to vary from lowest to highest by a factor of 1.5, 1.6, 1.7, and 1.9, respectively, among provenances of *Pinus banksiana, Picea glauca* (MIKSCHE 1968), *Picea sitchensis* (MIKSCHE 1971) and *Pseudotsuga menziesii* (EL-LAKANY & SZIKLAI 1971). Furthermore, the increase in DNA supposedly shows a clinal pattern from south to north in the geographic range of the species. The maximum variation by a factor of 2.2, without a clinal pattern, was detected in 20 seed sources of *Pinus resinosa* (DHIR & MIKSCHE 1974). TEOH & REES (1976), however, failed to repeat MIKSCHE's observations in *Picea glauca*, collected from the same range. A constancy

in DNA amount was observed, except for some differences between provenances due to variable number of B-chromosomes. Similar uniformity in DNA values was also observed in *Pinus contorta* and *Picea engelmannii* (TEOH & REES 1976), and in five populations of *Pinus rigida* (DHILLON & al. 1978). Such constancy of DNA values among different seed sources also has been demonstrated in the present study for *Pinus taeda, P. mugo* and *Cupressus sempervirens*. Remarkably, two provenances of *Pinus wallichiana*, one from the extremely dry area of the Northwest Himalayas with an annual precipitation of 10 to 20″, and the other from the wet eastern region (Bhutan) with an annual precipitation of upto 200″, also show no difference in DNA content (Table 1). Similar observations have also been made in 20 provenances of *P. roxburghii* from the western Himalayas (OHRI & KHOSHOO 1986, in prep.).

References

BENNETT, M. D., 1972: Nuclear DNA contents and minimum generation time in herbaceous plants. – Proc. Roy. Soc. London, Ser. B, **181**, 109–135.

– SMITH, J. B., 1976: Nuclear DNA amounts in Angiosperms. – Philos. Trans., Ser. B, **274**, 227–273.

– – HESLOP-HARRISON, J. S., 1982: Nuclear DNA amounts in Angiosperms. – Proc. Roy. Soc. London, Ser. B, **216**, 179.

CAVALIER-SMITH, T., 1978: Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. – J. Cell Sci. **34**, 247–278.

DARLINGTON, C. D., 1937: Recent Advances in Cytology. 2nd ed. – London: Churchill.

DHILLON, S. S., BERLYN, G. P., MIKSCHE, J. P., 1978: Nuclear DNA in populations of *Pinus rigida*. – Amer. J. Bot. **65**, 192–196.

DHIR, N. K., MIKSCHE, J. P., 1974: Intraspecific variation of nuclear DNA content in *Pinus resinosa*. – Can. J. Genet. Cytol. **16**, 77–83.

EHRENDORFER, F., 1976: Evolutionary significance of chromosomal differentian patterns in Gymnosperms and primitive Angiosperms. – In BECK, C. B., (Ed.): Origin and Early Evolution of Angiosperms, pp. 220–240. – New York: Columbia University Press.

EL-LAKANY, M. H., SZIKLAI, O., 1971: Intraspecific variation in nuclear characteristics of Douglas-Fir. – Advancing Frontiers Pl. Sci. **28**, 363–378.

FRANCINI-CORTI, E., 1962: Ecology of the haploid generation in *Pinus*. – Advancing Frontiers Pl. Sci. **1**, 35–49.

GRANT, W. F., 1976: The evolution of karyotype and polyploidy in arboreal plants. – Taxon **25**, 75–84.

HESEMANN, C. U., 1980: Cytophotometrical measurement of nuclear DNA content in some coniferous and deciduous trees. – Theor. Appl. Genet. **57**, 187–191.

KHOSHOO, T. N., 1959: Polyploidy in Gymnosperms. – Evolution **13**, 24–39.

– 1962: Cytogenetical evolution in Gymnosperms–karyotype. – Proc. Summer School, Darjeeling, Govt of India, 119–135.

LEVIN, D. A., WILSON, A. C., 1976: Rates of evolution in seed plants: Net increase in diversity of chromosome numbers and species numbers through time. – Proc. Nat. Acad. Sci. U.S.A. **73**, 2086–2090.

MEHRA, P. N., KHOSHOO, T. N., 1956: Cytology of Conifers I. – J. Genet. **54**, 165–180.

– – 1956: Cytology of Conifers II. – J. Genet. **54**, 181–185.

MIKSCHE, J. P., 1968: Quantitative study of intraspecific variation of DNA per cell in *Picea glauca* and *Pinus banksiana*. – Can. J. Genet. Cytol. **10**, 590–600.

– 1971: Intraspecific variation of DNA per cell between *Picea sitchensis* (BONG.) CARR. provenances. – Chromosoma **32**, 343–352.

– HOTTA, Y., 1973: DNA base composition and repetitious DNA in several Conifers. – Chromosoma **41**, 29–36.

NAGL, W., 1978: Endopolyploidy and Polyteny in Differentiation and Evolution. – Amsterdam: North-Holland.

OHNO, S., 1970: Evolution by Gene Duplication. – New York: Springer.

OHRI, D., KHOSHOO, T. N., 1986: Plant DNA–contents and systematics. – In DUTTA, S. K., (Ed.): DNA Systematics. Vol. II, Plants. – Florida: CRC Press.

– – 1986: Constancy of nuclear DNA amount in 20 provenances of *Pinus roxburghii* SARG. – (In preparation).

PEDERICK, L. A., 1970: Chromosome relationships between *Pinus* species. – Silvae Genet. **19**, 171–180.

PRAGER, E. M., FOWLER, D. P., WILSON, A. C., 1976: Rates of evolution in Conifers (*Pinaceae*). – Evolution **30**, 637–649.

PRICE, H. J., SPARROW, A. H., NAUMAN, A. F., 1973a: Evolutionary and developmental considerations of the variability of nuclear parameters in higher plants I. Genome volume, interphase chromosome volume and estimated DNA content of 236 Gymnosperms. – Brockhaven Symp. Biol. **25**, 390–421.

– – – 1973b: Correlations between nuclear volume, cell volume and DNA content in meristematic cells of herbaceaous Angiosperms. – Experientia **29**, 1028–1029.

RAKE, A. V., MIKSCHE, J. P., HALL, R. B., HANSEN, K. M., 1980: DNA reassociation kinetics of four Conifers. – Can. J. Genet. Cytol. **22**, 69–79.

SAYLOR, L. C., 1964: Karyotype analysis of *Pinus* Group *Lariciones*. – Silvae Genet. **13**, 165–170.

– 1972: Karyotype analysis of the genus *Pinus* subg. *Pinus*. – Silvae Genet. **21**, 155–163.

– 1983: Karyotype analysis of the genus *Pinus* subg. *Strobus*. – Silvae Genet. **32**, 119–124.

SPARROW, A. H., NAUMAN, A. F., 1976: Evolution of genome size by DNA doubling. – Science **192**, 524–529.

STEBBINS, G. L., 1950: Variation and Evolution in Plants. – New York: Columbia Univ. Press.

TAYLOR, T. N., 1976: Introduction: Patterns in Gymnosperm evolution. – Rev. Paleobot. Palynol. **21**, 1–3.

– DELEVORYAS, T., 1982: Introduction: Gymnosperms, Paleozoic and Mesozoic. – Rev. Paleobot. Palynol. **37**, 1–5.

TEOH, S. B., REES, H., 1976: Nuclear DNA amounts in populations of *Picea* and *Pinus* species. – Heredity **36**, 123–127.

VAN'T HOF, J., 1965: Relationships between mitotic cycle duration, S period duration and the average rate of DNA synthesis in root meristem cells of several plants. – Exp. Cell. Res. **39**, 48–58.

Wilson, B. F., 1964: A model for cell production by the cambium of Conifers. – In Zimmermann, M. H., (Ed.): The Formation of Wood in Forest Trees. – London: Academic Press.
– 1966: Mitotic activity in the cambial zone of *Pinus strobus*. – Amer. J. Bot. **53**, 364 – 372.

Authors' addresses: Dr. D. Ohri, Cytogenetics Laboratory, National Botanical Research Institute, Lucknow-226001, India. – Dr. T. N. Khoshoo, Distinguished Scientist (CSIR) Tata Energy Research Institute, 7 Jor Bagh, New Delhi-110003, India.