Theor Appl Genet (1992) 83:273–278

THEORETICAL AND APPLIED GENETICS

Variation of genome size and organization within hexaploid *Festuca arundinacea*

M. Ceccarelli [1], E. Falistocco [2], and P.G. Cionini [1,*]

[1] Department of Plant Biology of the University, [2] Center of Studies for the Genetic Improvement of Forage Plants of C.N.R., Borgo XX Giugno 74, I-06100 Perugia, Italy

Received June 19, 1989; Accepted June 11, 1991
Communicated by F. Mechelke

Summary. Cytophotometric, karyological, and biochemical analyses were carried out in the meristems of seedlings obtained from seeds collected from 35 natural populations of hexaploid *Festuca arundinacea* in Italy. Highly significant differences between populations were observed in the amount of nuclear DNA (up to 32.3%). These changes are linked to variations in the amount of heterochromatin and in the frequency of repeated DNA sequences, and particularly of a fraction of them. Differences between populations in the arm ratios and total length of the chromosomes were also observed. The genome sizes of the populations are correlated positively with the mean temperature during the year and with that of the coldest month at the stations, and correlate negatively with their latitudes. The intraspecific genome changes observed are discussed in relation to other pertinent data to be found in the literature and in relation to their possible role in environmental adaptation.

Key words: *Festuca arundinacea* – Intraspecific DNA changes – DNA cytophotometry – Karyology – Environmental adaptation

Introduction

It is known that the C-value (the DNA content of the unreplicated haploid chromosome complement) of eukaryotes spans several orders of magnitude (Sparrow et al. 1972; Bennett and Smith 1976). Differences in the frequency of noncoding, repeated DNA sequences have been demonstrated to account for this. Thus, the relationship between the number of genes and the basic nuclear DNA content differs greatly from species to species and this forms the basis of the C-value paradox (Thomas 1971). An increasing number of reports in the literature concerning both animals and plants suggests that this relationship and, hence, the C-value may differ not only among species, but even within species (see Cavallini and Natali 1991 for plants).

The study of the intraspecific variation in genome size and organization is of great interest for many reasons. These changes may be related to the divergence and evolution of species or may represent programmed responses to developmental and environmental stimuli, and they may help explain the extraordinary plasticity at both the morphological and physiological levels which is a common feature of plants. Such a study is of paramount importance for the genetic improvement of species of agricultural interest, because of possible linkages with environmental adaptation and/or changes in the phenotypic characters, as have been seen, e.g., in flax (see Cullis 1983). Moreover, should certain correlations be proved, they would shed further light on the functional role of fractions of nuclear DNA, such as repeated sequences, many aspects of which are being currently debated.

Different ploidy levels (tetraploid, hexaploid, octoploid, decaploid) are found in *Festuca arundinacea* Schreber, an important herbage crop in temperate regions. In this paper, we report the results of a study on the genome size and organization of 35 hexaploid natural populations, distributed all along the Italian peninsula which, according to morphological and anatomical observations, should be assumed to belong to one hologamodeme (Cenci et al. 1990).

Materials and methods

Preparation of the material

Seeds (caryopses) were collected from 35 natural populations of *Festuca arundinacea* on the Italian peninsula, between 45°33′

* To whom correspondence should be addressed

Table 1. Feulgen absorption of early prophases (=4C) and DNA content calculated per 1C in 35 hexaploid natural populations of *Festuca arundinacea* in Italy. Twenty prophases were analyzed in the root meristem of each of five seedlings obtained from seeds of each population

Seed provenance and district code	Latitude north	Altitude (m a.s.l.)	Feulgen absorption (mean ±SE)	Mean C-value (pg)
Montecchio (VI)	45°33′	225	3,270± 51	8.26
Mazzano (BS)	45°32′	130	2,564± 94	6.47
Sirmione (BS)	45°28′	60	3,231± 52	8.16
Cadilana (MI)	45°24′	100	3,154± 71	7.96
Dolo sul Brenta (VE)	45°24′	10	2,518± 58	6.36
Rubano PD)	45°24′	15	2,689± 89	6.79
Borgo Vercelli (VC)	45°20′	55	2,929± 93	7.40
Chioggia (VE)	45°14′	0	3,251± 95	8.21
Poirino (TO)	45°00′	290	2,689± 92	6.79
Cesenatico (FO)	44°12′	5	2,397±134	6.05
Pistoia (PT)	43°56′	365	3,356± 86	8.47
Gragnano (LU)	43°51′	410	3,413± 98	8.62
Gualdi Urbania (PS)	43°40′	455	2,713± 33	6.85
Città di Castello (PG)	43°37′	295	2,854± 95	7.21
Arcidosso (GR)	42°54′	660	2,901± 49	7.33
Abbadia S. Salvatore (SI)	42°53′	830	2,515± 35	6.35
Sellano (PG)	42°50′	715	3,154± 48	7.96
Castelviscardo (TR)	42°45′	305	3,062± 82	7.73
Alba Adriatica (TE)	42°45′	0	3,194± 63	8.07
Bolsena (VT)	42°39′	305	3,235± 68	8.17
Montefiascone (VT)	42°32′	510	2,959± 66	7.47
Pescara (PE)	42°28′	0	2,915± 70	7.36
Rieti (RI)	42°24′	405	2,834±112	7.16
Principina (GR)	42°21′	10	3,297±124	8.33
Borgorose (RI)	42°18′	750	2,584± 71	6.53
Roccacasale (AQ)	42°07′	300	2,873± 88	7.26
San Severo (FG)	41°41′	10	2,961± 76	7.48
Rionero Sannitico (IS)	41°40′	820	3,191±109	8.06
Baronissi (SA)	40°47′	105	3,278± 49	8.28
Marina di Castellaneta (TA)	40°37′	0	3,134± 93	7.91
Riva dei Tessali (TA)	40°31′	0	2,866± 49	7.24
Papasidero (CS)	39°52′	450	2,959± 41	7,47
Trebisacce (CS)	39°52′	10	3,078± 67	7.77
Cosenza (CS)	39°17′	300	3,537±163	8.93
Lido di Catanzaro (CZ)	38°49′	5	3,448±106	8.71

Source of variation	*F*	*P*
Populations	2.58	≤0.001
Plants within a population	0.33	–
Nuclei within a plant	0.92	–

and 38°49′ of latitude north, at altitudes ranging from 0 to 830 m above sea level. These seeds were germinated on wet paper in petri dishes under sterile conditions at 20 °C, and the seedlings were used as experimental material.

DNA cytophotometry

Root or shoot apices, fixed in ethanol-acetic acid 3:1 (v/v) or in 10% neutral formalin, were squashed under a coverslip in a drop of 45% acetic acid, after treatment with a 5% aqueous solution of pectinase (Sigma) for 1 h at 37 °C. The coverslips were removed by the solid CO_2 method and the preparations were Feulgen-stained after different hydrolysis durations in 1*N* HCl at 60 °C: 8 min for those made with materials fixed in ethanol-acetic acid, and 20 min for those made with formalin-fixed materials. After staining, the slides were subjected to three 10-min washes in SO_2 water prior to dehydration and mounting in DPX (BDH). Since simultaneous processing was not possible due to the large number of preparations to be analyzed, squashes made with the root tips of a single plantlet of *Vicia faba* (4C= 53.31 pg; Bennett and Smith 1976) were concurrently stained for each group of slides and used as standards, in order to make the results comparable and to convert relative Feulgen units into picograms of DNA. All notable differences in Feulgen/DNA absorption between preparations observed using the above method of comparison were further checked by analyzing preparations that were made again and processed all together. Feulgen/DNA absorptions in individual cell nuclei were measured at the wavelength of 550 nm, using a Leitz MPV 3 microscope photometer equipped with a mirror scanner and an HP 85 computer.

Table 2. Feulgen absorption (a.u.; mean ± SE) at different thresholds of optical density of interphase 4C (G_2) nuclei in the root meristems of seedlings obtained from seeds collected in two populations, largely differing in the amount of nuclear DNA. The differences between absorptions at successive thresholds of optical density and their ratios are also given

Threshold of optical density (a. u.)	Populations				Ratio between absorption differences
	Gragnano		Papasidero		
	absorption	difference	absorption	difference	
4	3,443 ± 108		2,934 ± 96		
		586		533	1.10
10	2,857 ± 131		2,401 ± 82		
		1,130		1,194	0.95
15	1,727 ± 124		1,207 ± 51		
		824		755	1.09
18	903 ± 40		452 ± 38		
		481		224	2.15
21	422 ± 31		228 ± 19		
		321		177	1.81
24	101 ± 12		51 ± 16		
		89		51	1.75
27	12 ± 4		0		
		12		–	–
30	0		0		

With the same instrument and at the same wavelength, the Feulgen/DNA absorptions of chromatin fractions with differing condensation were determined by measurements on one and the same nucleus, after different thresholds of optical density were selected in the instrument. The instrument does not read all parts of the nucleus where optical density is lower than the preselected limit, but rather regards them as a clear field.

Karyology

For the karyological analyses, the meristems of roots, treated with a 0.05% aqueous solution of colchicine (Sigma) for 4 h at room temperature and fixed in ethanol-acetic acid, were Feulgen-stained and squashed as described above. The lengths of metaphase chromosomes and their arm ratios were determined on the basis of measurements taken on microphotographs.

Chemico-physical DNA characterization

DNA was extracted from seedlings and purified according to Maggini et al. (1978). Thermal denaturation was performed in 0.1 × SSC using a Gilford 250 E spectrophotometer equipped with a temperature program controller, and the increase of hyperchromicity at 260 nm was continuously followed by a linear recorder. In order to study reassociation kinetics, DNA was sheared by sonication in an MSE sonicator at medium energy output for 5 × 5 s, with a 10-s interval, at 4 °C. Sedimentation in neutral sucrose gradients according to Clewell and Helinsky (1969) revealed that the fragments of all DNA samples analyzed were of a relatively homogeneous length of ca. 400 bp. Sheared DNA, dissolved in 0.12 *M* phosphate buffer at a concentration of 50 µg/ml, was denatured for 10 min at 103 °C and allowed to renature according to Britten et al. (1974). The reassociation process was monitored at 260 nm by a linear recorder, using the same equipment employed for the analysis of the thermal denaturation kinetics. *E. coli* DNA (Sigma) was used as a standard after shearing under the same conditions as above. The data obtained were subjected to Scatchard-type analysis (Marsh and McCarthy 1974).

Results

Cytophotometry

The results obtained on materials fixed in ethanol-acetic acid were comparable to those obtained on formalin-fixed materials. The mean Feulgen absorptions of early prophases (=4C) in the root meristems of seedlings, obtained from seeds collected from each of 35 natural populations of *F. arundinacea,* and the C-values calculated are given in Table 1. Highly significant ($P \leq 0.001$) variations in the amount of DNA occur between populations; taking the most variant values, a 32.3% difference can be calculated between the population at Cosenza (C-value = 8.93 pg) and that at Cesenatico (6.05 pg), with the former as standard. In contrast, the genome size of individual plants within a single population does not differ significantly. Neither do significant differences exist between the DNA contents in the root and shoot meristems of one and the same plant, as proven by comparisons between the respective Feulgen absorptions made in a number of seedlings belonging to different populations (not shown).

The results of cytophotometric measurements, taken on interphase nuclei at different thresholds of optical density (Table 2), indicate that the differences in the Feulgen absorptions observed are mainly due to DNA fractions contained in comparatively more spiralized, optically dense chromatin (heterochromatin). Indeed, at relatively high thresholds of optical density, when only nuclear parts with dense chromatin are read by the instrument, clear-cut differences in Feulgen/DNA absorptions are

Table 3. Number of chromosomes with different arm ratios and total length of the complement (means ± SE) in colchicine-treated root meristems of seedlings obtained from seeds collected from two populations differing in the amount of nuclear DNA. For each population, a total of 20 metaphases was scored in five seedlings

Arm ratio	No. of chromosomes per metaphase	
	Lido di Catanzaro	Papasidero
1.00–1.20	13.20 ± 0.37	13.10 ± 0.67
1.21–1.40	6.40 ± 0.51	12.65 ± 0.46
1.41–1.60	7.25 ± 0.37	9.45 ± 0.36
1.61–1.80	8.05 ± 0.31	4.75 ± 0.33
1.81–2.00	7.10 ± 0.44	2.05 ± 0.22
Total length of the complement (μm)	204.94 ± 4.48	179.88 ± 2.71

obtained in seedlings belonging to two populations differing in the size of their genome. These differences do not increase with the lowering of the threshold of optical density, which makes it possible to read less condensed chromatin (euchromatin), too.

Karyometry

All populations studied were hexaploid (2n = 6x = 42). Neither the presence of supernumerary chromosomes, already reported in *F. arundinacea* (see Jones and Rees 1982), nor the occurrence of such phenomena as aneuploidy or recurrent aneusomaty, which are found with relative frequency in polyploid species, were observed.

Table 3 contains data on the arm ratios of the chromosomes and the length of the complement obtained by studying root meristems of seedlings belonging to two populations differing in DNA content: Lido di Catanzaro (C-value = 8.71 pg) and Papasidero (7.47 pg). The total chromosome length is significantly higher in seedlings obtained from seeds collected at Lido di Catanzaro than in those from seeds collected at Papasidero. The chromosomes with given arm ratios clearly differ in number in the two populations; it is interesting to note, in particular, that the number of those with relatively high arm ratios is much lower in the population with a smaller genome size than in the other. This seems to suggest that the DNA loss producing variations in genome size has occurred mainly in the long arm of less metacentric chromosomes.

Biochemical analyses

A chemico-physical characterization of the DNAs extracted from seedlings obtained from seeds of the populations at Cesenatico (C-value = 6.05 pg; Table 1), Castelviscardo (7.73 pg), and Baronissi (8.28 pg) was carried out. No significant difference was found as far as the

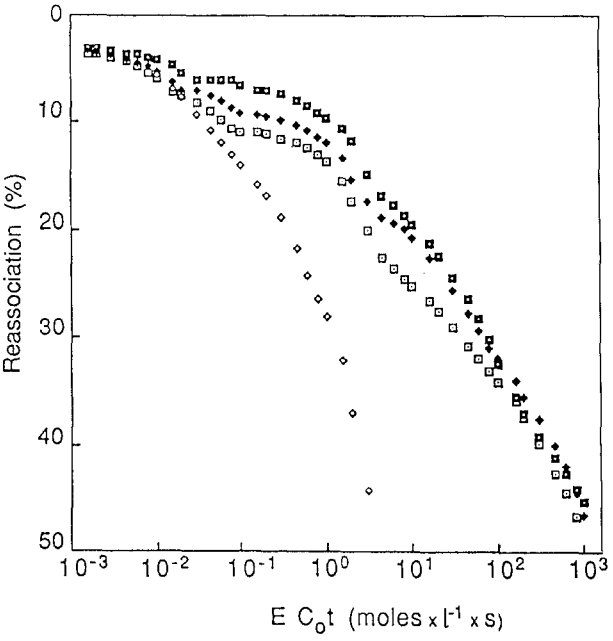

Fig. 1. Reassociation kinetics of the DNAs extracted from seedlings obtained from seeds of the populations at Cesenatico (■), Castelviscardo (◆), and Baronissi (⊡). Each point is the mean of the values obtained in four repetitions: two for each of two different DNA extractions. *E. coli* DNA (◇) was used as a marker

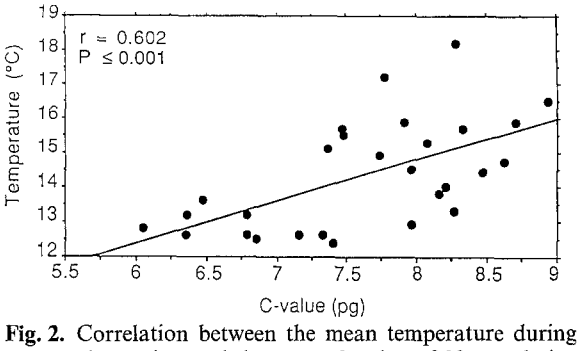

Fig. 2. Correlation between the mean temperature during the year at the station and the mean C-value of 28 populations

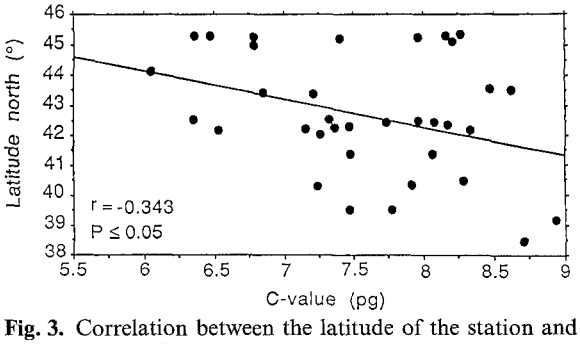

Fig. 3. Correlation between the latitude of the station and the mean C-value of 35 populations

Table 4. Scatchard-type analysis of the reassociation curves shown in Fig. 1. Two families of highly repeated DNA sequences (HR I and HR II) and a family of medium repeated sequences (MR) are taken into account

DNA sequences	Populations	Frequency	C_0t 1/2 observed (moles $\times l^{-1} \times s$)	C_0t 1/2 pure (moles $\times l^{-1} \times s$)	Kinetic complexity (nucleotide pairs)	Redundancy
HR I	Baronissi	0.073	1.73×10^{-3}	1.26×10^{-4}	1.44×10^{2}	3.86×10^{6}
	Castelvisc.	0.068	1.90×10^{-3}	1.29×10^{-4}	1.47×10^{2}	3.29×10^{6}
	Cesenatico	0.061	2.21×10^{-3}	1.35×10^{-4}	1.54×10^{2}	2.22×10^{6}
HR II	Baronissi	0.037	4.75×10^{-2}	1.75×10^{-3}	1.99×10^{3}	1.42×10^{5}
	Castelvisc.	0.025	5.64×10^{-2}	1.41×10^{-3}	1.61×10^{3}	1.10×10^{5}
	Cesenatico	0.009	1.51×10^{-1}	1.36×10^{-3}	1.55×10^{3}	3.23×10^{4}
MR	Baronissi	0.109	1.74×10^{0}	1.89×10^{-1}	2.15×10^{5}	3.86×10^{3}
	Castelvisc.	0.097	1.71×10^{0}	1.66×10^{-1}	1.89×10^{5}	3.65×10^{3}
	Cesenatico	0.086	1.89×10^{0}	1.62×10^{-1}	1.85×10^{5}	2.58×10^{3}

Tm values are concerned (86.8 ± 0.21, 87.4 ± 0.29, and 87.0 ± 0.18, respectively).

The three DNAs, and particularly their repetitive fractions, that are most likely to be involved in the variations of the genome size, as suggested by the changes observed in the amount of heterochromatin (Table 2), were also studied by means of reassociation kinetics. The reassociation curves in Fig. 1 reveal three fractions of repetitive DNA: two of them, which reassociate within an equivalent C_0t of 2×10^{-1}, are considered by us to consist of highly repeated sequences (HR I and HR II); the third fraction is considered to represent medium repeated sequences (MR). Scatchard-type analysis of the reassociation experimental data, detailed in Table 4, indicates that all these DNA fractions are differently represented in the three DNAs; the lower the genome size, the lower their frequency. This is particularly clear in the case of HR II sequences; their redundancy in the genome is 4.4 times greater in the population at Baronissi (1.42×10^5) than in that at Cesenatico (3.23×10^4).

Correlations with environmental parameters

As shown in Fig. 2, a highly significant ($P \leq 0.001$) positive correlation exists between the C-values of the populations and the mean temperature during the year at the stations where these data were available. The correlation with the mean temperature in the coldest month is equally significant (not shown). As expected, the C-values are also negatively correlated with latitude (Fig. 3). The lower level of significance ($P \leq 0.05$) is readily explained by the different altitudes of the stations (from 0 to 830 m above sea level). In contrast, the C-values do not correlate significantly with rainfall (not shown).

Discussion

Mutually supporting one another, our cytophotometric, karyometric, and biochemical results indicate that changes in the nuclear DNA, affecting the C-value and the relative proportions between genome fractions, differentiate Italian populations of hexaploid *Festuca arundinacea* (Table 1). These changes consist of frequency variations of repeated DNA sequences, and in particular of a fraction of them (Fig. 1 and Table 4), which are located mainly in the heterochromatin (Table 2). As a consequence, variations in the length of given chromosome arms and in that of the entire complement also occur (Table 3).

Differences in chromosome size between populations of *F. arundinacea*, suggesting variations in DNA content, have already been reported (Malik and Thomas 1966), and a significant difference in the amount of nuclear DNA at the hexaploid level between a cultivar and a natural population of this species was assessed cytophotometrically by Seal (1983). The possibility of intraspecific variations in the amount of nuclear DNA due to changes in the relative proportions of genome fractions is now generally accepted. The view that fluid domains exist in the genome also seems to be supported by the observation that even its sometimes massive changes, which are involved in the divergence and evolution of species, appear to be restricted to DNA fractions with a particular composition and organization (Hutchinson et al. 1980).

The correlations between the genome size of our populations and a climatic factor (Fig. 2) or the latitude (Fig. 3) of their stations suggest that nuclear DNA changes play some role in environmental adaptation. Intraspecific variations of genome size and organization, which may be hypothesized to play such a role since they correlate with given environmental parameters, have been found while studying other species. *Zea mays* is a ready example: in this species, lines from the higher latitudes of North America have significantly lower amounts of DNA and heterochromatin than those from lower latitudes; correlations (even if not always in the same direction) between altitude and the amounts of

both heterochromatin (C-bands) and DNA have also been reported (Porter and Rayburn 1990; Rayburn and Auger 1990 and references therein). Similarly, nuclear DNA content correlates with latitude or altitude in other species (see Cavallini and Natali 1991), and the variations in the nuclear DNA that changing environmental conditions induce in plastic varieties of flax are well known (Cullis and Cleary 1986).

As in North American *Zea mays* lines, the amount of nuclear DNA of hexaploid Italian fescues increases progressively from north to south. It seems worth noting that a similar gradient can be found when considering the ploidy level of populations of *F. arundinacea* distributed over a wider geographical area. Indeed, tetraploids are found in France and hexaploids prevail in Italy, Spain, and Portugal, while octoploids and decaploids are confined to Northern Africa (Borrill et al. 1971). This might suggest an adaptive mechanism operating through nucleotypic effects (Bennett 1987). The variations in the amount of nuclear DNA we have observed in our populations might have the same significance.

Acknowledgements. The authors are grateful to the Centro di Studio per l'Olivicoltura, C.N.R., Perugia, for laboratory facilities, and to Mr. A. Bolletta for his skillful technical assistance. Research supported by National Research Council of Italy, Special Project RAISA, Subproject N. 2, Paper N. 188.

References

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. Philos Trans R Soc London Ser B 274:227–274

Borrill M, Tyler B, Lloyd-Jones M (1971) Studies in *Festuca*. I. A chromosome atlas of *bovinae* and *scariosae*. Cytologia 36:1–14

Britten RJ, Graham DE, Neufeld BR (1974) Analysis of repeating DNA sequences by reassociation. Methods Enzymol 29:363–405

Cavallini A, Natali L (1991) Intraspecific variation of nuclear DNA content in plant species. Caryologia 44:93–107

Cenci CA, Ceccarelli M, Pasqualini S, Falcinelli M, Cionini PG (1990) *Festuca arundinacea* Schreber (*Gramineae*) in Italy: morphological, anatomical, karyological and biochemical analyses. Webbia Rac Scr Bot 44:255–270

Clewell DB, Helinski DR (1969) Supercoiled circular DNA-protein complex in *Escherichia coli:* purification and induced conversion to an open circular DNA form. Proc Natl Acad Sci USA 62:1159–1166

Cullis CA (1983) Environmentally induced DNA changes in plants. CRC Crit Rev Plant Sci 1:117–131

Cullis CA, Cleary W (1986) Rapidly varying DNA sequences in flax. Can J Genet Cytol 28:252–259

Hutchinson J, Narayan RKJ, Rees H (1980) Constraints upon the composition of supplementary DNA. Chromosoma 78:137–145

Jones RN, Rees H (1982) *B* Chromosomes. Academic Press, London

Maggini F, Barsanti P, Marazia T (1978) Individual variation of the nucleolus organizer regions in *Allium cepa* and *A. sativum*. Chromosoma 66:173–183

Malik CP, Thomas PT (1966) Karyotypic studies in some *Lolium* and *Festuca* species. Caryologia 19:167–196

Marsh JL, McCarthy BJ (1974) Effect of reaction conditions on the reassociation of divergent deoxyribonucleic acid sequences. Biochemistry 13:3382–3388

Porter HC, Rayburn AL (1990) B-chromosome and C-band heterochromatin variations in Arizona maize populations adapted to different altitudes. Genome 33:659–662

Rayburn AL, Auger JA (1990) Genome size variation in *Zea mays* ssp. *mays* adapted to different altitudes. Theor Appl Genet 79:470–474

Seal AG (1983) DNA variation in *Festuca*. Heredity 50:225–236

Sparrow AH, Price HJ, Underbrink AG (1972) A survey of DNA content per cell and per chromosome of prokaryotic and eukaryotic organisms: some evolutionary considerations. Brookhaven Symp Biol 23:451–494

Thomas CA (1971) The genetic organization of chromosomes. Annu Rev Genet 5:237–256