Theor Appl Genet (1988) 75:319–329

Constraints upon the organisation and evolution of chromosomes in *Allium*

R. K. J. Narayan

Department of Agricultural Botany, University College of Wales, Aberystwyth, Dyfed SY23 3DD, UK

Received January 28, 1987; Accepted August 23, 1987
Communicated by B. R. Murty

Summary. In terms of chromosome morphology, karyotype organisation, taxonomy and genetic relationship as judged from chromosome pairing in the F1 hybrid, *A. cepa* and *A. fistulosum* are two closely related species. But large variation in nuclear DNA amounts has occurred during the evolution of the two species. A comparison of the molecular composition of DNA in the two species has confirmed that the excess DNA acquired during evolution was predominantly repetitive sequences (sequences which do not encode genetic information). However, its distribution within the chromosome complements was equal in all chromosomes irrespective of the differences in chromosome size. The even distribution of the excess DNA within complements suggests strong constraints underlying evolutionary changes in genome organisation. The nature of the constraints is discussed, and it is shown that such constraints can influence the direction of karyotype evolution during speciation.

Key words: *Allium* – DNA – Chromosome evolution – Genomic constraints

Introduction

Nuclear characters involved in the organisation of a chromosome complement are considerably more stable than the characters which make up the external morphology of a species. Nuclear DNA amount, chromosome number, chromosome shape and karyotype arrangement are invariant within most species of higher organisms. Strong correlations are found between several nuclear characters: the total chromosome volume for example, is directly correlated with the total DNA amount. Evolution, on the other hand, is accompanied by changes in the amounts of nuclear constituents and in the organisation of the chromosome complements. Strong constraints upon evolutionary changes in chromosomal DNA are reported in *Lathyrus*. A comparison of the DNA distribution within the complements of several *Lathyrus* species ($2n = 14$) which embraced a large variation in nuclear DNA amount has confirmed that the extra DNA acquired during evolution were distributed evenly in all chromosomes irrespetive of the significant differences in chromosome sizes (Narayan and Durrant 1983). The genus *Allium* (Liliaceae) has diploid species with basic chromosome numbers $2n = 14$, 16 or 18. *A. cepa* and *A. fistulosum* are two closely related diploid species with sixteen chromosomes. They do not hybridise in nature, but interspecific hybrids may be formed with relative ease. Chromosome pairing in the hybrid is effective and extensive (Jones and Rees 1968). Despite the close genetic relationship as judged by taxonomy and chromosome pairing in the F_1 hybrids, *A. cepa* has a significantly greater amount of nuclear DNA in its genome than *A. fistulosum*. The experiments described below were carried out to determine the nature and location of chromosomal DNA changes which accompanied the evolution of the two species. Comparisons were also made on the karyotype organisation in related species using multivariate statistical methods. This paper describes the nature of the constraints underlying chromosome organisation in *Allium* and its influence on karyotype evolution.

Materials and methods

Chromosome measurements

Chromosome measurements were made in the Feulgen-stained metaphase plates prepared from root meristems. The lengths of the chromosome and width of the chromatids were measured at C-mitosis using a micrometer eye piece. The volumes of chromosomes were estimated as described by Narayan (1982). The mean volume for each chromosome was obtained from measurements from at least five different metaphase plates. Metaphase preparations stained with Giemsa according to the C-banding procedure (Vosa 1974) was used for measuring separate volumes of heterochromatin and euchromatin in each chromosome.

Nuclear DNA measurements

The amount of DNA in the 2C nuclei of root meristems was measured by Feulgen photometry (McLeish and Sunderland 1961). All DNA measurements were made on a Vickers M85 microdensitometer. For the measurement of DNA contained in chromosomes, mitotic metaphase chromosomes were stained quantitatively with Feulgen. Well-spread metaphase chromosome preparations were made in 45% acetic acid. With the aid of the masking device available with the M85 microdensitometer DNA content was measured separately for each chromosome. At least three full metaphase plates were measured in each plant and the mean values were calculated. Using a similar method, DNA contained in eight meiotic bivalents at M_1 was measured in the F_1 hybrid between *A. cepa* and *A. fistulosum*. Chromosome bivalents were markedly asymmetric in the hybrid, the larger half bivalent coming from *A. cepa*. The DNA content of each half bivalent was measured with the aid of the masking device as described above. The mean DNA value for each half bivalent was calculated using data obtained from three full metaphase plates. Data from two separate experiments were used in the statistical analysis.

DNA in C band heterochromatin and euchromatin

The method used was described previously by Narayan and Durrant (1983). Using spot microdensitometry it was established that the heterochromatin is on average 1.66 times denser than euchromatin. Using separate volumes of heterochromatin and euchromatin and their relative densities, the amounts of DNA contained in each fraction was calculated separately for each chromosome. The sum of the two fractions made up the total DNA in each chromosome.

The Cot reassociation of nuclear DNA

The percentage reassociation of dissociated DNA sequences was monitored optically at regular Cot intervals. The method was the same as described by Narayan and Rees (1976). The purified DNA was sheared to an average fragment size of 450 base pairs. The reassociation was carried out at 62 °C (Tm-20). The Cot reassociation was done in 0.04 M phosphate buffer (equimolar mixture of Na_2HPO_4 and NaH_2PO_4, pH 6.8) between Cot values 0.001 and 0.1, in 0.12 M buffer between Cot 0.1 and 1,000, and in 0.4 M buffer between Cot 1,000 and 20,000. All Cot values were then standardised for 0.12 M phosphate buffer.

Restriction endonuclease analysis

Purified nuclear DNA (5 µg) was digested with different restriction endonucleases. The DNA fragments were separated by electrophoresis and the DNA bands of interest were cut out from the gel under UV. The DNA fragments were extracted by electrolution and recovered by ethanol precipitation (Maniatis et al. 1982).

In situ hybridisation of ^{3}H-labelled complementary RNA (cRNA) to the chromosomal DNA

cRNA was transcribed from DNA sequence using *E. coli* RNA polymerase. Unlabelled nucleotide were purchased from Boehringer Ltd. and ^{3}H-labelled nucleotides (UTP, 46 Ci/mMol, ATP 32 Ci/mMol) were purchased from Amersham International Ltd. The labelled cRNA had a specific activity of 5.7×10^7 cpm/µg. The labelled cRNA was hybridised in situ to the denatured chromosomal DNA using the method described by Dennis et al. (1980).

Southern blot hybridisation of ^{32}P-labelled DNA sequences to the genomic DNA

Genomic DNA of *Allium* species digested with HaeIII restriction enzyme was electrophoresed on agarose gel slab. The restriction fragments were denatured with NaOH and blotted onto intracellulose filters (Southern 1975). The ^{32}P-labelled probe DNA sequences were prepared by nick translation. They were hybridised to the filter-bound genomic DNA according to the procedure described by Maniatis et al. (1982). The filters were washed thoroughly to remove non-specifically bound labelled DNA sequences. Autoradiography was done using Kodak X-omat AR film. An intensifying screen was used to sharpen the image in the X-ray film.

Multivariate statistical analysis

Factor analysis. Factor analysis is a method of multivariate statistical analysis useful for describing the intercorrelations between several biological characters and to describe their causal influences upon speciation and evolution. The centroid method suggested by Holzinger and Harman (1941) was used in this analysis. The extraction of factors involved a series of iterations and the estimates of communalities were obtained by an iterative procedure starting with a trial vector (Arunachalam 1967). The method was programmed on an IBM computer.

Results

The genome of *A. cepa* contains 33.5 pg nuclear DNA, which is 28% more than the amount in *A. fistulosum*. The variation is also reflected in their relative chromosome volumes. *A. cepa* chromosomes are on average 12% bigger than the chromosomes of *A. fistulosum*. The eight chromosomes in each complement were arranged in the order of decreasing chromosome size and the largest chromosome was ranked 1 (Fig. 1). Despite significant DNA differences between the two species, the chromosome complements are markedly similar in chromosome shapes and karyotype arrangements. Moreover, the distribution of constitutive heterochromatin as revealed by C-banding is also similar, being located at both telomeres of all chromosomes. Large-scale changes in DNA amount and chromosome vol-

Table 1. Total chromosome volume, total DNA amounts and DNA separated into amounts in euchromatin and heterochromatin in the eight ranked chromosomes

Species	Chromosomes								Means	Variance
	1	2	3	4	5	6	7	8		
Chromosome volume (cu · μ)										
A. cepa	21.94	20.96	19.98	19.23	18.96	17.96	16.84	15.40	18.87	4.59
A. fistulosum	19.75	19.40	18.36	17.46	16.72	15.97	14.41	13.29	16.92	5.27
Mean	20.85	20.18	19.17	18.35	17.71	16.97	16.63	14.35		
Total DNA (pg)										
A. cepa	4.89	4.62	4.42	4.29	4.15	3.99	3.75	3.41	4.19	0.22
A. fistulosum	3.81	3.76	3.55	3.39	3.24	3.10	2.81	2.60	3.29	0.18
Mean	4.35	4.19	3.99	3.84	3.70	3.56	3.28	3.01		
DNA in euchromatin (pg)										
A. cepa	4.44	4.28	4.09	3.84	3.82	3.65	3.42	3.08	3.83	0.20
A. fistulosum	3.50	3.45	3.24	3.05	2.93	2.71	2.51	2.29	2.96	0.19
Mean	3.97	3.89	3.66	3.44	3.37	3.18	2.96	2.69		
DNA heterochromatin (pg)										
A. cepa	0.45	0.34	0.33	0.48	0.34	0.34	0.34	0.33	0.37	0.003
A. fistulosum	0.31	0.31	0.31	0.35	0.31	0.42	0.30	0.32	0.33	0.001
Mean	0.38	0.32	0.32	0.41	0.32	0.37	0.32	0.33		

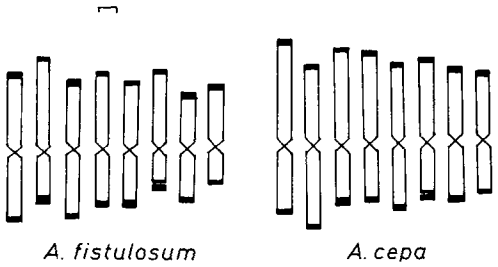

Fig. 1. C-banded idiograms of *A. fistulosum* and *A. cepa*. Bar, 1 μm

Table 2. Regression slopes for chromosome volume, total DNA, euchromatin DNA and heterochromatin DNA

Species	Total nuclear DNA	Regression coefficients			
		Chromosome volume	Total DNA	Euchromatin DNA	Heterochromatin DNA
A. cepa	33.50	0.97	1.05	1.01	1.37
A. fistulosum	26.30	1.04	0.95	0.98	0.64

ume on the one hand, and the astonishing similarity in chromosome organisation on the other would suggest constraints upon evolutionary chromosome changes. The molecular composition and the chromosomal distribution of the excess DNA implicated in evolutionary chromosome changes were investigated.

DNA distribution within the complements of A. cepa and A. fistulosum

Total DNA. The total amount of DNA contained in each of the eight ranked chromosomes of the two species are given in Table 1, as well as their constituent amounts of euchromatin and heterochromatin. Each value is a mean of at least two measurements from different plants which provided the experimental error. The table also contains the volumes of each ranked chromosome.

In Fig. 2a the DNA amounts are regressed on to the mean DNA amounts for the eight ranked chromosomes of the two species. The slopes of the two regression lines are individually significant and virtually parallel, and their regression coefficients approximate to unity (Table 2). The analysis of variance of the regression lines appear in Table 3. Ranking of chromosomes in the order of their DNA content may have reduced the residual mean squares, giving a larger variance ratio for the differences between means. In spite of this possible bias there is no significant difference between slopes when tested against the residual variation about the regression lines. In *Allium*, as in several other genera of higher plants, the total chromosome volume is directly correlated with DNA content (Jones and Rees 1968). In Fig. 2b the volumes of the eight ranked chromosomes of *A. cepa* and *A. fistulosum* are regressed onto the mean volumes of the eight chromosomes for the two species. The results of

Table 3. Analyses of variance on the differences between the regression slopes given by the eight ranked chromosomes

	Chromosome volume		Total DNA		DNA in euchromatin		DNA in heterochromatin	
	DF	MS	DF	MS	DF	MS	DF	MS
Means	1	17.08	1	3.27	1	3.00	1	0.006
Slopes	1	0.08	1	0.0065	1	0.001	1	0.964
Residual	12	0.02	12	0.0014	12	0.0013	12	0.001

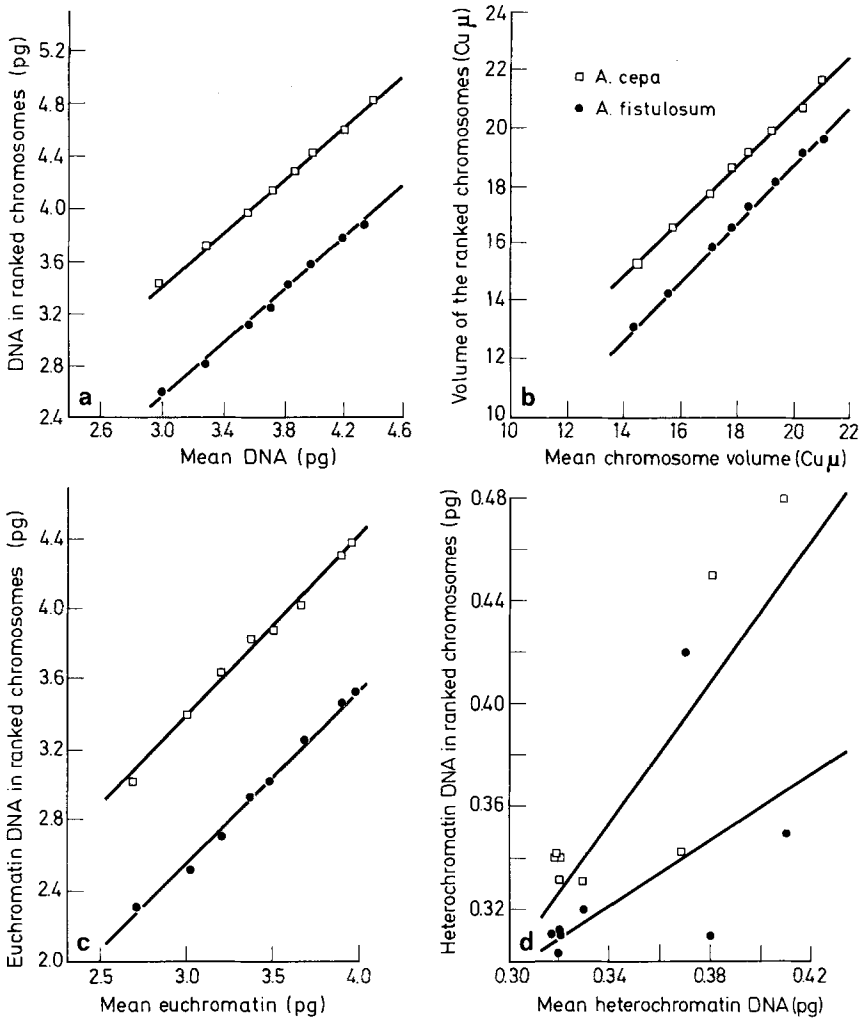

Fig. 2a–d. Linear regressions of the amounts of DNA, chromosome volume, euchromatic DNA, and heterochromatic DNA in the eight ranked chromosomes to the mean amounts for the two species: **a** total DNA; **b** chromosome volume; **c** euchromatin DNA; **d** heterochromatin DNA

the variance analysis were almost identical to that obtained when DNA amounts were used (Tables 2 and 3).

If *cepa* and *fistulosum* chromosomes with identical rankings were homoeologous to each other, then the DNA increments from *A. fistulosum* to *A. cepa* were approximately the same for all chromosomes. If the larger chromosomes gained proportionately greater amounts of DNA then the regression slopes would diverge, the slopes increasing with increase in DNA amounts. The homoeologous relationship between the ranked chromosomes was confirmed by DNA measurements in the F_1 bivalents of the hybrid between the two species (see below).

Heterochromatin and euchromatin constituents of DNA. Similar analyses were made on the DNA in euchromatin and heterochromatin. The euchromatin DNA was regressed onto the mean euchromatin DNA and the heterochromatin DNA to mean heterochromatin DNA for the eight ranked chromosomes. In Table 1 the chromosomes are left in their original

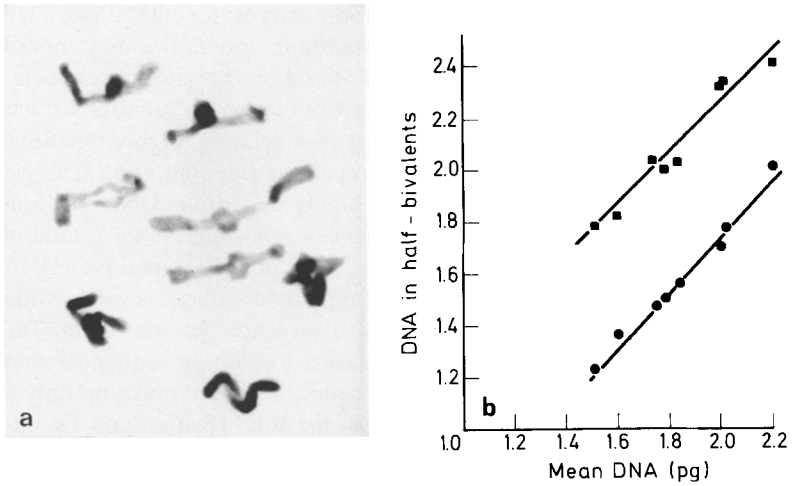

Fig. 3a, b. DNA distribution in the meiotic bivalents and half bivalents of the *cepa*×*fistulosum* hybrid: **a** asymmetric bivalents at metaphase 1 of meiosis; **b** DNA contained in the *cepa* and *fistulosum* half bivalents are plotted separately against the mean for the two half bivalents. ■—■, *A. cepa;* ●—●, *A. fistulosum*

rankings. The division of total DNA into euchromatin and heterochromatin components did not break the rankings for euchromatin. The heterochromatin DNA, on the other hand, broke the rankings to a very large extent. In Fig. 2c the regression slopes for euchromatin DNA are individually significant and their regression coefficients approximate to unity (Table 2). There is no significant difference between slopes when tested against residual variation (Table 3). For heterochromatin DNA (Fig. 2d) the regression slope is significant for *A. cepa* ($P < 0.01$) but not for *A. fistulosum.* The best fitting lines diverge, and in the analysis of variance (Table 3) the difference between slopes is very highly significant. The results suggest that the larger chromosomes of *A. cepa* were accumulating greater amounts of heterochromatin than smaller ones.

Homoeology between the ranked chromosomes of A. cepa and A. fistulosum

Meiotic chromosome pairing is regular and extensive in the F_1 hybrids between *A. cepa* and *A. fistulosum* (Fig. 3a). Nuclear DNA contained in each bivalent was measured using Feulgen photometry. The bivalents were ranked one to eight in the order of decreasing DNA amounts. In Table 4 the DNA values of the ranked chromosomes of *A. cepa* and *A. fistulosum* are compared with the ranked DNA values of the F_1 bivalents. If chromosomes with identical rankings were homoeologous to each other, they would be expected to form bivalents with similar DNA rankings in the F_1 hybrid. Moreover, the mean of two homoeologous chromosomes will be equal to the DNA value of the F_1 bivalent if all three have the same ranking in their respective complements. Variance analysis was done to test whether the observed DNA values of the F_1 bivalents were significantly different from the values that would be expected from homoeologous pairing

Table 4. Nuclear DNA in the chromosomes of *A. cepa, A. fistulosum,* their F_1 hybrid and in the meiotic half-bivalents of the hybrid

Chromosomes	*A. cepa* (P_1)	*A. fistulosum* (P_2)	Cepa × fist. (H)	Half bivalents	
				Cepa	*Fistulosum*
1	4.89	3.81	4.47	2.44	2.03
2	4.62	3.76	4.12	2.35	1.77
3	4.42	3.55	4.02	2.32	1.70
4	4.29	3.39	3.68	2.12	1.56
5	4.15	3.24	3.56	2.07	1.49
6	3.99	3.13	3.52	2.04	1.48
7	3.75	2.81	3.20	1.82	1.37
8	3.41	2.60	3.02	1.79	1.23
Total	33.52	26.29	29.59	16.95	12.63

Table 5. Analysis of variance

	SS	DF	MS	VR
Total	7.797	47		
$P_1 + P_2$	6.5340	1	6.5340	53.12**
$P_1 + P_2 - 2$ H	0.0165	1	0.0165	–
Error (a)	0.0370	3	0.123	–
Chromosomes	8.9161	7	1.2737	48.25***
CX ($P_1 - P_2$)	0.0603	7	0.0086	–
CX ($P_1 + P_2 - 2$ H)	0.0661	7	0.0094	–
Error (b)	0.5554	21	0.0264	–

Significant at: ** 1% level; *** 0.1% level

according to chromosome ranks. The results are shown in Table 5. As would be expected, the parental species have significantly different amounts of DNA and there are significant differences between the ranked chromosomes. On the other hand, differences in the amount of DNA between the two species are constant over all homologous chromosomes, and the mean of each homoeologous pair is not significantly different from the

hybrid value. The DNA amounts in the half-bivalents are also given in Table 4. The larger half-bivalent presumably has come from *A. cepa* and the smaller one from *A. fistulosum*. In Figure 3 b the DNA amounts of the ranked *cepa* half bivalents and *fistulosum* half bivalents are plotted separately against the mean DNA values of the two half bivalents. The regressions are linear and highly significant ($P < 0.01$). There is no heterogeneity between the two regression slopes. The results give additional evidence that equal amounts of DNA has accrued on all chromosomes irrespective of the differences in chromosome size.

DNA composition

Much of the nuclear DNA gained or lost during speciation and evolution is made up of sequences which have no known genetic function. Both non-repetitive and repetitive sequences are involved, but preponderantly the latter (Rees and Narayan 1977; Flavell et al. 1977).

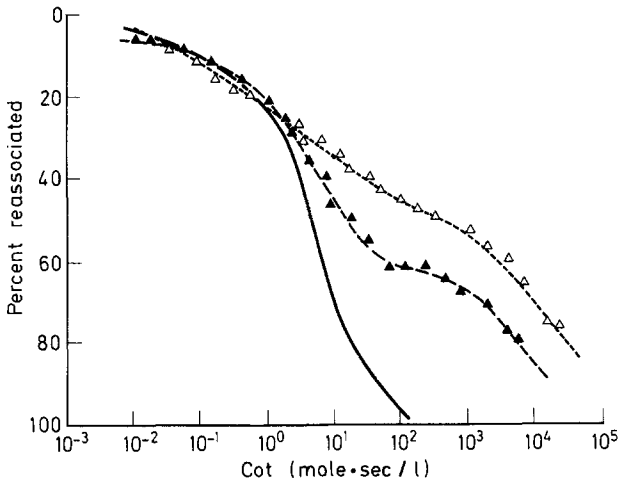

Fig. 4. The Cot reassociation curves for the total nuclear DNA of *A. cepa* (▲——▲) and *A. fistulosum* (△——△) and for the DNA of *E. coli* (——) as standard

The Cot reassociation curves for the dissociated DNA of *A. cepa*. *A. fistulosum*, and *E. coli* as standard are compared in Fig. 4. Based on the rates of reassociation estimated from the Cot curves, the total DNA was classified into: (1) a fast reassociating, highly repetitive fraction; (2) a middle repetitive fraction; and (3) single copy sequences. The highly repetitive DNA fraction comprised DNA sequences repeated up to a million times, or even more. The middle repetitive DNA consisted of families of repetitive sequences with a wide range of base sequence divergence between them. The single copy DNA is known to contain sequences that code for the structural genes, but these make up only a very small proportion of the total (Britten and Davidson 1971). Most of the non-repetitive sequences obtained by this method are presumably short interspersed DNA sequences which have undergone extensive base sequence divergence during evolution (Thompson and Murray 1980). The DNA amounts of the three fractions in the two species are compared in Table 6 as proportions to the total nuclear DNA as well as in absolute amounts (pg). The two species have remarkably similar amounts of highly repetitive sequences. In absolute amounts the non-repetitive sequences are also similar, although they make up a greater proportion of the total DNA in *A. fistulosum*. In proportional terms as well as in absolute amounts the middle repetitive sequences are increased substantially in *A. cepa*. In fact, the variation in the middle repetitive DNA alone would account for the nuclear DNA variation between the two species.

The average kinetic complexity of the middle repetitive DNA is given in Table 6. The complexity values were derived from the reassociation data for the middle repetitive fraction using *E. coli* DNA (genome size 4.6×10^6 bp) as a standard (Laird and McCarthy 1969). The average kinetic complexity is a measure of the heterogeneity between families of middle repetitive sequences. The heterogeneity results from base substitutions, rearrangements, mutations, and large-scale inter-

Table 6. Composition of the nuclear DNA in *A. cepa* and *A. fistulosum*

Species	Repetitive DNA							Non-repetitive DNA	
	Highly repetitive DNA		Middle repetitive DNA						
	As % of the total	In pico-grams	As % of the total	In pico-grams	Cot ½	Av. kinetic[a] complexity in base pairs	Av. degree of repetition	As % of the total	In pico-grams
A. cepa (33.5 pg)	5.5	1.84	56.5	18.93	3.0	2.3×10^6	0.8×10^4	38	12.73
A. fistulosum (26.3 pg)	6.0	1.57	45.0	11.83	0.6	0.46×10^6	2.5×10^4	49	12.88

[a] *E. coli* (genome size 4.6×10^6 bp) used as standard had a Cot ½ value of 6 by the present method

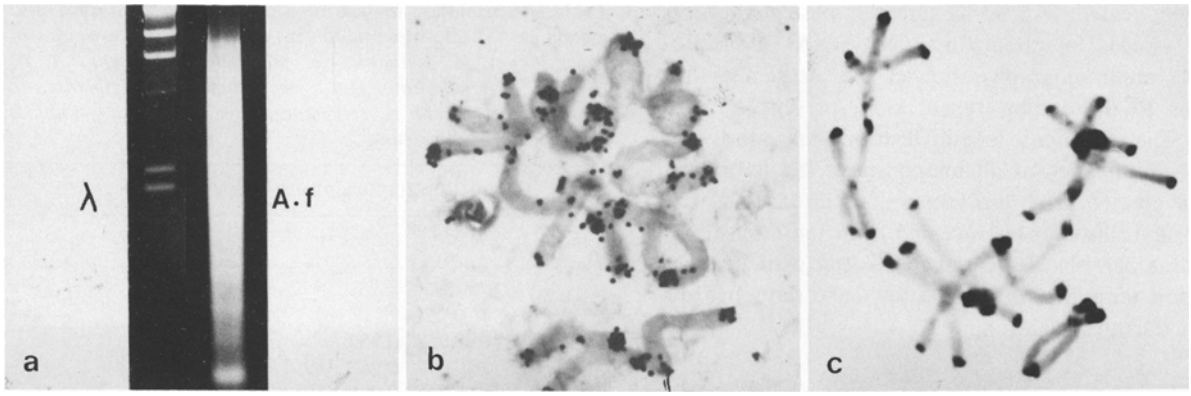

Fig. 5a–c. Chromosomal distribution of a specific (145 bp) repetitive DNA sequence in *A. fistulosum:* **a** Hae III digests of the DNA of *A. fistulosum* is compared with Hind III digests of lambda DNA used as marker; **b** 145 bp repeat sequence hybridised in situ with the chromosomal DNA of *A. fistulosum;* **c** C-banded chromosomes of *A. fistulosum*

spersion of DNA sequences during speciation. The average kinetic complexity is approximately five times greater for *A. cepa* than for *A. fistulosum.* The conclusion would be that DNA proliferation and base sequence divergence have progressed concurrently during the evolution of *Allium* species.

Chromosomal location of DNA changes

New families of repetitive sequences arise suddenly (on an evolutionary time scale) by tandem replicative events termed saltatory replications (Britten and Khone 1968 a). In investigating the chromosomal location of DNA changes, repetitive DNA sequences with very low base sequence complexity are effective as molecular probes. Satellite DNA sequences isolated from plants and animals are highly repetitive. The reassociated satellite DNA duplexes often show very high thermal stability which denotes a low level of base sequence divergence. Similarly, specific restriction bands may also represent repeated sequences which are homogenous in base sequence composition and are of relatively recent origin.

It was not possible to isolate a satellite DNA fraction from the nuclear DNA of either *A. cepa* or *A. fistulosum.* Total nuclear DNA of *A. cepa* and *A. fistulosum* were digested with a set of different restriction endonucleases. HaeIII digests of the genomic DNA of *A. fistulosum* showed a particularly prominent band with a basic repeat length of 145 bp (Fig. 5 a). A restriction band of similar repeat length or a multiple seriation of this fragment length was not seen in *A. cepa* under ethidium bromide fluorescence. Yet the close genetic and taxonomical affinity between the two species would suggest that the sequences ancestral to this repetitive sequence may be common to both species. The preferential amplification in *A. fistulosum* must have been recent on an evolutionary time scale (i.e. subsequent to the interspecific divergence between *A. fistulosum* and *A. cepa*). The reassociated duplexes of this repetitive DNA sequence showed very high thermal stability, with a Tm value similar to that of the native DNA. The distribution of this repetitive DNA sequence (hereafter referred to as 145 bp repeat) in the chromosome complements of *A. cepa* and *A. fistulosum* was investigated using in situ hybridisation. The pattern of distribution of DNA sequences homologous to the 145 bp repeat in the genomes of *A. cepa* and *A. fistulosum* was also compared by Southern blot hybridisation.

The ^{3}H labelled cRNA transcribed from the 145 bp repeat sequences was hybridised in situ to the denatured chromosomal DNA of *A. cepa* and *A. fistulosum.* In Fig. 5 the hybridised metaphase chromosomes of *A. fistulosum* are compared with the c-banded metaphase chromosomes. Fig. 5 shows that the probe repetitive sequences are located in the c-band heterochromatin at both telomeres of all chromosomes. The labelled cRNA did not hybridise with the chromosomal DNA of *A. cepa,* although few silver grains were detected in the interphase nuclei. The conclusions are 1) the DNA changes during evolution must have occurred as discrete saltatory replications, and 2) the newly evolved sequences are distributed at specific sites evenly in all chromosomes irrespective of the differences in chromosome size. The small variation in the number of silvergrains between chromosomes was not related to chromosome size.

The ^{32}P labelled 145 bp repeat DNA sequences were hybridised to the HaeIII digests of the total DNA of *A. fistulosum* and *A. cepa* in Southern blots. The experiment was done at three different reaction temperatures (75 °C, 60 °C, 45 °C), each representing a different level of stringency for molecular hybridisation. At 75 °C the stringency was the greatest and only sequences with a minimum of base sequence diver-

gence reannealed. At 45 °C greater base pair mismatches were tolerated in DNA-DNA hybridisation. The autoradiographs showed that sequences homologous to the 145 bp repeat were present in both species. The fragment length distributions were also broadly similar. Yet, at all temperatures the hybridisation was greater in *A. fistulosum* than in *A. cepa.* The extent of hybridisation as revealed from the intensity in the autoradiographs decreased in *A. cepa* with increase in reaction temperature, suggesting base sequence divergence during speciation.

Multivariate analysis on the evolution of chromosomes in Allium

Among species of *Allium,* chromosome size is directly correlated with nuclear DNA amounts. An analysis of the covariance between chromosome lengths will give us additional and meaningful information about the constraints which underlie the evolution of chromosomes. The lengths of individual chromosomes from 20 species were used in this investigation. The species were grouped into two classes with seven or eight pairs of chromosomes in the complements. The chromosomes were ranked as before in the descending order of chromosome lengths. The length of each chromosome was a mean of at least four measurements from four different plants which provided a measure of experimental error. The data has been previously reported by Jones (1967) and Jones and Rees (1968).

The variance-covariance matrix for the two groups of *Allium* species were calculated using their ranked chromosome lengths. The total correlation matrices derived from the variance-covariance matrices are given in Tables 7 and 8. In both tables the correlation coefficients are individually significant and most values approximate to unity.

Factor analysis explains observed relations between numerous variables in terms of simpler relations (Cattel 1965). A factor model assumes that a set of 'p'-correlated variables follow a multivariate normal distribution, and that their intercorrelations can be adequately described by K factors (K < P) which are linear and additive. Such an approach is important in investigations on biological evolution where very little information is available on the causal influences which underlie speciation. In the present analysis, ranked chromosomes were grouped into clusters of correlations using factor analysis. The factors F_1 and F_2 given in Tables 9 and 10 are essentially uncorrelated. The factor coefficients (loadings) are the weights given to chromosomes in each factor. The magnitudes of the factor coefficients describe the relative importance of that chromosome in interspecific divergence. Total communality is a measure of the amount of variance of the chromosomes accounted for by the common factors together. The factors and the communalities for species with eight chromosomes are in Table 9. Two factors account for 97.5% of the total communality, of which Factor 1 alone accounts for 95.4%. Factor 1 coefficients

Table 7. Correlation matrix for *Allium* species with $n = 8$ chromosomes. Species included in the analysis are *A. ostrowskianum, A. stellatum, A. pulchellum, A. cepa, A. fistulosum, A. galanthum, A. jesolianum, A. dicipiense, A. azureum, A. roseum, A. schoenoprasum, A. odoratissimum, A. darwasicum, A. sibiricum*

Chromosome	Correlation coefficients for 8 chromosomes							
	1	2	3	4	5	6	7	8
1	0.954							
2	0.954	0.954						
3	0.902	0.952	0.952					
4	0.850	0.912	0.947	0.951				
5	0.853	0.915	0.906	0.951	0.951			
6	0.852	0.890	0.867	0.910	0.950	0.950		
7	0.794	0.858	0.807	0.869	0.896	0.910	0.919	
8	0.804	0.862	0.826	0.865	0.871	0.891	0.919	0.919

Table 8. Correlation matrix for *Allium* species with $n = 7$ chromosomes. Species included in the analysis are, *A. moly, A. cernuum, A. neapolitanum, A. fuscum* and *A. subhirsutum*

Chromosomes	Correlation coefficients for 7 chromosomes						
	1	2	3	4	5	6	7
1	0.926						
2	0.926	0.977					
3	0.910	0.977	0.977				
4	0.906	0.944	0.958	0.958			
5	0.878	0.926	0.926	0.941	0.953		
6	0.903	0.926	0.926	0.930	0.953	0.953	
7	0.778	0.838	0.876	0.860	0.815	0.894	0.894

Table 9. Factor loadings and communalities in the centroid factor analysis for species with $n = 8$. 95.4% of the total variation is explained by factor 1

Chromosomes	Common factor coefficients		Communalities	
	F_1	F_2	Original	Calculated
1	0.921	−0.299	0.954	0.960
2	0.966	−0.186	0.954	0.967
3	0.947	−0.182	0.952	0.958
4	0.960	0.018	0.951	0.963
5	0.965	0.091	0.951	0.954
6	0.955	0.148	0.950	0.960
7	0.923	0.234	0.919	0.926
8	0.921	0.176	0.919	0.930

Table 10. Factor loadings and communalities in the centroid factor analysis for species with $n=7$. 96.1% of the total variation is explained by factor 1

Chromosomes	Common factor coefficients		Communalities	
	F_1	F_2	Original	Calculated
1	0.932	−0.216	0.926	0.928
2	0.975	−0.125	0.977	0.979
3	0.981	−0.049	0.977	0.979
4	0.973	0.015	0.958	0.946
5	0.957	0.062	0.953	0.921
6	0.971	0.067	0.953	0.970
7	0.891	0.246	0.894	0.859

are almost identical and therefore equally significant. In other words, all chromosomes within the complement have undergone an equal degree of change during speciation. Almost identical results were obtained from the analysis of species with seven chromosomes (Table 10).

Another interesting observation is the manner in which the relative magnitudes and directions of the loadings in the second factor differs from that of the corresponding factor loadings in the first factor. Although the variation accounted for by the second factor is only a very small proportion of the total variation, the consistency in the size and in the direction of the loadings in both groups of species should be noted. The loadings in the second factor are negative for the three largest chromosomes (1, 2 and 3), and their magnitudes decrease with decreasing chromosome size. In the smaller chromosomes (6, 7 and 8) the directions are positive. The changes in the magnitude is minimal in chromosomes 4 and 5 (Table 9). An analogous situation is found in Table 10. The conclusion would be that the second factor denotes a balancing effect which sets a constraint upon larger increases in the bigger chromosomes as compared to the smaller ones. The consequences of this phenomenon and the mechanisms involved have to be further investigated.

The inferences from the factor analysis are similar to the conclusions drawn from the detailed DNA measurements in the chromosome complements of *A. cepa* and *A. fistulosum*. Strikingly similar patterns of evolutionary chromosome changes are reported in *Lathyrus* (Narayan 1982; Narayan and Durrant 1983) and *Vicia* (Raina and Rees 1983).

Discussion

The above investigations clearly show that there are strong constraints upon DNA changes associated with the evolution of *Allium* species. The strong covariance between chromosome size as seen in the multivariate analysis is an index of the strength of the underlying constraints.

Developmental constraints, defined as "biases on the production of variant phenotypes or limitations on phenotypic variability caused by structure, character, composition or dynamics of the developmental systems" (Maynard Smith et al. 1985), can restrict the range of morphology by rendering certain phenotypes more accessible than others. Developmental constraints can therefore affect the evolutionary outcome by biasing the probability of entering onto one evolutionary lineage rather than another. The questions discussed below are: (1) Are the constraints upon chromosomal DNA changes due to selection? (2) What influence has it upon the outcome of karyotype evolution?

Each species represents a stable functional state of a chromosome complement. The orderly apearance and behaviour of chromosomes during mitotic and meiotic cell cycles reflects precise interrelationships between chromosomes. That chromosome size and shapes may be important in the spatial distributions of chromosomes during cell division is shown by Bennet (1983). To determine whether the constraints which dictate an equal increment of DNA in chromosomes is caused by selection, we must first examine the composition of the DNA sequence involved and consider whether it contributes to the genetic variation in species. When genetic variation is absent or extremely small, selection has no effect upon that trait.

The DNA difference between *A. cepa* and *A. fistulosum* is 7.2 pg, a value many times greater than the total DNA content in several angiosperm species. The Cot reassociation experiments have shown that this massive change in nuclear DNA amount is the result of an increase in the amount of repetitive sequences. With the exception of multigene sequences, the repetitive DNA do not encode structural genes. They do, however, make up a very large proportion of the genome and are integral to the structure and organisation of the chromosome complements. What evidence that is available shows that the gain or loss of repetitive sequences has very little effect upon the genetic variation in a species (Hutchinson et al. 1979). Nor is there any evidence to suggest that DNA changes influence the recombination at meiosis. Recombination varies independently of DNA amounts (Cavalier-Smith 1985). In short, the massive DNA changes associated with the evolution of *Allium* chromosomes do not seem to have contributed directly towards the genetic variation of the species.

Although the mechanisms involved in the proliferation of repetitive sequences remain unresolved, its distribution within the complements appears to be

governed by the constraints inherent to the organisation of the genome. The constraints themselves may be preserved by natural selection due to its bearing upon the reproductive fitness of the species.

Consequences upon the evolution of the karyotype

The equal distribution of DNA in all chromosomes ensures that the DNA differences between two chromosomes is the same in the two species if they have identical ranks (chromosome numbers). This is most obvious from the parallel arrangement of the regression lines in Fig. 2a. If the DNA increments were proportional to their respective chromosome size then the regression lines would diverge, the slopes increasing with increase in mean DNA amounts. As a result the relative sizes of the chromosomes would be more similar in a complement with greater DNA amount. In other words, chromosome complements will become progressively more symmetrical with increasing amounts of nuclear DNA.

The molecular mechanism which ensures an even distribution of DNA among individual chromosomes is not clear. Since the repetitive sequences acquired during evolution have no known genetic function, their accretion in a genome may be due to chance events. On the assumption that each repetitive DNA sequence has an equal chance of becoming the template for further proliferation, larger chromosomes in a complement have a greater chance of acquiring more DNA than smaller ones. The experiments described above have revealed that the accretion of DNA in individual chromosomes is not related to their DNA contents. The in situ hybridisation experiments have yielded no results suggesting that larger chromosomes have a greater number of hybridisation sites than the smaller ones. Moreover, a balancing mechanism opposing uncontrolled increase in chromosome size appears to be present, as seen from the magnitude and direction of factor loadings in the second factor.

Equal increment of DNA on all chromosomes must be due to (1) continued random accretion and dispersion where each chromosome, irrespective of differences in chromosome size, has an equal chance of acquiring additional amounts of DNA; (2) strong developmental constraints which ensure that equal increments occur in all chromosomes; or (3) stabilising selection against unequal increments.

The same pattern of evolutionary chromosome changes occurring in diverse genera of plants is significant. Genera *Lathyrus* and *Vicia* belong to the order *Rosales* of Dicotyledonea. Genus *Allium* (order *Liliales*) belong to the class Monocotyledonea. In genus *Nicotiana* (order Polymomiales) also from Dicotyledoneae, amphiploidy and karyotype rearrangements subsequent to interspecific hybridisation are major features of chromosome evolution. However, within subgeneric sections *Repandae* and *Noctiflorae,* chromosome shapes and karyotype arrangements are strikingly similar (Goodspeed 1954). A survey of the DNA amounts in *Nicotiana* (Narayan 1987) has revealed that the DNA increase is two- to three-fold within the two sections. DNA contained in the individual chromosomes of different *Nicotiana* species is not available at present, but the pattern of evolutionary chromosome changes is virtually the same as that found in *Lathyrus, Allium* and *Vicia.* The recurrence of a similar pattern of chromosome evolution among species which are remotely related in phylogeny may be due to a similar type of constraint in the organisation of the genome.

Acknowledgements. I am most grateful to Dr. A. Durrant for valuable help in the statistical analysis of the data and interpretation of the results. The *Allium* hybrid used in this investigation was a gift from Dr. R. N. Jones in our department. I thank Dr. B. R. Murty for helpful suggestions in the interpretation of the results from the factor analysis.

References

Arunachalam V (1967) Computer programs for some problems in biometrical genetics – 3. Indian J Genet Plant Breed 27:381–391

Bennet MD (1983) The spatial distribution of chromosomes. In: Brandham PE, Bennet MD (eds) Kew chromosome conference vol. 2. Allen and Unwin, London pp 71–79

Britten RJ, Khone DE (1968a) Repeated sequences in DNA. Science 161:529–540

Britten RJ, Davidson EH (1971) Repetitive and non-repetitive DNA sequences and a speculation on the origin of evolutionary novelty. Rev Biol 46:111–137

Cattel (1965) Factor analysis, an introduction to essentials-1. Biometrics 21:190–215

Cavalier-Smith T (1985) In: Cavalier-Smith T (ed). The evolution of genome size. Wiley and Sons London, pp 1–37

Dennis ES, Gerlach WL, Peacock WJ (1980) Identical polypyrimidine-polyuridine satellite DNA's in what and Barley. Heredity 44:349–366

Flavell RB, Rimpau J, Smith DB (1977) Repeated sequence DNA relationship in four cereal genomes. Chromosoma 63:205–222

Goodspeed TH (1954) The genus *Nicotiana.* Chronica Botanica. Massachusetts, Waltham, pp 536

Holzinger KJ, Harman HH (1941) Factor analysis. University of Chicago Press, Chicago

Hutchinson J, Rees H, Seal AG (1979) An assay of the activity of supplementary DNA in *Lolium.* Heredity 43:411–421

Jones RN (1967) PhD Thesis, University College of Wales, Aberystwyth (UK)

Jones RN, Rees H (1968) Nuclear DNA variation in *Allium.* Heredity 23:591–605

Laird CD, McCarthy BJ (1969) Molecular characterisation of the *Drosophila* genome. Genetics 63:865–882

McLeish J, Sunderland N (1961) Measurement of deoxyribonucleic acid (DNA) in higher plants by Feulgen photometry and chemical methods. Exp Cell Res 24: 527–540

Maniatis T, Fritsh EF, Sambrook J (1982) Molecular cloning. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY, pp 164–165

Maynard Smith J, Burian R, Kauffman S, Iberch P, Campbell J, Goodwin B, Lande R, Raup D, Wolpert L (1985) Developmental constraints and evolution. Q Rev Biol 60: 265–287

Narayan RKJ (1982) Discontinuous DNA variation in the evolution of higher plant species. The genus *Lathyrus.* Evolution 36:877–891

Narayan RKJ (1987) Nuclear DNA changes, genome differentiation and evolution in *Nicotiana.* J Plant Syst Evol 157:161–180

Narayan RKJ, Durrant A (1983) DNA distribution in chromosomes in *Lathyrus.* Genetica 61:47–53

Raina SN, Rees H (1983) DNA variation between and within chromosome complements of *Vicia* species. Heredity 51: 335–346

Rees H, Narayan RKJ (1977) Evolutionary NA variation in *Lathyrus.* In: Chapelle, A de la; Sorsa, M (eds) Chromosomes today, vol 6. Elsvier/North-Holland, Amsterdam, pp 131–139

Southern E (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Thompson WF, Murray MG (1980) Sequence organisation in pea and mung bean DNA and a model for genome evolution. In: Davies DR, Hopwood DA (eds) Plant genome. John Innes Charity, Norwich, pp 31–47

Vosa CG (1974) The basic karyotype of Rye (*Secale cereale*) analysed with giemsa and fluorescence methods. Heredity 33:403–408