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Triploidy in *Puschkinia libanotica* L. and Chromosome Size

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The chromosome size, mass and DNA content of diploid and triploid forms of *Puschkinia* were estimated. Triploids did not show any variation in chromosome size, and its mass as compared to diploids. Both diploid and triploid exhibited a virtually constant DNA amount per genome. In other words, there was no detectable change in chromosome size, mass or DNA in conjunction with polyploidy. The significance of these findings was discussed in relation to presence or absence of significant amount of heterochromatin with special reference to B chromosomes in *Puschkinia*.

Introduction

Evidences indicate that, in general, change in chromosome size is associated with polyploidy, though in several cases such a correlation is not met with (Sharma & Sharma 1968, 1970). It is now inferred that change in chromosome size consequent to polyploidy is the result of coiling and uncoiling of heterochromatin (Sharma 1972). Such inference has been drawn from the observations of chromosomes having significant amount of heterochromatic segments. A question which arises as to what extent, if any, the chromosomes of diploid species without significant amount of heterochromatin would have changed subsequent to polyploidy. To answer this question three types of comparisons, viz., the size of chromosomes their total dry mass and the DNA content of chromosome mass, between the chromosomes of the diploids and polyploids can usefully be made.

In the present study comparisons have been made between naturally occurring triploids of *Puschkinia* and its diploids. *Puschkinia* is characterised by easily distinguishable five pairs of chromosomes and thus allows a precise measurement of individual chromosomes. No significant amount of heterochromatin has so far been detected in the chromosomes of *Puschkinia* (Vosa 1969, Barlow & Vosa 1969a). Estimates of size were based on the chromosome volume since the length alone as a measure of chromosome size does not take into account the variation attributable to differences in chromosome coiling. Measurements of the dry mass of chromosomes provide further and more precise information

about structural changes, such as large scale diminution or, for that matter, accretion of chromosome material that may have taken place during this evolutionary process. In conjunction with DNA measurements the estimates of dry mass of the chromosomes also make it possible to ascertain whether the components of the chromosome mass, the DNA and non-DNA material, vary in the diploids and their polyploid descendants, i.e. they permit a qualitative and quantitative comparison between the diploids and triploids.

Materials and Methods

Bulbs of *Puschkinia libanotica* L. were supplied by Wallace and Barr Ltd., Kent. A few triploids were isolated from these bulbs. Their chromosomal length and volume, chromosome mass and DNA content were estimated and compared with diploids.

Estimation of chromosome volume: The volume of individual chromosome was estimated from the chromosome length and chromatid width measured at metaphase by means of a Vickers Moving Scale Micrometer Eye Piece considering chromatids as cylindrical in form. The calculation was done using the formula: $1.571w^2l$, where w is the chromatid width and l the chromosome length. Metaphases were scored in five root tips, each taken from different bulbs.

Roots were pretreated with 0.5% Colchicine for 2 hr and fixed in acetic-alcohol. They were hydrolysed in 1N HCL for 8 min and stained with Feulgen. All the chromosome measurements were done from temporary preparations.

Estimation of Chromosome Dry Mass: Estimates of chromosome dry mass were made by interference microsocopy (Davies 1958) in nuclei isolated from root tips following the method of McLeish (1963). All estimates of chromosome volume and dry mass were made from roots of 10 days' growth.

Estimation of Nuclear DNA by Feulgen Photometry: The staining procedure was that described by McLeish and Sunderland (1961). Measurements were made on a Barr and Stroud Integrating Microdensitometer. All measurements were done on 2C telophase and early interphase nuclei.

Observations

Chromosome Length: Total chromosome length in triploid was 1.5 times that in the diploid. This was expected in case of genome duplication provided no change in chromosomes took place following polyploidisation. In fact, chromosome length per genome in diploid and triploid did not differ significantly. Measurement of individual chromosome lengths both in diploid and triploid further indicated that there was no variation in chromosome length for each individual chromosome (table 1 and figures 1 & 2).

Cromosome Volume: No significant change was observed when volume of each chromosome was compared between diploid and triploid, and so chromosome volume per genome in each case remained unchanged. Total chromosome volume was 50% more in triploids (table 1).

Chromosome mass: Assuming no variation in chromosome mass subsequent to polyploidy, it is expected that triploids would have 50% more chromosome mass than diploids. Observations did not show any significant variation from this expectation. Furthermore, chromosome mass per genome was worked out to be 9.68×10^{-11}g and 9.91×10^{-11}g for diploid and triploid respectively, showing no

Table 1 *Average chromosome length and chromosome volume in diploids and triploids (Each value is ihe mean of five observations)*

Chromosome	Length (in microns)					TCL	CL/g
	1	2	3	4	5		
Diploid	9.88	8.90	8.78	6.76	4.41	76.86	38.43
	9.81	8.85	8.73	6.42	4.52		
Triploid	9.72	8.82	8.60	6.29	4.89	115.01	38.33
	9.85	8.91	8.68	6.36	4.94		
	9.70	8.77	8.57	6.23	4.67		
	Volume (in cubic microns)						
						TCV	CV/g
Diploid	28 51	26.86	25.37	18.11	12.00	218.38	109.38
	28.42	25.48	23.84	17.16	12.63		
Triploid	27.61	26.02	23.94	17.27	12.66	324.13	108.04
	28.25	26.77	24.99	17.75	12.98		
	27.45	26.72	23.41	16.92	11.99		

*Chromosome are arranged in descending order showing two homologues for diploid and three for triploid

CL/g = Chromosome length/genome

CV/g = Chromosome volume/genome

TCL = Total chromosome length in somatic complement

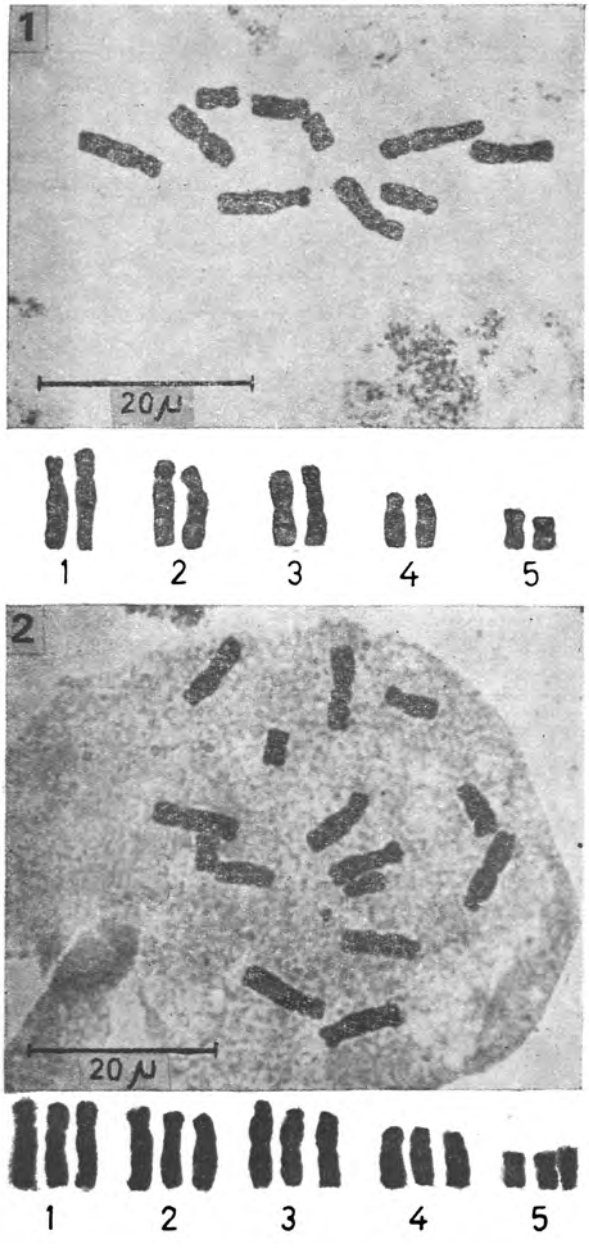
TCV = Total chromosome volume in somatic complement

significant change in chromosome mass in triploid as compared to diploid. Similarly, no variation was observed in their total mass. Triploids as expected showed 50% more total mass than diploids. However, nucleolar mass did not show similar rate of change in triploid table 2. It can be concluded that comparisons of chromosome mass; like those of chromosome volume showed no detectable change in the material subsequent to polyploidy.

DNA content: Amount of DNA per 2C nucleus was on an average 50% more in triploid. Estimates of DNA per genome showed no variation between diploid and triploid. This indicated a constancy of DNA (table 3).

Discussion

Study indicates that triploids of *Puschkinia* (family Liliaceae) do not show any variation in chromosome size and its



Figures 1 and 2 Chromosomes at metaphase from a plant of diploid (2n=10) and triploid (3n=15) *Puschkinia* with their karyotype given below

Table 2 *The total mass, the nucleolar dry mass and the chromosome mass in isolated 2C nuclei (Data from 10 nuclei in each of 4 root tip meristems)*

	Chromosome mass ($\times 10^{-11}$g)	Nucleolar mass ($\times 10^{-11}$g)	Total dry mass ($\times 10^{-11}$g)
Diploid	19.37	7.24	26.61
Triploid	29.73	8.28	38.01

Table 3 *DNA content in isolated 2C nuclei (arb. units) (Figures are mean of 10 nuclei)*

	Replicate			Mean	DNA/ Genome
	1	2	3		
Diploid	18.64	18.68	19.01	18.77	9.38
Triploid	27.90	27.45	27.36	27.57	9.19

mass as compared to diploids. On the other hand, several Liliaceous genera such as *Trillium*, *Fritillaria* and *Paris* showed diminution of chromosome size (Darlington & Lacour 1940) and their chromosomes carry significant amount of heterochromatin. Lacour's (1951 observations in *Trillium tschonoskii* are also significant, where reduction of heterochromatic segments was noted in tetraploids. Sharma (1972) also observed significant differences in polyploids of *vicia faba* having prominent heterochromatic segments in diploids. Polyploids of *V. sativa*, on the other hand, are not characterised by any difference in chromosize as compared to diploids some which do not have significant amount of heterochromatin. In this content, a critical examination of *Puschkinia* B chromosomes would have an important bearing. It was observed that at late prophase

B chromosome is more compact than A chromosomes and this difference may be due to precocious coiling of long arm of B chromosome (Barlow & Vosa 1969a). However, some of the A chromosomes at late prophase show a gradient of condensation which might suggest the presence of heterochromatin in normal A chromosomes; but this cannot be considered as conclusive evidence. It is only the B chromosomes, and especially the long arm of B's that are highly condensed throughout interphase and appear to be easily identifiable as darkly stained bodies, and A chromosomes, on the other hand, never exhibit such a characteristic (Barlow & Vosa 1969a). The pattern of replication between them is distinctly different. The long arm of the B chromosome replicates its DNA at a high rate at the end of the DNA synthetic period of interphase (Barlow & Vosa1969b). Indeed, B chromosomes of *Puschkinia* possess clearly defined heterochromatic mass (Vosa 1969). It can be assumed that A chromosomes of *Puschkinia* are not characterised by significant amount of heterochromatin, as distinct and well-defined as B chromosomes. No variation in chromosome size in *Puschkinia* subsequent to polyploidy would therefore, corroborate the observations made in case of *Vicia sativa*. This further implies that the effect of polyploidy is different in chromosomes with significant amount of heterochromatin from those without.

As regards DNA values, no significant variation in DNA amounts between *Puschkinia* chromosomes of diploid and triploid forms, was observed. This fact might be interpreted as suggesting that little or no variation in DNA amount has occurred in triploids subsequent to their formation from parent diploid species. Smith and Bennett (1975) in their study on DNA variation in the genus *Ranunculus* observed that in case of *R. facaria*, diploid, triploid and tetraploid forms had a virtually constant DNA amount per genome. No real decrease in DNA content per genome in cells at higher ploidy levels was also reported in colchicine-induced endopolyploid nuclei of *Vicia faba* L. (Bennett& Jellings 1975). The relationship between nuclear DNA content and ploidy level as reflected by the present study in which increase in ploidy level results in directly proportional increase in nuclear DNA content, therefore, follows the general rule of DNA constancy viz., the nuclear DNA content is constant for a given set of chromosomes within a species. With rare exceptions (see Miksche 1968) the concept of DNA constancy is true. Thus the haploid set at G_1 during interphase is 1C, the diploid 2C, the autotriploid 3C and so on. Similarly, it would be expected that the DNA content of allopolyploids should equal the sum of the DNA amounts of their constituent diploid genomes. This is the situation reflected by results in the Triticinae (Rees & Walters 1965, Nishikawa & Furuta 1969, Pegington & Rees 1970), in *Avenae* (Bullenaad & Rees 1972), in *Nicotiana* (Narayan & Rees 1974) and in *Brassicae* (Verma & Rees 1974).

Comparisons between three characters, namely, chromosome volume, chromosome mass and nuclear DNA content indicated that triploid showed a constant rate of 50% increase over diploid for these characters as expected in case of genome duplication. In other words, as the level of ploidy increased a proportionate increase of these characters was realised. This then, clearly indicates that all these characters are very closely related. There is, therefore, a strict parallel in the variation of the characters

relative to one another a constancy with respect to the ratios, chromosome mass/chromosome volume, DNA/chromosome volume and DNA/chromosome mass; a constancy pertaining both to diploid and triploid (table 4). However, nucleolar mass in triploid did not show a similar rate of change. Without more information about the chemistry of nucleolar activity it would be unprofitable to speculate on the significance of this observation.

Table 4 *Ratio between chromosome mass (CM), chromosome volume (CV) and DNA*

	CM/CV	DNA/CV	DNA/CM
Diploid	0.088	0.086	0.969
Triploid	0.091	0.085	0.927

From the above discussion it becomes evident that triploid forms of *Puschkinia* do not exhibit any variation in chromosome size and mass as compared to diploids. Both diploid and triploid show virtually a constant DNA amount per genome. In other words, there is no detectable change in chromosome volume, mass or DNA in conjunction with the polyploidy. The question which becomes very significant is that why chromosomes with significant amount of heterochromatin would show variation in chromosome size as a consequence to polyploidy but not the chromosomes without significant heterochromatic segments as observed in *Puschkinia*. Possibly an insight into the mechanism can be obtained if the role of the heterochromatin is taken into account. It is possible that heterochromatic segments needed at the diploid level become condensed in polyploids because of their redundancy. Such condensation of heterochromatic segments would result in a decrease in chromosome size without involving a decrease in DNA content. It may be suggested that heterochromatic segments, being not needed, are rendered non-functional in polyploids. Such non-functional state may be achieved by condensation of heterochromatic segments as gene activity involves uncoiling and separation of the polynucleotide columns in the DNA double helix. On the other hand, no variation in chromosome size in *Puschkinia* subsequent to polyploidy was observed. In this case, it would be possible to conceive a probable mechanism if accessory B chromosomes are considered. In *Puschkinia* as many as 7 B chromosomes are recorded and they are characterised by well-defined significant amount of heterochromatin (Vosa 1969, Barlow & Vosa 1969). In contrast, in triploids not more than 1 B chromosome is observed (Dass 1977). These highly heterochromatic B's are therefore, gradually eliminated at higher level of ploidy. Consequently no further diminution in chromosome size is expected. Similar observation is made in *Allium stracheyii* (Sharma & Aiyangar 1961). This species is characterised by 14 A and as many as 8 B chromosomes and it becomes converted into polyploids with the loss of the B chromosomes. The heterochromatic regions are represented by B's and are therefore, absent in polyploids. In other words, adaptability afforded by polyploidy is associated with the elimination of B chromosomes. It is assumed that selective value in adaptability conferred by B chromosomes in *Puschkinia* is no longer needed by polyploids where increased gene dosages allow wider tolerance to environmental differences.

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