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Chromosome size variation during pollen grain development in *Scilla sibirica*

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Summary. The first pollen grain mitosis in *Scilla sibirica* takes place within three weeks after the completion of meiosis. Within one anther the duration of the first pollen grain mitotic cycle varies substantially. The duration of the mitotic cycle affects the length of chromosomes at metaphase of the first pollen grain mitosis. In grains which divide "early" the chromosomes at metaphase are longer, up to twice the length, of the chromosomes in grains dividing "late". The diminution in length with increase in the mitotic cycle is due to more intensive coiling which, in turn, is explained by a lengthening of G2 and of prophase. The relationship between the duration of the mitotic cycle and chromosome length at metaphase would account, at least largely, for the variation in chromosome length between different tissues within organisms. It explains also why the chromosome at metaphase of mitosis are shorter in polyploids than in their diploid ancestors.

Key words: Chromosome size – Duration of mitotic cycles – Pollen development – Diploids and polyploids

Introduction

The size of the chromosomes may vary considerably between species. Such variation is to be expected in view of the large differences in their DNA content.

For example, in the genus *Lathyrus* the chromosomes of *L. tingitanus* ($2n = 14$) are three times larger than the chromosomes of *L. miniatis* ($2n = 14$). The size difference is correlated with the difference in nuclear DNA amounts, which are 20.51 pg and 6.86 pg per 2 *C* nucleus respectively (Narayan 1982). The same situation is found in many other flowering plant genera (Rees and Jones 1972).

There is, however, variation in chromosome size that is independent of variation in nuclear DNA amount. In rye seedlings the size (volume) of metaphase chromosomes in root meristems doubles during the first three weeks of development. Large differences in chromosome size may also be induced by growing seedlings in culture solutions with varying phosphorus content (Bennett and Rees 1969). Polyploidy also affects the size of chromosomes. Darlington (1937) refers to observations which show that the chromosomes of polyploid species at metaphase of mitosis are generally shorter than the chromosomes of their diploid ancestors. Work by Pegington and Rees (1970) confirms these observations. They showed that the chromosomes of polyploid wheat species are shorter than those of their wild diploid progenitors. This work confirms, also, that the difference in length is not due to a change in DNA amount.

A possible explanation for the reduction in chromosome length such as is found in polyploids is as follows.

1. The mitotic cycle and its constituent phases, prophase, metaphase, anaphase, G1, S and G2 are of longer duration in polyploids than in their diploid ancestors, no doubt because the polyploids have more nuclear DNA (Evans et al. 1970).

2. Since G2 and the prophase of mitosis are longer in polyploids than in diploids the chromosomes in polyploids are subjected to a longer period of contraction with the result that the chromosomes at metaphase would be shorter in the polyploids than in the diploids.

A way of testing the hypothesis is provided by an investigation of pollen grain development in the Siberian squill, *Scilla sibirica*. In the pollen mother cells of this, as in many other, flowering plant species meiosis is synchronised within anthers. About three weeks after the completion of meiosis the resulting haploid pollen grains undergo the first pollen grain mitosis. However, some pollen grains within an anther reach the metaphase of mitosis well before others. In other words there is some variation in the duration of their mitotic cycles. If the above hypothesis is correct, namely that the longer the mitotic cycle the shorter the chromosomes at metaphase of mitosis, we should expect the pollen grains dividing late within an anther to have shorter chromosomes than those dividing early.

Materials and methods

Scilla sibirica Andr. is a diploid with $2n=2x=12$ chromosomes. The bulbs were obtained from Wallace and Barr of Kent, U.K. Pollen grains were stained by the Feulgen method after fixing whole anthers in alcohol : acetic acid.

The pollen grain index

The anthers in which a few pollen grains have undergone the first pollen grain mitosis will have mainly grains with one nucleus (ON). In anthers at a later "developmental age" an increasing number of grains will have two nuclei (TN). A simple formula provides an useful measure of the "age" of the anther, namely (100%−%ON+%TN)/2 (Karp et al. 1982). The pollen grain index (PGI) will be low for "young" anthers, high for "older" anthers. The PGI was estimated from 200 pollen grains in one anther from each of six flower buds on each day. The youngest bud in each bulb was chosen for fixation.

Metaphases in anthers with a low PGI will clearly be those in pollen grains dividing early, as compared with metaphases in anthers with a high PGI. It is possible therefore to make a straightforward comparison between chromosomes in grains dividing early within an anther with those dividing late, i.e. in grains with short and, relatively, long mitotic cycles.

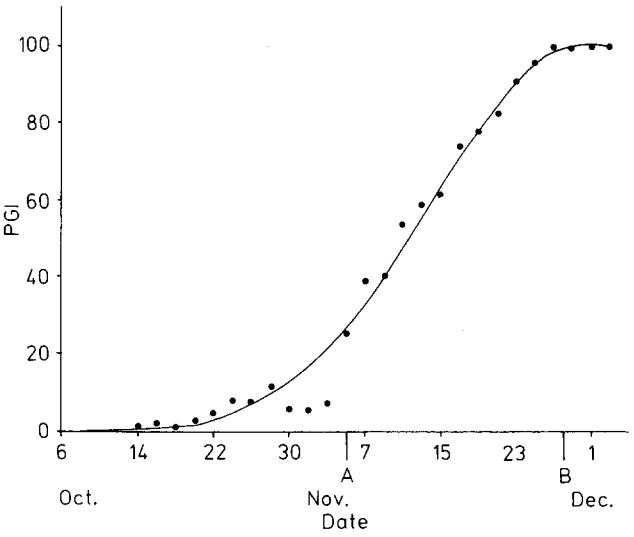

Fig. 1. PGI plotted against date of fixation. Results were pooled over two day intervals. *A* denotes the last day on which meiosis was observed. *B* marks the date on which the first pollen grain division was completed. Data from 30,200 grains

Fig. 2. Metaphases of the first mitosis in pollen grains of *Scilla sibirica*. Note variation in chromosome lengths between grains. Horizontal bar ca. 20 μ

Results

1 The mitotic cycle

Before dealing with the relationship between chromosome length and the duration of the mitotic cycle it is useful to establish a time scale for the cycle and its component phases.

In Fig. 1 the PGI values are plotted against the dates of fixation. The graph shows, first, an increase in PGI with time as would be expected. It reaches 100 on November 27th and is maintained at 100 thereafter. At this date all the anthers sampled have completed the first pollen grain mitosis. Second, the last stage of meiosis, namely tetrads, was observed on November 5th. The interval between the end of meiosis and the completion of the first pollen mitosis is 22 days. This represents the average duration of the first pollen mitotic cycle.

The duration of division. An estimate may also be made of the duration of the division phase, i.e. of the interval between the onset of prophase and the end of telophase. On November 2nd 80% of the pollen grains had one nucleus, 3% contained two nuclei, the remaining 17% were in division. The divisions in this 17% of cells were completed on November 6th, when 20% of grains contained two nuclei. The interval, 96 h, is an estimate of the maximum time taken for the nuclear division.

The times taken in prophase, metaphase and anaphase/telophase were derived as follows. In a random sample of grains undergoing division the ratio of cells at these three phases of division were 74 : 19 : 7. These figures reflect the proportion of time spent at each phase. Since the complete cycle takes 96 h the duration at prophase, metaphase, anaphase/telophase are 71, 18 and 7 h, respectively. These are, of course, estimates of maximum times.

2 Chromosome length variation in relation to the PGI

In Table 1 are the average lengths of chromosomes in microns of the haploid complement at metaphases from a random sample of anthers fixed on various dates and with varying PGI. It will be observed that there is a very considerable variation in the average lengths of chromosomes, from 45 μ–18 μ. These differences are reflected also in Fig. 2.

In Fig. 3 the average lengths are plotted against the PGI. It is clear that the chromosome length decreases with increasing PGI. A linear regression analysis of variance confirms that the decrease in length with increase in PGI is highly significant ($P=<0.01$). The results are in agreement with expectation, namely that the chromosomes are shorter at metaphase in anthers with a high PGI. It is reasonable to conclude therefore that the chromosome length depends, in accord with

Table 1. PGI values, chromosome lengths and volumes, chromatid widths at metaphase of the first pollen grain mitosis in *Scilla sibirica*

PGI	Average chromosome length (μ)	Average chromatid diameter (μ)	Average chromosome volume (μ)
25	45	1.35	795
25	38	1.42	862
31	35	1.65	870
32	29	1.65	857
32	28	1.65	819
40	30	1.65	813
40	30	1.65	780
40	27	1.73	843
42	30	1.65	814
42	29	1.65	851
44	38	1.65	879
44	26	1.65	800
44	26	1.65	817
44	26	1.73	784
44	23	1.80	785
45	24	1.80	784
45	26	1.65	860
47	36	1.73	886
52	31	1.73	795
52	30	1.73	804
52	26	1.75	851
55	26	1.80	869
56	26	1.80	797
56	23	1.95	789
66	26	1.95	835
66	25	1.95	820
67	22	1.95	755
67	22	2.02	817
67	20	2.02	855
69	23	1.88	781
69	25	1.88	790
69	23	1.95	837
69	21	2.02	860
70	28	1.80	842
70	26	1.88	852
70	24	1.88	855
75	24	1.88	788
79	22	2.02	872
80	23	1.95	807
80	22	1.95	817
84	23	1.95	850
88	20	2.10	865
88	19	2.10	820
88	19	2.17	875
90	18	2.17	860

Table 2. The average chromosome lengths at metaphase, in microns, in grains with similar PGI (40–45) in anthers fixed on different days

PGI	40	40	40	42	42	44	44	44	44	45	45
Date (November)	9	9	9	9	9	9	18	18	22	15	15
Chromosome lengths	30	30	27	29	30	26	26	23	26	34	26

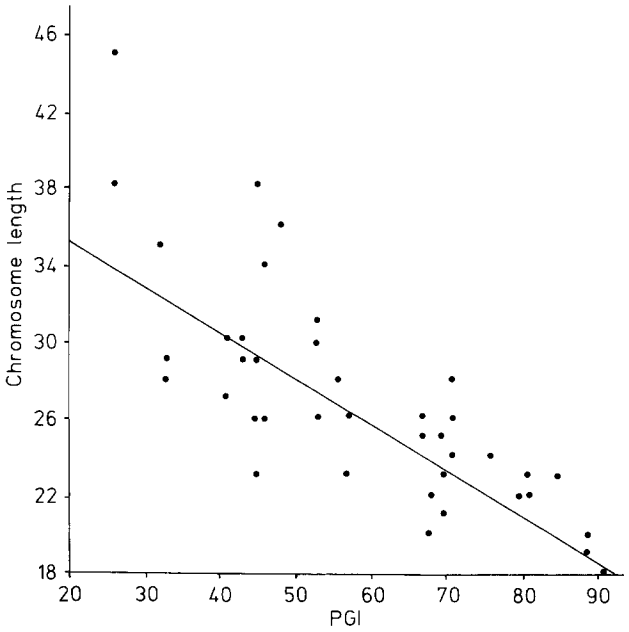

Fig. 3. Average chromosome lengths at metaphase plotted against the PGI

hypothesis, upon the duration of the mitotic cycle. There is, however, one possible objection to this conclusion. Figure 1 showed that PGI changes with date. It might be argued, therefore, that the chromosomes in anthers with a high PGI were shorter not because they were found in grains with longer mitotic cycles but because the cells were fixed at later dates when, for example, environmental conditions might be different from those at earlier dates. It is of course unlikely that environmental conditions would change substantially within a laboratory over such a short period. Nevertheless, it is necessary to determine whether the date of fixing is in itself of significance.

Measurements from anthers with different PGI fixed on the same day. In Fig. 4 the average chromosome lengths at metaphase are plotted against the PGI in pollen grains fixed on the same day (November 15th). Although the sample is small, there is undoubtedly a decrease in chromosome length with increase in PGI. The regression is significant ($P = < 0.05$). The evidence indicates that the date of fixation does not directly account for the change in chromosome length. In other words the chromosome length depends upon change in PGI independently of the date of fixation.

Measurements on different days and similar PGI. The independence of chromosome length from the date of fixation was further confirmed by the results in Table 2. There is no indication of change in length due to date of fixation in anthers with similar PGI. The two sets of results confirm that the chromosome length at metaphase is directly related to the PGI. In pollen grains

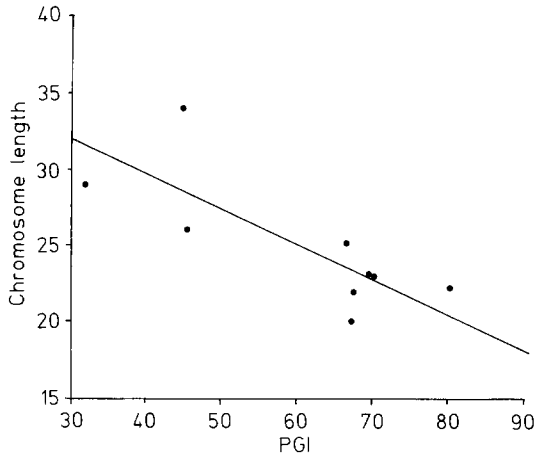

Fig. 4. The average chromosome lengths plotted against PGI in anthers fixed on the same day

which divide late within an anther and, hence, having a longer mitotic cycle, the chromosomes are shorter than in grains dividing earlier and with shorter mitotic cycles.

3 Chromosome volumes

If the reduction in the length of chromosomes with increase in PGI is the result of contraction due to coiling we should expect an increase in the thickness of chromatids without change in the volume of the chromosomes.

The volume of the metaphase chromosomes was calculated assuming the chromatids to be cylindrical in form. The formula used was: the total length of chromatids $x\Pi r^2$; where r is the average radius of the chromatids. The average volume per chromosome was obtained by dividing the total by six. The chromosome volumes and PGI are given in Table 1. It is clear from the table that there is no change in volume with change in PGI. This was confirmed by a regression analysis of variance ($P = > 0.20$).

The data in Table 1 confirm also that the variation in length with increase in PGI is due to a thickening of chromatids due to coiling. The regression of chromatid with width onto the PGI is highly significant ($P = < 0.001$).

4 Individual chromosomes

The results show that, overall, the chromosomes are shorter at metaphase in cells with the longer mitotic cycles. The question arises as to whether the shortening applies equally, and at the same rate, to all chromosomes within the complement. In particular, is the lengthwise contraction the same for the short (S) and for the long (L) chromosomes within the *Scilla* complement? It will be seen from Fig. 2 that there is a considerable difference in length between the longest

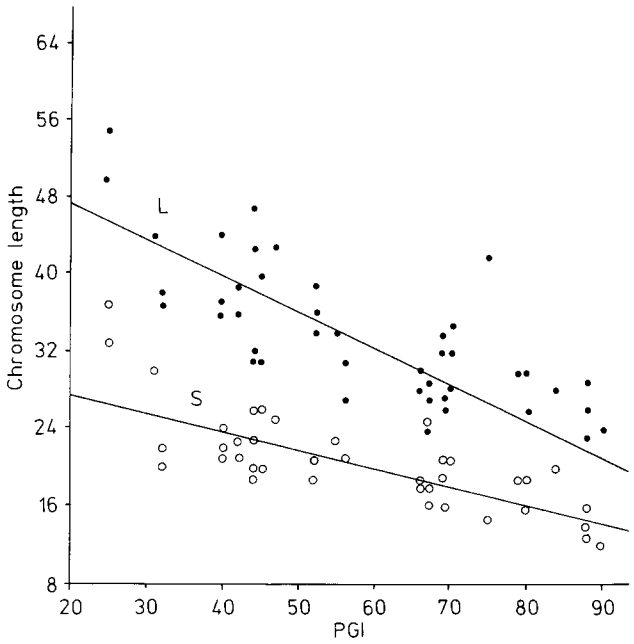

Fig. 5. The lengths of L and S chromosomes plotted against PGI

and the shortest chromosomes and that L and S are readily identified at metaphase.

a) The L chromosome. The lengths of the L chromosome in pollen grains with different PGI are plotted in Fig. 5. The regression is significant ($P = < 0.001$), the slope (b) is -0.30.

b) The S chromosome. The lengths of the S chromosome in pollen grains with different PGI are also plotted in Fig. 5. The length decreases with increasing PGI ($P = < 0.001$) and the slope (b) is -0.19.

c) L and S chromosomes compared. The analyses above show that both L and S chromosomes decrease in length with increasing PGI. The analyses show, also, that the slopes of the regressions differ substantially. The difference is significant ($P = < 0.05$).

In absolute terms the diminution in the length of the L chromosome, over the same period of time, is clearly greater than that of S. From Fig. 5 it will be seen that between PGI 20 and 90 the length of the L chromosome drops from 47 μ–23 μ, i.e. a change of 24 μ. During the same period S changes from 27–13 μ, a drop of only 14 μ. If, however, we look at the rate of change in length in relative terms the lengths of L and S chromosomes have been reduced by almost exactly one half between PGI 20 and PGI 90. The relative rates of change in the L and S chromosomes are similar.

Discussion

In part the size of the chromosomes depends directly upon the genotype, i.e. upon the DNA content. Chro-

mosomes containing large amounts of DNA will be larger than chromosomes with a lower DNA content (Raina and Rees 1983). The correlation, however, is not complete. The chromosome size at metaphase of mitosis in cells of different tissues varies substantially, despite the fact that the DNA amount is the same in these tissues. Moreover the chromosome size may vary very considerably between cells within the same tissue. An example, referred to earlier, comes from the work of Bennett and Rees (1969) which shows a large change in chromosome size in metaphases of root meristems of rye seedlings of different age. This kind of information shows that the chromosome phenotype depends in part upon the cell environment as well as upon the chromosome genotype, i.e. the DNA content.

One of the first demonstrations of change in chromosome phenotype due to the cell environment was by Pierce (1937). He showed that changes in chromosome and nuclear volumes could be induced by varying the concentration of phosphorus supplied to the roots of a violet species, *Viola conspersa*. The chromosomes in root meristems grown in high concentrations of phosphorus were twice the size of those grown in a 'normal' culture solution. While these observations are of long standing comparatively little work has been devoted to explaining the causes of such variation.

It has been argued that the variation in chromosome size might be of adaptive importance. Darlington (1937) maintained that the diminution in chromosome size in polyploid species may be adaptive in the sense that the larger number of chromosomes within the polyploid cells may be more readily accommodated on the mitotic and meiotic spindles. Also, from an adaptive standpoint, there is evidence from Bennett and Rees (1970) that induced differences in chromosome length increase the amount of recombination at meiosis. The evidence for this was as follows. Increasing the phosphorus content of culture solutions generates an increase in chromosome length in rye inbred lines. In turn the increase in chromosome length is accompanied by an increase in the frequency of chiasmata at meiosis.

Leaving aside the question of adaptation the object of the present work was simply to attempt to establish the cause of the alteration in chromosome size or, more specifically, the alteration in chromosome length that is associated with change in the cell environment. The proposition was that the chromosome length could be directly determined by the duration of the mitotic cycle within the cell. Since the contraction of the chromosomes at metaphase is, at least largely, due to coiling the argument was that with the lengthening of the cycle and, consequently, the lengthening of G2 and of prophase, the coiling would be that much protracted with the result that the chromosomes at metaphase would be shorter than in cells with a shorter cycle. The proposition was suggested by observations that the duration of mitotic cycles in polyploids is longer than in their diploid ancestors (Evans et al. 1970) and that the chromosomes at metaphase of mitosis in the polyploids are shorter than the same chromosomes at metaphase in their diploid progenitors.

The use of pollen grains for this purpose is particularly convenient and straightforward because the time taken between the end of meiosis and the metaphase of the first pollen grain mitosis varies between grains within the same anther. The results from measurements in these grains were in agreement with the proposition. The longer the mitotic cycle the shorter the chromosomes at metaphase. The *Scilla* material was useful also in the sense that individual chromosomes, the large L, and small S, chromosomes could be readily identified. This made possible a comparison of changes in length, consequent upon change in the mitotic cycle, between chromosomes of different sizes.

The overall conclusion was that the chromosome length at metaphase is largely dependent upon the duration of the mitotic cycle (cf. Karp et al. 1982). The evidence goes a long way to explain, therefore, the paradox that chromosome lengths vary in different cell environments quite independently of their DNA content.

Finally, since chromosome length at metaphase varies as a result of variation in the mitotic cycle a survey of chromosome lengths at metaphase in different tissues and organs might well be useful as an index of the duration of the mitotic cycles in different tissues and organs during development, a factor of importance and significance from the standpoint of morphological and physiological differentiation during growth.

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