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3. Estimates of the replication patterns of individual chromosomes.

In MGCNL 42:175-178 (1968) we presented a preliminary study of the nuclear cycle in maize root tip nuclei. This investigation has been extended to determine ^{3}H-thymidine incorporation patterns in individual chromosomes of maize root tip nuclei.

The stock used was a chromosome 3 tester in which each cell contained one B chromosome. Autoradiographs were prepared in the manner previously reported (MGCNL 42:175-178, 1968) with the following modifications:

1) root tips were fixed 8, 9 and 10 hours after pulse labeling.
2) root tips were immersed in 0.002 M 8-hydroxyquinoline for 3 hours prior to fixation.

After photographing labeled cells the silver grains were removed in the following manner:

1) Slides were soaked in absolute ethanol until the coverslips were removed, then were passed through an alcohol series to distilled water.
2) Slides were transferred to 7.5% $K_3Fe(CN)_6$ for 3 min. and then to 20% $Na_2S_2O_3 \cdot 5\ H_2O$ for 3 mins.
3) Following 3 changes of distilled water, slides were passed through an alcohol series to absolute ethanol and mounted in Eupanol.

Cells previously photographed were rephotographed and individual chromosomes were identified on these photographs.

Mean silver grain counts over each chromosome (Table 1) were plotted against time (between fixation and pulse labeling). A linear regression coefficient was calculated for each chromosome (Table 2). We interpret a regression coefficient of a chromosome to represent its mode of replication during that portion of the S period in which the chromosome is replicating. Thus the chromosomes demonstrating initial rapid uptake of label will possess the most positive coefficients.

Table 1

Frequency data from ^{3}H-thymidine pulse labeling (30 min) in maize root tip nuclei

Chromosome	Mean number of Silver Grains		
	8 hr	9 hr	10 hr
1	6.82	5.00	3.75
2	5.94	4.67	5.44
3	6.02	3.83	4.38
4	6.06	3.67	3.69
5	5.23	3.17	3.75
6	3.97	1.50	3.06
7	3.97	3.17	3.19
8	4.09	2.83	2.87
9	4.23	3.50	3.19
10	3.54	1.83	2.62
B	6.67	1.25	5.33

Table 2

Values calculated from the data recorded in Table 1

Chromosome	Regression Coefficient	Standard Error
1	-1.54	±0.11
2	-0.23	±0.33
3	-0.86	±0.44
4	-1.22	±0.38
5	-0.37	±1.02
6	-0.51	±0.61
7	-0.40	±0.13
8	-0.57	±0.21
9	-0.53	±0.07
10	-0.48	±0.41
B	-1.12	±0.53

Even though we are reporting limited data (28 cells), certain relationships are seen to emerge: Chromosomes 1, 3, 4 and the B chromosome appear to possess the most negative coefficients. Additional data from this study suggest that the B chromosome is delayed beyond mid-S in initiating uptake of label. Further, the data suggest that the terms late and early replication are ambiguous when used to describe the replication of chromosomes. The terms indicate the time within the S period at which a chromosome begins replication, but are not necessarily descriptive of the rate or mode of replication once DNA synthesis has been initiated within a chromosome.

Differences in coefficients can be tested by appropriate tests. Large standard errors, such as the one associated with chromosome 5, may indicate curvilinear as well as linear relationships. Regression analysis was possible only because of our ability to identify the somatic chromosomes in maize, and is unique in that it quantifies the patterns of replication of specific chromosomes and allows quantitative comparisons of these patterns. We are employing cytogenetic modifications of specific chromosomes in an attempt to alter the replication patterns. In addition, we can determine gross alternatives of replication patterns in chromosome segments.

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4. An abnormal chromosome 6 in maize.

One stock of maize was found having two secondary constrictions located in the distal portion of the short arm of chromosome 6 at somatic metaphase. Two tandem satellites were observed on each homologue (Fig. 1d). If in the normal stocks the secondary constriction at somatic metaphase corresponds to the heteropycnotic nucleolar organizer at pachynema, the stock described here may have two nucleolar organizers on chromosome 6. The following observations from meiosis and mitosis seem to suggest that this abnormal chromosome may have originated from a normal 6 through a paracentric inversion with one breakpoint in the nucleolar organizer and the other proximal to the organizer (Fig. 1). To conform with maize terminology, we have assigned "A6" to this abnormal no. 6 chromosome.