

Table 3
Standard deviations of the nuclear cycle component estimates
in the primary root-tips of Zea mays L. (KYS) at 25°C

Phase	Hrs.
G ₁ + Mitosis	0.53
S	0.13
G ₂ + ½ Prophase	0.05
Total nuclear cycle	0.55

Initially, consideration was given to the view that the nuclear cycle was under specific genetic (gene) control. It was proposed that maize was an excellent material in which to test the hypothesis of specific genetic control, inasmuch as a wide variety of markedly different agronomic and genetic stocks was available. If the duration of the nuclear cycle was related to the growth characteristics of a stock, judicious choice of a few stocks should permit the identification of different nuclear cycles under identical controlled conditions.

Starting with 'Seneca 60', KYS, 9 tester and W23 stocks, all possible F₁, F₂, BC₁ and BC₂ stocks were developed at our field station. Upon discovering that no differences in the nuclear cycle of the different stocks at 25°C could be described, the analysis of the F₁, F₂, BC₁ and BC₂ hybrids was discontinued. It remains to be shown whether or not stock differences can be described at temperatures other than 25°C, or under the influence of other environmental conditions, as a prerequisite for heritability studies; at the moment, we must conclude that these differences do not exist and that further information would not be contributed by analyzing the F₁ and subsequent generations.

Ram S. Verma

3. Reassociation of interchange and interstitial segments.

It has already been demonstrated (Jancey and Walden, 1972) that significant departures from an equidistribution of 'breaks' occur in the

chromosome arms of maize. In this report, it will be shown that a striking reassociation pattern also exists, beyond that attributable to the previous inequality. Data were drawn from the reports of Longley (1958, 1961) and Burnham (1969).

The hypothesis was erected that the reassociation between chromosome segments, pooled for long and short arms, did not differ from an equidistribution when corrected for known 'breakage' frequency per segment. Data were expressed in micron units from measurements made at pachynema; arms were divided into 5u classes. If the pooled data are divided into n frequency classes, and if f = the observed break frequency per class, then the expected frequency of reassociation (F), for the i th row and j th column intersection in the $n \times n$ symmetric matrix of reassociation frequencies, will be:

$$F_{ij} = \frac{\sum_{i=1}^n f_{ij}}{\sum_{j=1}^n f_{ij}} \left(\frac{\sum_{i=1}^n f_{ij}}{\sum_{i=1}^n \sum_{j=1}^n f_{ij}} \right)$$

From this, a test of significance of departures from expectation can readily be obtained using X^2 values.

The frequencies of reassociation of chromosome 'breaks' were used to compute deviations from expected values as described earlier. Chi square contributions resulting from these deviations are presented in three forms, in Tables 1, 2 and 3. In Table 1, the 10 columns of X^2 contributions correspond to class intervals along the chromosome arms. Thus 'breaks' occurring between 0.0u and 4.9u show a X^2 contribution of 107.6 resulting from an excess of observed reassociations with interchange segments whose residual, complementary interstitial segments were also of 0.0u to 4.9u. In the same column it will be seen that the identical interstitial class shows a X^2 contribution of 7.7, obtained from the deficit in observed reassociations in the class of interstitial segments which were 5.0 - 9.9u in length. Subsequent columns of Table 1 similarly show relatively large X^2 contributions resulting from excess reassociation where the interstitial segment (class I) is of the same length as the interstitial segment of the second chromosome involved in the translocation (class II). This relationship between segments of like length can be seen by examining the values around the top left to lower right diagonal of Table 1. Chi square con-

Table 1
 Pooled data for total chromosome complement. χ^2 contributions for reassociation frequencies. Classes based on mid pachytene lengths expressed in microns.

		INTERSTITIAL SEGMENT LENGTH										Total χ^2 contribution interstitial segment
		0.0- 4.99	5.0- 9.99	10.0- 14.99	15.0- 19.99	20.0- 24.99	25.0- 29.99	30.0- 34.99	35.0- 39.99	40.0- 44.99	45.0- 49.99	
RELATED INTERSTITIAL SEGMENT LENGTH	0.00- 4.99	107.6°	7.7	3.0	7.6	5.0	2.0	0.0	0.4°	2.9	0.0	136.2
	5.00- 9.99	7.7	37.1°	0.3°	2.4	3.4	4.1	3.2	3.0	0.7	0.0	61.9
	10.00- 14.99	3.0	0.3°	0.6°	2.6°	0.2°	1.3	0.3	1.4	0.2°	0.0	9.9
	15.00- 19.99	7.6	2.4	2.6°	0.5	7.8°	2.7°	1.2°	0.1°	0.4	0.0	25.3
	20.00- 24.99	5.0	3.4	0.2°	7.8°	0.9°	3.6°	0.0	0.1	0.4	0.0	21.4
	25.00- 29.99	2.0	4.1	1.3	2.7°	3.6°	4.9°	0.2°	0.0	5.0°	0.0	23.8
	30.00- 34.99	0.0	3.2	0.3	1.2°	0.0	0.2°	0.0	10.3°	15.6°	0.0	30.8
	35.00- 39.99	0.4°	3.0	1.4	0.1°	0.1	0.0	10.3°	15.3°	0.2	0.0	30.8
	40.00- 44.99	2.9	0.7	0.2	0.4	0.4	5.0°	15.6°	0.2	0.1	0.0	25.5

° χ^2 contributions resulting from reassociations in excess of expected frequencies.

tributions away from the diagonal tend to be small; the larger values are, without exception, derived from negative departures from expected values in the direction of fewer events than expected rather than the reverse.

Table 2 presents X^2 contributions derived from expected reassociation frequencies based on the same data, but expressed in terms of interchange segment classes reassociating with interstitial segment classes. In the first column it will be seen that the interchange class containing 'breaks' 0.0 - 4.9u from the telomere shows X^2 values resulting from an excess of reassociation over expected values for the larger interstitial segment classes, with a high X^2 contribution (13.0) from the interstitial segment class 25.0 - 29.9u. There is a corresponding lack of observed reassociations with the shorter interstitial segment classes, e.g., the X^2 contribution (9.4) for the interstitial class 5.0 - 9.9u. The distinction is less marked than that seen in the first column of Table 1. This may be attributed to the differing total lengths of chromosome arms, which, when breaking within 4.9u from the centromere, will give rise to a variety of interchange segment lengths. In other words, for both chromosomes involved, only the distance of the break from the centromere has relevance. This latter point is clearly made in Table 3, which is similar to Table 1 except that both segments are expressed in terms of distance of 'breaks' from the telomere, i.e., interchange segments. The pattern of excess and deficit of reassociation is still less clear in this case, except when close to the telomeres (top left of table) where the pattern of preferential reassociation of like lengths is again seen. This is as would be expected if a phenomenon involving interstitial segments is being twice diffused in its demonstration by the variability of arm length among the chromosome complement. The contribution of 18.3 from a positive deviation for class 25.0 - 29.9u corresponds to the top left cell of Table 1 as revealed by a reference system based on the telomere and observed by those based on the centromere.

The relationship between the data as recovered and original reassociation events has been discussed elsewhere (Jancey and Walden, 1972). The values derived from reassociation frequencies demonstrate that the most important feature in predicting the preferential areas of reassociation is the distance of the 'breaks' from the centromere (see Table 1). As would

Table 2
 Pooled data for total chromosome complement. χ^2 contributions for reassociation frequencies. Classes based on mid pachytene lengths expressed in microns.

	INTERCHANGE SEGMENT LENGTH										Total χ^2 contribution interstitial segment
	0.0- 4.99	5.0- 9.99	10.0- 14.99	15.0- 19.99	20.0- 24.99	25.0- 29.99	30.0- 34.99	35.0- 39.99	40.0- 44.99	45.0- 49.99	
0.00- 4.99	0.9	1.1	0.7	3.2	1.5	0.6°	13.2°	4.6°	5.0°	3.0°	33.8
5.00- 9.99	9.4	6.5	2.5	0.2°	3.3°	13.9°	1.2°	0.1°	0.1°	0.0	37.2
10.00- 14.99	0.3	0.0	3.2°	0.1°	0.0	0.1°	0.2	1.9	1.0	0.4	7.2
15.00- 19.99	0.2°	3.4°	0.2	2.6°	0.7	1.8	1.1	0.0	0.4	0.4	10.8
20.00- 24.99	1.4°	5.4°	2.4°	0.2°	3.6	4.4	4.6	0.1°	0.2°	0.4°	22.7
25.00- 29.99	13.0°	0.5°	0.2	0.1°	2.4°	8.0	4.1	1.2	0.9	0.2	30.6
30.00- 34.99	3.9°	0.8°	1.0°	0.7°	0.8°	4.9	1.4	0.3	1.3	0.2	15.3
35.00- 39.99	3.2°	0.4°	0.4	0.0	3.1	0.0	0.2°	0.6	0.2	0.0	8.1
40.00- 44.99	0.7°	0.4°	2.5°	0.0	0.1	0.7	1.7	0.6	0.2	0.0	6.9

° χ^2 contributions resulting from reassociations in excess of expected frequencies.

Table 3
 Pooled data for total chromosome complement. χ^2 contributions for reassociation frequencies. Classes based on mid pachytene lengths expressed in microns.

	INTERCHANGE SEGMENT LENGTH										Total χ^2 contribution interstitial segment
	0.0- 4.99	5.0- 9.99	10.0- 14.99	15.0- 19.99	20.0- 24.99	25.0- 29.99	30.0- 34.99	35.0- 39.99	40.0- 44.99	45.0- 49.99	
0.00- 4.99	12.1°	1.6°	1.3°	0.0	2.9	6.3	0.2	3.4	0.5	0.3	28.6
5.00- 9.99	1.6°	0.8°	0.9°	0.4°	0.0	2.6	3.7	0.0	1.2	0.5	11.7
10.00- 14.99	1.3°	0.9°	1.4	0.2	0.0	1.1	0.4°	0.0	0.1	0.4	5.8
15.00- 19.99	0.0	0.4°	0.2	3.0°	0.1°	4.3	0.0	0.2	0.3	0.4	8.9
20.00- 24.99	2.9	0.0	0.0	0.1°	0.2°	0.9°	0.3	0.2°	0.1	0.4	5.1
25.00- 29.99	6.3	2.6	1.1	4.3	0.9°	18.3°	3.3°	0.4	6.3°	2.3	45.8
30.00- 34.99	0.2	3.7	0.4°	0.0	0.3	3.3°	0.3	6.0°	0.5	1.6	16.3
35.00- 39.99	3.4	0.0	0.0	0.2	0.2°	0.4	6.0°	2.1°	0.0	0.4	12.7
40.00- 44.99	0.5	1.2	0.1	0.3	0.1	7.6°	0.0	0.0	0.5	0.0	10.3
45.00- 49.99	0.3	0.5	0.4	0.4	0.4	2.3	1.6	0.4	0.0	0.0	6.3

° χ^2 contributions resulting from reassociations in excess of expected frequencies.

be expected, reassociation frequencies, expressed wholly or partially in terms of interchange segment length, still show some significance because of the apparent correlation which exists between the lengths of interchange and interstitial segment. Were all the chromosome arms of the same physical length, the correlation would of course be perfect. Its imperfection permits the demonstration of the primary role played by the interstitial segment lengths, and also the importance of telomere related length for telomeric events.

The deviations of observed frequencies of reassociation from expected values are of particular interest. Along the whole length of the chromosome arm an excess of reassociations occurs between other chromosomes in which the 'break' has occurred at a similar distance from the centromere. Both of these results would be compatible with the hypothesis of chromosome attachment by their centromeres to some limited area of the nuclear membrane, and telomere attachment to the nuclear membrane with possibly a greater mutual spatial separation between them than in the case of the centromeres. Evidence from electron micrographs for such attachment has been presented recently from several laboratories for several species. The excess of reassociations involving breaks of similar distance from the centromere would also suggest that the chromosome arms are extended in the nucleus in a parallel manner rather than the classical concept of a complex, intertwined mass.

We have been tempted to use these reassociation data to predict an organizational 'geography' of the interphase nucleus. Computer simulation has been moderately successful and we hope to report on this extension of our analysis shortly.

References:

- Burnham, C. R. 1969. In IV. Report on maize cooperative by R. J. Lambert. Maize Genet. Cooperation Newsletter 43: 230.
- Jancey, R. C. and D. B. Walden. 1972. Analysis of pattern in distribution of breakage points in the chromosomes of Zea mays L. and D. melanogaster Meigen. Can. J. Genetics & Cytol. 14 (2), 429-442.
- Longley, A. E. 1958. Breakage points for two corn translocation series maintained at the California Institute of Technology. U.S. Dept. Agr. Res. Serv. ARS 34-4.

Longley, A. E. 1961. Breakage points for four corn translocation series and other corn chromosome aberrations. Crop Res. U.S. Dept. Agr. Res. Serv. ARS 34-16.

R. C. Jancey
D. B. Walden

4. Intervarietal differentiation of maize pollen.

Considerable differences in values for quantitative characters of maize pollen and pollen of maize-related taxa have already been demonstrated (Tsukada and Rowley, 1964, Banerjee and Barghoorn, 1970). Attempts to discriminate between the pollen of maize and that of Euchlaena mexicana (teosinte) and Tripsacum spp. are complicated by the wide range of values for any given character. Pollen diameter, pore-axis ratio, spinule density and spinule distribution have proven to be useful characters when taken together. Little work has been done, however, on variation at the varietal level.

Rumbaugh and Whalen (1972) reported that significant size differences exist among pollen grains from some maize genotypes, particularly in the case of tetraploid varieties.

This report outlines some preliminary aspects of a study being carried out to determine the feasibility of characterizing the pollen on the basis of multivariate analysis of a number of characters.

Pollen samples were collected from plants of 11 stocks (see Table 1) grown in the field or in the greenhouse. Samples were immediately transferred to a deep freeze where they were stored at -10°C .

Micrographs of pores and of areas of the spore wall were taken at a magnification of 5000, 10,000 and 20,000 diameters in a Cambridge Mark 2a scanning electron microscope. For this purpose, samples were fixed to glass and coated with a gold-palladium alloy.

Spinule density was calculated for 5 to 8 pollen grains per stock by counting the number of spinules in a 176 sq. cm. area from 20,000X micrographs. This corresponded to an actual area on the pollen of $44\mu^2$. The mean basal diameter of spinules was calculated from 20,000X micrographs and based on 40 spinules per grain. Data for 5 to 8 pollen grains were recorded for each stock.