Table 6

Distribution of reassociation pairs, including centromeres, recognising the difference in break frequency between arms and centromeres*

	Obs	Expected	$\frac{(f - F)^2}{F}$
Between arms	962	928.28	1.22
Between arms and centromeres	8	82.84	67.61
Between centromeres	43	1.86	909.94
	1013	1012.98	978.77
D.F. = 2		$p < 0.005$	

*Expected values are on the basis of pachytene length (see text) and corrected for the demonstrated higher breakage frequencies in the centromeres.

It has been demonstrated that in maize the hypotheses of random breakage and random reassociation are not supported by the data. Inasmuch as these data represent the most extensive collection, further analyses may reveal new concepts concerning the composition and organisation of nuclear chromatin.

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4. Principal Components Analysis applied to addition segments in chromosome 9.

In an attempt to quantify differences within chromosomal parameters on a biometrical basis, several types of tests, both parametric and non-parametric, were performed. These types of repetitive testing lead ultimately to the acceptance or rejection of the null hypothesis of no difference. Other types of analyses are available (see Jancey, this News Letter). Of particular noteworthiness for our ongoing interest in karyotype analysis are principal components analysis (PCA) and graph theory.

The source material for our study consisted of six reciprocal interchange stocks. The meiotic post-interchange length of 9S in these

stocks ranged from 0% to 50% increments in steps of about 10% of the standard length. Two normal stocks, seneca '60' a commercial single cross ($\underline{su}_1/\underline{su}_1$) and a chromosome 9 tester stock ($\underline{yg}_2$ $\underline{C}$ $\underline{sh}_1$ $\underline{bz}_1$ $\underline{wx}$), also were included.

Crosses were performed to obtain the interchange stocks in both the T/N and T/T condition. Mitotic karyotypes were prepared from 25 cells with non-overlapping chromosomes in each of the 14 stocks. The mitotic chromosome arms were measured and values for the following parameters were calculated and included: total length; arm ratio; centromeric index; and homologue differences between all the preceding parameters. Each cell was considered an individual in the PCA yielding 140 characters (variables) per cell. Any type of mensuration data might be included, for example, labelling counts or density measurements. The only restriction on the number of variables used in any one analysis is the capacity of the computer facilities.

The print out from the analysis allows the investigator to examine the data both numerically and pictorially. The absolute values of the eigen vectors represent the contributions of the character used; that is, for any particular axis, comparatively high vector values represent primary or important contributors and comparatively low vector values represent characters which are of less importance in the separation of the individuals for a particular axis. Factor scores are printed out for each axis. The factor scores of an individual (cell) on any given component axis is the sum of the individual's (cell's) standardized value for each character multiplied by the contribution of that character (i.e., the vector value) to the axis. The analysis has, to some extent, a built in checking system. For example, if one or two particular cells of a specific population of cells are separated off by themselves, one can go back to the eigen vector values and see which characters are responsible for this separation. The original data can then be checked for possible errors.

Some of the more striking results from the P.C.A. applied to the 14 stocks include:

1) The characters 'arm-ratio' and 'centromeric-index' were identical contributors. Thus, the use of one rather than the other

appears to be unwarranted. Further, these two indices had a correlation of 1.0 in the correlation matrix. Thus, one or the other may be used but not both simultaneously.

2) The pictorial spatial relationships demonstrated that the T/T was separated more completely than the T/N. This would be expected since the T/N condition involved two altered chromosomes and the T/T involved four.

3) In the larger chromosomal alterations (40% and 50%), the altered chromosomes were the major contributors in stock separation. In other stocks, however, chromosomes not involved in an interchange were, in many cases, the important contributors to stock separation. These observations, that specific chromosomes within particular stocks were of major importance, indicates the existence of between stock differences, i.e., values of specific chromosome parameters may be unique to certain stocks. This was very apparent in the complete separation noted for the two normal stocks. These two stocks have a divergent pedigree in which we estimate there has been no interbreeding for at least 50 generations and probably many more.

4) The absolute changes in pachytene length (microns) between the interchanged chromosomes used and chromosomes of the normal maize complement are presented in Table 1. Included in Table 1 is a comparison between the size (microns) of these changes in pachytene and mitotic metaphase. The entries for mitotic metaphase are based on a 13x reduction from the pachytene values.

In maize, in chromosomes 9 and 10, which are submetacentric chromosomes, a change of 0.58 μ resulted in stock separation. Increments to 9S of less than 0.58 μ did not result in stock separation and a decrement of 0.46 μ in 10L was not detected via the P.C.A. However, the addition of 0.24 μ to 5L, a metacentric chromosome, was detected. Overall, our results suggest a fortiori that a percent change (x) may be easier to detect mitotically if it is involved in a decrement rather than an increment. Further, the ability to detect an anomaly may be dependent upon the type of chromosome altered (i.e., metacentric, sub-metacentric or acrocentric).

Table 1

Changes in Chromosome 9S		
Stock Code	Pachytene Length (microns)	Somatic Metaphase Length (microns)[a]
9-5 4871	0.5	0.04
9-7 a	1.6	0.12
9-5 a	3.1	0.24
9-2 c	4.7	0.36
9-10 8630	6.0	0.46
9-10 b	7.6	0.58

a) reduction factor = 13

The problem of somatic chromosome identification of an aberration such as an interchange can now be readdressed. One hypothesis suggests that a simple relationship exists between the ability to identify an anomaly and the size of the chromosomal alteration; that is, the greater the anomaly, the greater the chance of detection. Our observations, however, indicate that a relatively short alteration may be detected in some chromosomes. Further, the ability to detect an anomaly may be dependent upon it being an increment or a decrement to a chromosome. These two criteria may also be interdependent. In order to explain the non-linearity observed between increase and decrease, the following hypothesis is offered: the interstitial segment plus the exchanged piece does not always present a 'simple additive model' in somatic metaphase.

Many factors could account for this 'non-additivity', such as: (1) differential chromosome contraction; (2) addition or subtraction of a specific segment at meiosis may not be expressed in the same relative magnitude during mitosis (possibly interdependent upon the 13x reduction and differential contraction); (3) effects may be chromosome specific, arm specific or both (the data suggest that a metacentric chromosome may show more pronounced differences with small alterations than chromosomes of a more submetacentric or acrocentric type).

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