

Table 2
Cytological data from T 9 - 2 c/N

	Diakinesis			Pollen	
	⊙ 4	Chain 4	Pairs	Abortion	Ratio : 1 + / wx
frequency	756	64	0	36.1%	1.69

At present, two more tests are being carried out; (a) intercross data from T/N x T/N, subsequent scoring of wx, bz₁, sh₁, and the occurrence of the three types of chromosomal combinations (TT, TN, NN); (b) screening of a large number of wx, bz₁, sh₁ genotypes in an attempt to recover crossover events between the waxy locus and the breakpoint. Recovery would permit the reciprocal test, namely the recessive alleles on the translocated chromosome and the dominant alleles on the non-translocated chromosome. Nuffer's data using the Wx allele as a marker on N, indicated a higher proportion of normal chromosomes were transmitted through the ♀. However, 9 - 2 c displays the reverse. By changing the allele-chromosome combination, some insight into the abnormal chromosome segregation and its relationship, if any, to the waxy or adjacent loci may be forthcoming.

W. G. Filion

6. Biometrical analyses of somatic (root-tip) chromosomes.

The maize root-tip karyotype procedures provide an experimental system within which can be studied environmental and heritable factors affecting chromosome parameters. We have initiated a study in which we propose to examine the biometrical modifications:

1. on the entire complement, which might result from the influence of gene loci known to alter chromosome behavior;
2. on individual chromosomes as a result of cytogenetic alteration.

Below are presented representative data from one of the stocks used as a basis for several studies in this laboratory.

Table 1
 Summary of maize chromosome statistics compiled from 38 metaphase
 root-tip karyotypes of hybrid 'seneca 60'

	Chromosome									
	I	II	III	IV	V	VI	VII	VIII	IX	X
A. Mean Arm Ratio standard error	1.20 0.06	1.50 0.07	1.91 0.08	1.46 0.06	1.09 0.06	2.25 0.10	2.30 0.08	2.66 0.11	1.64 0.08	1.95 0.08
B. Mean Relative Length of <u>homologue</u> standard error	14.40 0.16	12.02 0.13	11.09 0.13	10.99 0.12	10.39 0.13	8.97 0.13	8.42 0.13	8.60 0.13	8.00 0.12	7.12 0.12
C. Mean Relative Length of <u>long arm</u> standard error	7.81 0.09	7.11 0.10	7.25 0.08	6.51 0.08	5.39 0.06	6.16 0.09	5.85 0.08	6.21 0.08	4.94 0.07	4.69 0.61
D. Mean Relative Length of <u>short arm</u> standard error	6.59 0.10	4.90 0.06	3.84 0.07	4.48 0.06	5.00 0.07	2.80 0.06	2.57 0.05	2.39 0.07	3.06 0.06	2.43 0.04
E. Variances										
Between homologues	1.96	0.96	0.51	0.90	0.60	1.05	0.47	0.71	0.55	0.43
Between long arms	0.66	0.65	0.39	0.58	0.29	0.63	0.34	0.47	0.38	0.24
Between short arms	0.96	0.27	0.37	0.21	0.23	0.27	0.12	0.33	0.30	0.10

Filion has commented (MGCN 42:175, 1968) on the use of computers in root-tip karyotype analysis. Technical difficulties with the maize system were discussed in relation to the use of the expensive and complex automated data input systems such as FIDAC, CYDAC, and the CHLOE Film Scanner.

Despite our inability to automate the mensuration of the chromosomes of maize, we feel that the opportunity to process large amounts of data in repetitious and complex calculations can still be useful for karyotype analysis. Using Fortran IV, we have written a simple but flexible program to allow rapid calculation of the various chromosome parameters for any number of chromosomes or cells. This program, now available on remote access call to our IBM 7040, may be obtained by writing the undersigned. The program accepts chromosome arm lengths and the centromere coordinate plots. The latter are obtained from a grid placed over the spread. All metaphase distributions are accepted by the program. The program permits continued evolution of the confidence limits employed to first identify and secondly, analyze the chromosomes. Presently, the chromosomes are assigned an identification (I-X) in the computer program on the basis of four criteria: relative length, arm ratio, long and short arm relative lengths. A subroutine classifies the satellited chromosome VI before identifying the rest of the complement. By integrating the normal curve for each chromosome I to X derived from previous experimentation, we can arrive at a probability expression summarizing the four criteria to yield the best possible fit for the unknown chromosome. Thus, the computer print out provides a numerical identity and an approximation of the reliability of this estimate. The program is useable whether or not all chromosomes of a metaphase spread are presented for analysis and, in addition, it is useable with aneuploid stocks.

Photographs of 38 metaphase spreads of 'Seneca 60' (x 3,000) were projected to give magnifications of approximately X 30,000. The more distinct chromatid of each chromosome was traced and measured. Following, these measurements of arm lengths were entered into the computer and processed. Table 1 contains the statistics for each chromosome. To eliminate errors due to magnification, relative lengths of arms and chromosomes per cell were indexed:

$$\frac{\text{length of segment}}{\text{total length of complement}} \times 50 = \text{relative length}$$

The arm ratio of chromosomes has been considered to be a rather stable chromosome parameter and although our data indicate variability of less than 10% for most chromosomes, chromosomes VI and VIII demonstrate unusual variability. The short arm of chromosome VI was measured in these studies to the end of the intact arm but not including the stalk and satellite. Reference to Table I indicates that excessive variation in chromosome VI is found in both arms. Likewise, chromosome VIII demonstrates considerable arm variation, particularly in the short arm. It is interesting to note that these same somatic chromosome arm ratios showed the greatest discrepancy with the published pachytene arm ratios. We are in the process of ascertaining the pachytene arm ratio of the 'Seneca 60' stock.

Several predictions can be derived from these chromosome parameters. Insofar as the corn system is "typical", this biometrical approach may be useful to investigators employing other organisms, particularly the organisms in which experimental possibilities are limited. For instance, the problem of detecting a translocation from only somatic metaphase chromosomes haunts the human cytogeneticists. The parameters presented in Table 1 can be used to provide ascertainment indices of the following magnitude:

1. The shortest minimal alteration segment capable of detection would occur in 3L. Appropriate biometrical tests will detect a difference as low as 2% of the length of 3L at a 95% confidence level based on a sample of 35 cells; if 3 is already identified.
2. The longest minimal alteration segment capable of detection would occur in 8S. A difference as low as 6% with sample size of 35 cells would be detected at the 95% confidence level if 8 is already identified.

We should point out that the two statements above do not imply identification of the chromosomes. Ascertainment of chromosome identity involves all chromosomes of the complement in our program; hence alterations in arm length may create new 'overlap' among the criteria used for

identification. In such cases, the resolving power for purposes of identification may have minimal estimates in excess of the 2% and 6% respectively, cited above.

We obtained an estimate of 35 as the number of nuclei which should be analyzed to obtain the level of significance needed to independently detect all chromosomes in the maize complement. This estimate will change as specific chromosome changes are introduced through experimental procedures.

The real value of these biometrical procedures lies not in whole nuclei analysis but in analysis of specific chromosomes. As few as 70 observations (one per 70 cells or two per 35 cells) for a single homologue will provide a discriminating power of greater than 90%.

We are extending these analyses to additional normal stocks and several aberrant stocks. Particular attention will be paid to segment specific variation as well as to the changes induced by the aberrant stocks.

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1. A description of R^{ch} alleles.

Even though R^{ch} was discovered more than twenty-five years ago (Anderson 1941, MGNL 15:4) no detailed description of its phenotype is available in the literature. In Dr. Brink's laboratory a good collection of R^{ch} stocks has been assembled; all of them have been repeatedly backcrossed to W22 and so possess a uniform genetic background. I worked with this collection for a considerable time and I hope the following description will be helpful to the future workers.

Three of the original collections, namely Peru Corongo 120 R^g , Peru Corongo 150 R^g , and Ecuador R^r , were not initially suspected to be cherry alleles and only during subsequent study was their ability to promote pericarp pigment discovered. Subsequently these alleles have