1. Genic control of chromosomal behavior.

Two new recessive genes affecting chromosomal behavior are under investigation. One of these is a gene for ameiosis. Both the male and female inflorescences are affected; the plants are completely male sterile and highly female sterile. The very infrequently occurring kernels have with one exception given rise to 3N plants. This exception was a diploid homozygous for ameiotic and arose by parthenogenesis. Cytological studies have been confined to the male inflorescence where it was found that the sporogenous cells underwent normal mitoses up to the time when PMC should be differentiated but the onset of meiosis fails to occur and the sporogenous cells degenerate. Presumably a similar behavior is present in the ovules since a vast majority of the ovules do not develop a functional embryo sac.

The second gene, designated as elongate, has been mere extensively studied. The following effects have been observed during microsporogenesis: (a) the chromonemata are uncoiled at MI, AI, MII and AII giving "elongated" chromosomes but disjunction is normal and only haploid spores have been observed; (b) misdivision of the centromere sometimes takes place at the 2nd meiotic division: and (e) neocentric regions are occasionally formed at MII. Plants homozygous for el are not copious pollen shedders. Some el plants have a considerable amount of aborted pollen but this is so variable that pollen abortion cannot be used to separate el from normal sibs. Ears borne on el plants have plump and shriveled kernels as well as aborted ovules. The relative frequencies of these vary greatly in different families and are evidently affected by modifying genes but, with the exception of one B.C. family, no difficulty was found in classifying for normal and el plants on the basis of ovule sterility and the formation of shriveled kernels. Although many of the shriveled kernels do not germinate, root tip counts of the chromosome number of plants from shriveled kernels have been made for 825 individuals. These data are as follows:

Chromosome No.	Frequency
25	1
26	1
27	2
28	17
29	71
30	676
31	46
32	10
33	1
	825

From these data it is evident that the el gene produces many functional eggs with an unreduced number of chromosomes as well as some with aneuploid members. The plump kernels gave rise to only diploid plants; to date no trisomes have been obtained from plump kernels.

The unreduced eggs could arise in the following ways: (a) somatic doubling in nucellar tissue thus forming 4N meiocytes; (b) doubling in the gametophyte generation; (e) doubling by a failure of the 1st meiotic division; and (d) doubling at the 2nd meiotic division. Genetic and cytological studies permit discrimination between these various hypotheses. Since observations of megasporogenesis have disclosed only 10 pairs of chromosomes at Dk and MI, it seems clear that nuceller doubling is not responsible for the diploid eggs. In support of this conclusion is the fact that the ratio of dominant to recessive phenotypes found in diploid eggs from heterozygous plants is not the 5:1 expected from tetravalents with two dominant and two recessive alleles.

The genetic studies are involved but illuminating. Diploid plants homozygous for el and heterozygous for the lg$_2$ and a$_1$ loci in coupling, both lying in the long arm of chromosome 3 with lg nearest the centromere, were crossed by pollen from recessive plants. Shriveled kernels found on these B.C. ears were germinated and the ensuing seedlings transplanted to the field. The chromosome number of each plant was determined; all were at the triploid level. These plants were scored phenotypically for the lg and a characteristics; the genotypic constitution of the diploid eggs was determined by testcrosses using the triploids as both egg and pollen parents.

A total of 204 triploid plants were successfully tested for their genotypic constitution. In terms of the constitution of their diploid eggs derived from the el parent the following classes with their frequencies were obtained:

Lg A/Lg A = 9
lg a/lg a = 4
Lg A/lg a = 66
Lg A/Lg a = 26
lg A/lg a = 37

Lg A/lg A = 21
Lg a/lg a = 16
lg A/Lg a = 22
lg A/lg A = 3

A similar experiment was run where el plants heterozygous for the Sh and Wx loci in coupling were crossed by sh wx pollen. These two loci lie in the short arm of chromosome 9 with Wx nearest the centromere. The genotypic constitution of 156 triploids was determined. The constitutions of the diploid eggs contributed by the el parent and their frequencies are as follows:

Sh Wx/Sh Wx = 24
sh wx/sh wx = 28
Sh Wx/sh wx = 30
Sh Wx/sh Wx = 35
sh wx/Sh wx = 33

Sh Wx/Sh wx = 4
sh wx/sh Wx = 2

In both the lg a and sh wx experiments many of the diploid eggs were heterozygous for one or both of the marked loci. Obviously such heterozygous diploid eggs could not have arisen by doubling in the gametophyte generation because, on such a mechanism, both homologues would carry the same alleles. Therefore hypothesis (b) is not the mechanism operating in el plants.

The essential difference between the remaining two hypotheses is that on (c) the doubling results from a failure of the 1st meiotic division followed by a normal 2nd division while in (d) the 1st meiotic division is normal but, an aberrant 2nd division produces a diploid megaspore. The genotypic constitution of the diploid eggs will not be the same on hypothesis (c) as with (d).

Let us consider first the lg a experiment. Region (1) marks the interval from the centromere to lg while region (2) denotes the lg-A interval. Hypothesis (c) assumes that an unreduced restitution nucleus is found at the 1st meiotic division while the 2nd division proceeds normally. If no crossi
crossover in (2) gives rise to four possible kinds of diploid eggs of which one is homoz
- i.e., homozygosity for both loci. Of the double exchanges in regions (1) and (2), the 3-strand doubles lead to equal frequencies of homozygosis for lg and a while with both 2- and 4-strand doubles one of the four possible combinations formed at AII is homozygous for lg and none is homozygous for a. Since the frequency of single exchanges in region (2) is between 2 and 3 times the frequency of double exchanges in (1) and (2), it follows that the percentage of homozygosis for the A locus should be greater than that for the Lg locus.

On hypothesis (d), where doubling occurs at the 2nd meiotic division after a normal first division, calculations similar to those made above show that diploid eggs homozygous for the A locus come only from noncrossover tetrads and from those with 2- and 4-strand double exchanges while homozygosis for the Lg locus results from noncrossover tetrads as well as from those with single exchanges in region (2). Therefore on hypothesis (d) the frequency of homozygosis for the more proximally placed locus (lg) should be greater than that of the more distal A locus. Likewise, considering the Sh Wx experiment, the frequency of homozygosis for the Wx locus should be greater than that of the Sh locus. Indeed, if there is no recombination between a locus and the centromere, 50% of the diploid eggs would be homozygous for the recessive allele and 90% homozygous for the dominant allele.

The pertinent data are as follows: In a total of 380 diploid eggs from el el Lg A/lg a plants, 69 (18.2%) were homozygous for the lg allele while only 31 (8.2%) were homozygous for the recessive a allele. (The 204 diploid eggs listed earlier are included in these 380; tests of the genotypic constitutions were incomplete for 176 of them). In the Sh Wx experiment the frequency of homozygosis for the wx allele was 39.1% and only 18.0% for the more distal sh allele. The data from both the Lg A and Sh Wx studies agree in showing that more proximally placed genes have a higher degree of homozygosis in diploid eggs than do more distally located loci. Therefore it may be concluded that hypothesis (c) is invalid and that hypothesis (d) is consistent with the genetic data.

There is a direct relationship between recombination values and the percentage of homozygosis. With no recombination between a marked locus and the centromere 50% of the diploid eggs are homozygous for the recessive allele and 50% for the dominant allele. With 10% recombination, 40% of the diploid eggs would be homozygous for the recessive allele and with 20% recombination, 30% of the eggs would be homozygous recessive, etc. Since 39.1% of the diploid eggs were homozygous wx it would follow that this locus is approximately 11 recombination units from its centromere, a value considerably greater than that reported by Anderson and Randolph from translocation studies. Using the percentage of homozygosis as the measure of recombination, there is 32% recombination between Sh and the centromere. The efficiency of this method for measuring recombination with the centromere can be tested since we know from other studies that the value for the centromere-lg region is about 36%. The observed 18% homozygosis for lg would be expected if there were 32% recombination between this locus and the centromere in el plants. The agreement is good and suggests that this a reliable way of determining recombination with the centromere.

The amounts of recombination found in the diploid eggs from el plants can be calculated from the genotypic determinations. The value of 24% for Sh-Wx is within the range normally found in haploid gametes of N plants. The recombination value for the Lg-A region from diploid eggs is 36% which is not far from the average value of 35%.

The el gene has been used to obtain an interesting series of polyploids. Triploids come frm the cross of el x N. Crosses of 2N el by 4N gave some plump kernels which were 4N. BY self pollination, 4N el stocks were obtained. When these were selfed, 6N plants arose from the union of unreduced 4N eggs with 2N pollen. These 6N plants came from shriveled kernels while the plump kernels on the same ears gave rise to 4N plants. Hexaploid plants homozygous for el have been obtained and self pollinated but no viable seed has yet been produced. If seed were produced, 9N plants should be formed. However plants at the 7N level have arisen from the cross of 4N el x 6N when unreduced 4N eggs were fertilized by 3N pollen. Pentaploids (5N) have come from crosses of 4N el x 6N where reduced 2N eggs were fertilized by 3N pollen. Vigorous plants are obtained up to and including the 5N level but the 6N and 7N plants are runts.