a single layer of cells at the periphery of the endosperm. Doubling the aleurone layer would increase the protein from this source.

From a recurrent selection study for high-amylose starch involving exotic strains into which the ae gene (amylose extender) had been introduced, several selections with high-amylose content and high-protein and several with high-amylose and low protein were found. While examining these selections microscopically for starch and protein characteristics, kernels with double-aleurone layers were found.

A summary of the aleurone composition for the parents and their progenies is given in Table 1. Reciprocal crosses between single and double aleurone layered selections indicate the double-aleurone layer character is dominant. Of additional interest is that some kernels with multiple aleurone layers were also found.

We assumed the source of the multiple aleurone layer characteristic was from the exotic strains. We, therefore, obtained as many of the exotics used in the recurrent selection study as possible. Among these, Peruvian 442 was found to have about 35% of its kernels with more than one layer of aleurone.

Inheritance studies of the multiple aleurone layer characteristic are in progress and we also plan to make comparative biological evaluations of the protein from single, double and multiple aleurone layered selections.

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2. Tetraploid gene segregation as studied with a repulsion phase marking system.

This system enables one to investigate the many factors which complicate tetraploid gene segregation such as double reduction, numerical non-disjunction, and the viabilities of aneuploid gametes and zygotes. The understanding of these factors is necessary to explain the partial sterility of most autotetraploids.

The system may be described as the following of two genes at the same time in the repulsion phase—each chromosome is marked with a dominant and a recessive gene. For example, plants with the genotypes of

A/A/a, A/A/a/a, and A/A/a/a/a give phenotypic ratios of A : a in their progeny that cannot be readily distinguished because they are similar. The frequency of A phenotypes is mostly independent of the number of chromosomes carrying a. However, if we use plants with the genotypes of Ab/Ab/aB, Ab/Ab/aB/aB, and Ab/Ab/aB/aB/aB, the plants can be easily distinguished by observing the ratio of B : b. Table 1 gives the theoretical gene segregation of fifteen different euploid and aneuploid genotypes.

Table 1

The theoretical gene segregation of euploid and aneuploid types of autotetraploids assuming random chromatid assortment

Genotype	Phenotypic ratio in backcross*			% a	% sh
	Ash	ASh	aSh		
Tetrasomic				21.43	21.43
Ash/Ash/aSh/aSh	6	16	6	3.57	53.57
Ash/Ash/Ash/aSh	15	12	1	53.57	3.57
Ash/aSh/aSh/aSh	1	12	15		
Trisomic				20.00	58.33
Ash/Ash/aSh	8	4	3	58.33	20.00
Ash/aSh/aSh	3	4	8		
Pentasomic				1.11	54.44
Ash/Ash/Ash/Ash/aSh	49	40	1	54.44	1.11
Ash/aSh/aSh/aSh/aSh	1	40	49	8.33	25.00
Ash/Ash/Ash/aSh/aSh	3	8	1	25.00	8.33
Ash/Ash/aSh/aSh/aSh	1	8	3		
Disomic				50.00	50.00
Ash/aSh	1	0	1		
Hexasomic**				0.00	54.54
Ash/Ash/Ash/Ash/Ash/aSh	6	5	0	54.54	0.00
Ash/aSh/aSh/aSh/aSh/aSh	0	5	6	2.73	25.45
Ash/Ash/Ash/Ash/aSh/aSh	9.3	26.3	1	25.45	2.73
Ash/Ash/aSh/aSh/aSh/aSh	1	26.3	9.3	9.09	9.09
Ash/Ash/Ash/aSh/aSh/aSh	1	9	1		

*No ash class is listed as its probability is very low, because of the close linkage between a and sh; also for a gamete to be ash it must have two crossover chromosomes or be monosomic which are rare events.

**Assuming 3 to 3 disjunctions.

Table 2 gives some data for seven different genotypes isolated so far. The data correspond roughly to theoretical expectations. Corrections need to be made for the frequencies of double reduction, numerical non-disjunction, and viability factors of the aneuploid gametes and zygotes. Additional data are needed to make a good estimate of the parameters of tetraploid gene segregation.

Table 2

Tetraploid gene segregation of seven different genotypes, euploid and aneuploid

Genotype	No. of plants tested	No. of gametes tested	Percent Ash	Percent aSh
Ash/Ash/aSh/aSh	155	25,556	19.86	20.68
Ash/Ash/Ash/aSh	14	1,867	48.90	2.52
Ash/aSh/aSh/aSh	21	3,128	2.40	52.17
Ash/Ash/Ash/aSh/aSh	4	468	31.83	8.97
Ash/Ash/aSh/aSh/aSh	5	498	9.84	28.11
Ash/Ash/aSh	7	932	46.03	18.56
Ash/aSh/aSh	5	586	22.69	48.29

The trisomics (Ash/Ash/aSh and Ash/aSh/aSh) and pentasomics (Ash/Ash/Ash/aSh/aSh and Ash/Ash/aSh/aSh/aSh) were isolated from crosses between 4n Ash and 4n aSh plants. The aneuploids are the result of numerical non-disjunction of quadrivalents at meiosis--3 to 1 separations of the chromosomes or from the trivalent-univalent configurations which should result in numerical non-disjunction about half of the time. In case of tivalent-univalent formation, it is possible that the disjunction may be 2 to 1 with one chromosome being lost. This would result in a higher frequency of 2n-1 gametes than 2n+1 gametes. The genotypes of 132 plants from 4n Ash and 4n aSh crosses are given in Table 3.

Table 3

Frequency of aneuploid genotypes in crosses of 4n <u>Ash</u>
by 4n <u>aSh</u> and reciprocal

Cross	4n <u>Ash</u> X 4n <u>aSh</u>	4n <u>aSh</u> X 4n <u>Ash</u>	Total
<u>Genotypes</u>			
Ash/Ash/aSh/aSh	25	97	122
Ash/Ash/Ash/aSh/aSh	1	3	4
Ash/Ash/aSh/aSh/aSh	1	2	3
Ash/Ash/aSh	1	1	2
Ash/aSh/aSh	0	1	1
			132

The 132 plants were formed by 264 gametes, 7 or 2.65% of which
were hyperploid for chromosome 3 and 3 or 1.14% of which were hypoploid.
The percentage of aneuploid gametes is 3.79%. The data are not adequate
for a precise estimate of numerical non-disjunction. If we take the
figure as a rough value then if all ten chromosomes behave similarly, the
frequency of aneuploid gametes is rather high. It is around $1 - (1 - .04)^{10}$
or 33.5%. The frequency of 40 chromosome eutetraploids in the progeny of
40 chromosome plants should be about 44% $(1 - .33)^2$. The figure observed
from the pooled data of Randolph, Kadam, and Catcheside is 60.6% but
this probably includes some numerically compensating types (3 of one and
5 of another).

The hexasomic and disomic types of aneuploid may be derived from
selfing the pentasomics and trisomics, respectively. One trisomic (<u>Ash/
Ash/aSh</u>) was selfed. It gave 7 tetrasomics (5 <u>Ash/Ash/aSh/aSh</u> and 2 <u>Ash/
Ash/Ash/aSh</u>) and 9 trisomics (5 <u>Ash/Ash/aSh</u> and 4 <u>Ash/aSh/aSh</u>) and no
disomics. We would expect 1/4 of the progeny to be disomic. It is possi-
ble that disomic tetraploids are not viable. If this is true, then it
would be a big factor in autotetraploid fertility particularly when tetra-
ploids are selfed.

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