(Schwartz, 1966, Proc. Nat. Acad. Sci. 56, 1431; Freeling and Schwartz, 1973, Biochem. Genet. 8, 27). All three sets of ADH can be induced by anaerobic treatment of primary roots (Freeling and Schwartz, 1973).

It was shown--from data to be reported elsewhere--that some anti-ADH1 antibodies specified against highly purified ADH1 subunits (Set I) also cross-react with ADH2 subunits (Set III). Competitive titrations and a two-dimensional immunoelectrophoretic technique (Schwartz, 1972, J. Chromatogr. 67, 385) were used. ADH1 and ADH2 subunits share some, but not all, antigenic sites. Homogenous ancestry is directly supported.

This result was not expected. The two subunits composing the major lactate dehydrogenases (LDH's) in animals are not immunologically similar although they do have considerable amino-acid sequence homology (see Kaplan, 1964, Brookhaven Symp. Biol. 17, 131). Compared to the animal LDH's, the original Adh duplication event reflected in contemporary maize may be recent. In any case, the Adh gene-system may prove phylogenetically useful. Quantitative immunological comparisons between the ADH's of maize and its relatives would be expected to yield evolutionary relationships. The antigenic similarity of ADH1 and ADH2 in maize, and presumably in maize relatives, may permit the quantitation of rate and extent of divergence of two, unlinked, duplicate genes.

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5. The functioning of the dissimilar sperm of high-loss plants in double fertilization.

Roman (1947, 1948) in his studies with TB-A translocations, where dissimilar sperm are formed by nondisjunction at the second microspore division, reported that the hyperploid sperm with two B^A chromosomes preferentially fertilized the egg while the hypoploid sperm with no B^A chromosome united with the polar nuclei. The fate of the two sperms is dependent on their genetic make-up and not on the segregation of the two B^A chromosomes into a specific nucleus with a preordained function in fertilization (Carlson, 1969). Carlson showed that preferential fertilization of the egg by sperm with two B^9 chromosomes did not occur when several intact B chromosomes were present (the so-called "swamping" effect). Any tendency toward

preferential fertilization was overcome by the predominating influence of the intact B's, which were randomly distributed to both sperm nuclei following nondisjunction. Dissimilar sperm are also formed at the second microspore division in high-loss plants; one sperm is hypoploid, while the other is usually euploid. Since chromosome elimination in high-loss plants occurs only when several B's are present, the potential ability of one of the sperm to undergo selective fertilization should be negated by the swamping effect of the B chromosomes, as suggested by Carlson.

The results of one test of preferential fertilization with a high-loss plant reported in the paper by Rhoades, Dempsey, and Ghidoni (1967) indicated approximately equal frequencies of deficient endosperms and embryos, although the deficient endosperms occurred with a somewhat higher frequency. These rather scant data suggested that the two dissimilar sperm, resulting from chromosome elimination at the second microspore division, were randomly involved in fertilizing the egg and polar nuclei, in agreement with Carlson's prediction. However, much more extensive data acquired subsequent to the 1967 report were at variance with this tentative conclusion. We stated in our 1972 paper in Genetics that "In general, deficient endosperms are found more frequently than are deficient embryos--i.e., selective fertilization does occur. However, the variation found in different crosses is so great that the phenomenon requires further study." This we have done and we now believe that the relative rates of loss in endosperm and embryo, ranging from no difference to frequencies at a much higher rate for the endosperm, reflect the tendency of deficient embryos to abort, producing either germless kernels or those with defective embryos unable to germinate. The triploid endosperm with its diploid genome from the female parent is buffered against the deleterious effects of genic unbalance and is able to develop normally, even though it possesses a deficient chromosome. In contrast, the deficient embryos are not so buffered by polyploidy and with varying frequency, depending upon modifying factors, cease to develop during embryogenesis.

The short arm of chromosome 9 is well suited for the detection of loss events in both endosperm and embryo. High-loss plants with both chromosomes 9 carrying a large terminal knob and the Yg and C alleles in the short arm were used. The consequences of breaks in the short arm of 9 are easily

discernible in the aleurone. Breaks between the C locus and the terminal knob result in a dicentric chromosome which undergoes the bridge-breakage-fusion cycle during endosperm development and produces kernels mosaic for colored and colorless tissue. Breaks proximal to C yield an acentric fragment with the C allele and a dicentric lacking the C locus. The acentric fragment is lost and, although the dicentric undergoes the bridge-breakage-fusion cycle, the aleurone is wholly colorless. The colorless kernels coming from breaks between C and the centromere cannot be distinguished from those arising by nondisjunction, a relatively infrequent phenomenon also occurring in high-loss microspores. In summary, nearly all types of loss affecting the short arm of 9 lead to recognizable aleurone phenotypes. The only exceptions are those rare breaks distal to the C locus in which healing of the broken end occurs or where there is a bridge-breakage-fusion cycle with breaks restricted to one region. On either alternative, a self-colored rather than a mosaic kernel would ensue and the deficiency would not be phenotypically expressed. A sperm with a deficient chromosome 9 coming from breaks anywhere in the short arm, save for the extremely short Yg-knob interval, would yield an F_1 plant with the recessive yg phenotype when it fertilizes the egg nucleus. From the above considerations, it is apparent that the consequences of breaks in the short arm, or loss by nondisjunction, are manifested in both the sporophyte and the endosperm. It follows that the loss of the C allele, measured by endosperm color, should equal the loss of Yg in the F_1 sporophyte if selective fertilization is not operating.

Three plants from the high-loss strain, $\frac{K9\ Yg\ C}{K9\ Yg\ C}$ in constitution, were used as male parents in crosses to yg c testers. The total progeny of 3196 included 2585 kernels with colored aleurone (no loss of C in the sperm uniting with the polar nuclei), 32 with a bridge-breakage-fusion pattern of C-c variegation and 579 colorless kernels. The 32 variegated kernels arose from breaks between C and the large terminal knob and the 579 colorless kernels stem from breaks between C and the centromere or come from nondisjunction. The sum of the variegated and colorless kernels (611 or 19.1% of the total population) represents the fraction of fertilizations in which the deficient sperm united with the polar nuclei.

The three classes of kernels were planted under a favorable environment in a sand bench and the resulting seedlings were scored for the green versus yellow-green trait. The 2585 C kernels produced a seedling population of 2180, consisting of 1823 Yg and 357 yg plants. The germination percentage of the C kernels was 84.3%, a fact which, as we shall see, is of some consequence.

The 611 variegated and colorless kernels gave rise to 546 green seedlings, a germination rate of 89.4%. As expected, none were yellow-green. The embryos all contained a normal chromosome 9 since the sperm with a deficient chromosome had united with the polar nuclei. The frequency of yg seedlings in the total seedling population of 2726 was 13.1%, a value significantly less than the 19.1% of kernels with loss of the C allele in the endosperm. Since breaks in 9S leading to deficient sperm should be as readily detectable in the embryo as in the endosperm, the data, at first glance, indicate that a deficient sperm is more apt to fertilize the polar nuclei than it is the egg nucleus. Two features of the above data are noteworthy. First, all of the yg seedlings came from the C class of kernels and second, the germination was higher in the exceptional colorless kernels (89.4%) than in the colored class (84.3%). It was possible that the decreased germination rate in the C kernels was caused by kernels with yg embryos which aborted, producing germless seeds. The germination rate of the colorless kernels was 89.4%; the 10.6% aborted embryos represent residual abortion most likely caused by deficiencies in other knobbed chromosomes. A similar rate of residual abortion should occur in the class from C kernels. Multiplying .894 X 2585 gives 2311, the number of kernels which should have germinated with no lethality of yg embryos. The difference between the expected number (2311) and the observed number (2180) of seedlings, or 131, is an estimate of the number of colored kernels which failed to germinate because their yg embryo had aborted. Combining the observed yg seedlings (357) and the estimated number of yg zygotes which aborted (131) gives a total of 488 yg zygotes in a seedling population of 2857 (2726 + 131) or 17.1% of yg zygotes. The 10.6% of kernels which failed to germinate (in C, mosaic and c classes) is assumed to include Yg and yg embryos in the same proportion as that determined for the 2857 population. Since the residual abortion is believed to be due either to unknown gm

factors or to loss of chromosomes other than 9, there is no reason to assume that the frequency of yg should differ from the frequency calculated for the estimated population of 2857. The percentage of 17.1 is in good agreement with the 19.1% of endosperms deficient for the C allele and is suggestive of random fertilization.

This conclusion was further tested in studies with chromosome 3. The A marker followed in the endosperm showed a much greater frequency of loss than did the Lg marker in the embryo. The chromosome 3 data are less amenable to a phenotypic analysis of loss events since breaks between Lg and A give rise to endosperm loss of A but not to embryo loss of Lg. However, the percentage of germination was higher among the kernels experiencing endosperm loss than among those with no loss of A. All of the deficient embryos occur in the latter group and presumably a fraction of these failed to germinate. When the data were corrected for the lethal embryos resulting from loss involving chromosome 3, the frequencies of loss in the endosperm and embryo were nearly the same, although the endosperm loss remained somewhat higher.

Dissimilar sperm arise at the second microspore mitosis in both TB-A translocations and high-loss plants, but they differ in their mode of origin. Nondisjunction of the B^A chromosome is unaffected by additional B's; hence, the fate of dissimilar sperm in double fertilization can be studied in plants with and without B's. The selective fertilization of the egg by the hyperploid sperm first found by Roman disappeared when the number of B's was great enough to insure that both sperm possessed them (Carlson, 1969). On the other hand, the dissimilar sperm of high-loss pollen arise only when the plants have several B's. Since dissimilar sperm are not produced in 0 or low B plants of the high-loss strain, it is impossible to determine whether or not selective fertilization would take place in the absence of B chromosomes. Carlson predicted that the dissimilar sperm of high-loss pollen would be randomly involved in double fertilization because of the swamping effect of B's and this appears to be the case when our data are corrected for lethal zygotes. The pattern of fertilization in high-loss plants without B's remains unknown.

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