plasma membrane, there is a connecting substance between them and the inner dense layer of the membrane. This point of attachment may anchor a portion of the microtubule so that any undulations or other displacement forces along its length would induce a streaming motion within the surrounding ground substance of the cytoplasm.

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17 February 1964

Tetrasporic Embryo-Sac Formation in Trisomic Sectors of Maize

Abstract. *Nondisjunction in mitotic divisions occurs spontaneously at a low frequency in somatic and germinal tissue in maize and results in sectors of trisomic cells. When this happens with chromosome 3 and in germinal tissue the embryo sac development is changed from the normal monosporic type to a tetrasporic type which is common in some species but not in maize.*

Under normal conditions the embryo sac in maize develops with eight nuclei from three divisions of a single basal megaspore. The position of these nuclei is such that two polar nuclei join to produce a $2n$ fusion nucleus, one becomes the egg nucleus, and the other five become the antipodal and synergid nuclei. Under these conditions and with certain rare exceptions the endosperm and embryo of a developing seed have the same genes.

In an experiment designed to test the effect of crossing-over on mutable loci, an apparent deviation from this scheme was observed. To determine the nature of factors taking part in mutable behavior at the A_1 locus on chromosome 3, a heterozygote composed of two distinguishable mutable alleles but lacking their respective mutators was prepared. One segment (α-*a-sh*) carried α (dilute aleurone), *a* (*Dt* responding, colorless aleurone), and sh_2 (shrunken endosperm) all within a map distance of 0.25 crossover units. The segment (a^m-*Sh*) carried either a^m-*1* (*Dt* responding), a^{m-3} (*Ac* responding), a^{m-4} (*Ac* responding), or a^s (nonresponding), depending on the experiment and Sh_2. The heterozygote was crossed by a recessive male stock (a^s-*sh*, *Dt*).

Progeny from this cross included the expected parental types and a variety of crossovers depending on the particular allele in the a^m-*Sh* segment (*1*). Among the α-a^m-*Sh* and α-*a*-*Sh* crossovers were some which on test had noncorresponding embryos of three types, namely, trisomic, (α-*a*-*sh*/a^m-*Sh*/a^s-*sh*) and both types of parentals (α-*a*-*sh*/a^s-*sh* or a^m-*Sh*/a^s-*sh*). The cultures carrying the alleles a^m-*1*, a^{m-4} and a^s all had a considerable number of both crossover and noncrossover cases but in the heterozygote carrying a^{m-3} all of the apparent crossover cases of the α-a^m-*Sh* and α-*a*-*Sh* types turned out to be noncorresponding cases.

The data (Table 1) clearly show these differences and also reveal that while the frequency of crossovers varies between alleles the frequency of noncrossover embryos of all three types is surprisingly constant (compare a^m-*1* and a^{m-3}, Table 1). Of further significance is the fact that several of the parent ears in this experiment had groups of apparent crossover seeds which on test were found to be noncorresponding and to include one or more of the embryo types already mentioned. A good example of this is presented in Fig. 1 where the position of the cases on the ear and the condition of the embryos for each case is indicated. The proximity of the α-*a*-*Sh* seeds and the low frequency of single occurrence of such cases in the rest of the population make it almost certain that all five have a related origin, an origin which precedes meiosis.

In seeking a reasonable explanation for the noncorresponding cases the expression of both chromosomes of the parent heterozygote in the endosperms, the occasional occurrence of these cases in groups or sectors, and the fact of noncorrespondence between endosperm and embryo in a large portion of these cases must be accounted for.

The first of these is fairly easy to explain as being due to nondisjunction with the production of an endosperm having both the α and a^m chromosomes. The occurrence of some of these in sectors (Fig. 1) is accounted for if we assume that nondisjunction may occur at various mitotic divisions including those preceding meiosis. Accounting for the third fact is more difficult. If nondisjunction has provided the megasporocyte with an extra chromosome thereby allowing (n + 1) nuclei carrying α and a^m as meiotic products, normal development of the megaspore into an eight-nucleate embryo sac will provide polar nuclei with the extra chromosomes which will give the observed type of endosperms. However, the egg from such development should also have an extra chromosome and therefore should generally produce a trisomic embryo. Since only one-third of the embryos are trisomic some other explanation is needed.

Additional nondisjunction in the three megaspore divisions could provide noncorrespondence. Unequal distribution of the extra chromosome (α or a^m) at the first megaspore division would provide one polar nucleus with two extra chromosomes (α α or a^m a^m) and the other polar nucleus with a normal complement. The fusion nucleus would be composed of one of each, and would be either α, α a^m a^m, or α α a^m, a^m; both would give the observed endosperm phenotype. The egg nuclei would be either α a^m a^m, α α a^m, α, or a^m. Fertilization of these would produce two tetrasomics; one α parental and one a^m parental. Nondisjunction at the sec-

Table 1. Distribution of all the dilute nonshrunken cases from the heterozygote α-*a*-*sh*/a^m-*Sh* pollinated by a^s-*sh*.

Allele	Seeds examined (No.)	Crossovers			Noncrossovers			Lost
						Parentals		
		α a^m *Sh*	α *a Sh*	α-*Sh*	Trisomic	α *a sh*	a^m *Sh*	
a^m-*1*	312,057	20	35	61	13	11	15	96
a^{m-3}	131,448	0	0	5	10	5	8	4
a^{m-4}	40,501	0	5	6	3	5	5	2
a^s	307,090	0	43	68	22	19	14	121

ond megaspore division would provide similar results except that the frequency of tetrasomics to parentals would be 6:1:1 and nondisjunction at the third division would give the same but the ratio would be 14:1:1. None of these fits the observed frequency (Table 1). Furthermore, tetrasomic plants can be distinguished from trisomics on the basis of frequency of types of gametes produced. The cases were confirmed as trisomics by the fact that they transmitted through male gametes all three kinds of chromosome 3 in equal numbers.

Another way to account for the apparent crossover endosperms with trisomic and noncorresponding embryos is to assume that all four products of meiosis take part in the development of the embryo sac and that the four nuclei divide at least once to form an eight-nucleate embryo sac which then functions normally except that the nuclei are of two different types. This eight-nucleate tetraspore type of megagametogenesis is called the *Adoxa* type by Maheshwari (*2*) and is common in some species of angiosperms but not in maize.

The events occurring and the consequences of such behavior involving the heterozygote for chromosome 3 are shown in Fig. 2. These events lead to the production of endosperms with α and a^m as expected. If similar embryo types are combined then 4 α a^m, 1 α α, 1 a^m a^m, 3 α and 3 a^m embryos result. When fertilized by a^s male the first will produce trisomics of the α a^m a^s type, the second and third trisomics of the α α a^s and a^m a^m a^s types, and the last two diploid parentals of the α a^s and a^m a^s types, respectively. If the trisomic phenotype in these cultures is not specifically being sought as was the case in recording the data for this experiment, the α α a^s cases would be classified as α parentals and the a^m a^m a^s cases as a^m parentals, so that the classes would be combined as four trisomics, four α parentals and four a^m parentals. Re-examination of several of the listed parental cases which had not been discarded showed that some of these were indeed α α a^s and a^m a^m a^s trisomics. There were not enough of these to provide an accurate measure of their frequency.

The diagrams cover only those situations where there is no crossing-over between these genes and the centromere. If the same hypothesis is worked through and random distribution of chromatids at the first meiotic division is assumed, the result is that some parental-type endosperms occur (these pass unrecognized) but that the same types of embryos occur in the same ratio as with complete linkage; therefore the 1:1:1 ratio still holds.

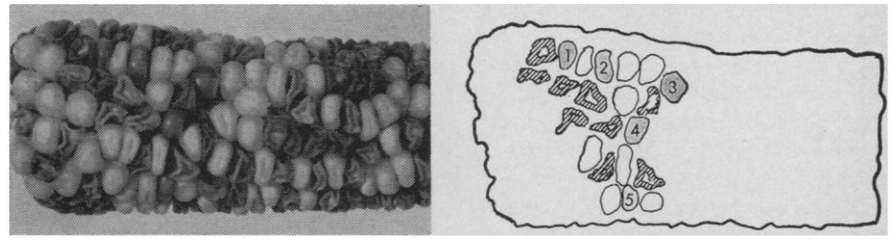

Fig. 1. Ear from the cross a-a-sh/a^m-Sh × a^s-sh showing a five-seed sector of apparent crossover a-Sh seeds. When tested seeds 1 and 2 had a-a-sh/a^s-sh embryos, seeds 3 and 5 had a^m-Sh/a^s-sh embryos and seed 4 had an a^m-Sh/a^m-Sh/a^s-sh trisomic embryo.

The frequency observed (48 trisomics, 40 α parentals, and 42 a^m parentals) shows a good fit to the expected 1:1:1 ratio. The expected value for each class would be 43.33, the calculated X^2 .8000, and the P value .68.

From the foregoing considerations it is possible to reconstruct the events which produced the peculiar type of behavior found. The heterozygous cultures used apparently have an occasional spontaneous occurrence of nondisjunction for chromosome 3 which produces sectors of trisomic tissue. If this occurs in the germ line the condition of trisomy for chromosome 3 changes the mode of development of the embryo sac so that all four meiotic products function. These megaspores will be of

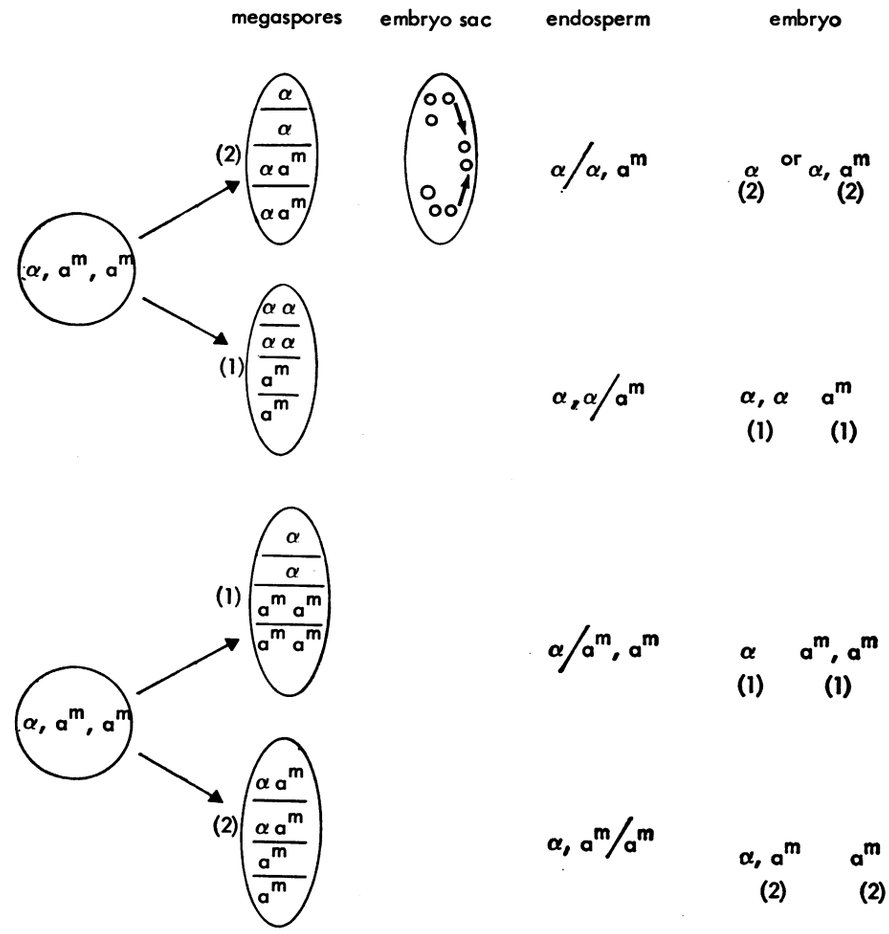

Fig. 2. The consequences of trisomy and tetrasporic embryo sac formation. The numbers in parentheses indicate the relative frequency of the events described.

two types, $(n + 1)$ and n; therefore the embryo sac will have two types of nuclei. The four nuclei will probably divide again to form eight (if they divide a second time to form 16 it will not change the result as long as the nuclei are oriented with the same polarity as that of the original four megaspores). At maturation the endosperm is formed from two unlike polar nuclei which are fertilized by a single pollen nucleus to produce a $(3n + 1)$ endosperm with both genetic makers, while the embryo is produced from a single nucleus which may be either $(n + 1)$ or n, but genetically of any one of five different types $\alpha\ a^m$, $\alpha\ \alpha$, $a^m\ a^m$, α, or a^m. These events provide the type of apparent crossover endosperms observed and account for the types of corresponding and noncorresponding embryos that were associated with them.

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27 March 1964

Water Turnover in Cattle

Abstract. *The half-life of the body water pool in cattle is unusually short, relative to their body size. The short half-life is not due to milk formation, since the same result is observed in nonlactating cattle as in cattle producing 23 kg (50 lb) of milk per day.*

A difference between cattle and other mammals became apparent during metabolic studies on rate of utilization of tritium-labeled compounds in cows (*1*). Richmond *et al.* (*2*) have established a correlation between water turnover and body weight with six mammalian species in which the half-life for body water ranged from 1 to 9 days. From this relationship one would predict a half-life of about 9 days for body water in the cow.

In contrast to this expectation we find that the half-life of water in cattle weighing 150 to 750 kg is only 3.5 ± 0.21 days, a value that is not significantly different from that obtained with 300-g rats (*2*).

Cattle were injected with tritium-labeled water or organic compounds through a plastic catheter inserted into their jugular vein. The amount of tritium injected in each trial and other characteristics of the experiments are listed in Table 1. Small samples of blood or milk or both were drawn at frequent intervals for several hours after the tritium-labeled compounds were injected and less frequently during a period lasting several days. Results reported here are for those samples collected after the tritium had equilibrated throughout the body water pool —later than 8 hours after injecting tritiated water and later than 24 hours after injecting tritiated organic compounds.

The blood was allowed to clot and, after syneresis, the serum was decanted off for lyophilization and collection of water. Three to four milliliters of water were collected in a cold trap from each blood serum or milk sample lyophilized. All samples were lyophilized to complete dryness to avoid any change in specific activity that would result from differences in rate of evaporation of tritiated versus unlabeled water molecules. A 0.1-ml aliquot of each water sample was transfered to 15 ml of liquid scintillation solution (containing dioxane for aqueous solutions) and counted for 10-minute intervals in the Packard Tricarb. The efficiency of the system (about 20 percent) was determined with an aliquot of a diluted standard prepared from the injected tracer. The efficiency factor was used to convert net counts per minute to millimicrocuries of tritium per liter of body water.

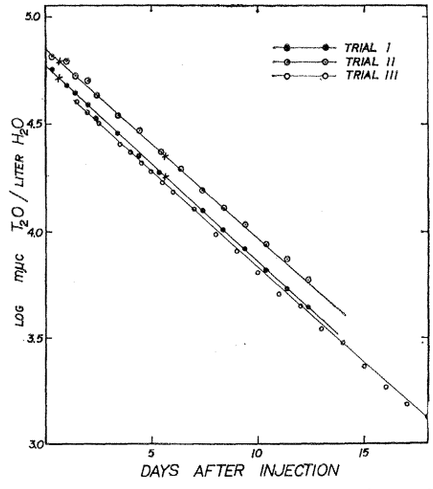

Fig. 1. Regression for concentration of tritium in body water of lactating cows after injecting tritiated water (trials 1 and 2) or tritiated acetate (trial 3). Experimental values are shown by circles; calculated regression points through which lines were drawn are indicated by X's.

When tritiated fatty acids were injected into cows, the blood or milk was made strongly alkaline by addition of dry powdered Na_2CO_3 prior to lyophilization. This treatment ensured that any tritiated acetate or propionate would remain in the residue and not influence the tritium level in the water collected.

The logarithm of tritium concentration in body water (millimicrocuries per liter) is plotted as a time series in Fig. 1, for three trials. The concentration decreased exponentially with time, the individual values fitting very closely the linear regression calculated by the method of least squares. The results for trials 1 and 2 show that the body water pool in cattle behaves kinetically as a single pool undergoing simple dilution at times greater than 8 hours after injecting tritiated water and during an interval extending almost 2 weeks.

The slope of the regression line, (k), multiplied by 2.3 (to convert to natural logarithms) represents the turnover rate of the body water pool, and from the relationship

$$0.693/k = T_{½}$$

the half-life $(T_{½})$ of body water was calculated.

Table 2 lists the half-life for body water found in several experiments with cattle. In dairy cows, trials 1 to 8, the half-life ranged from 3.0 to 3.9 days, with an average value of 3.54 ± 0.10 days. The Jersey cow used in trial 8 was not lactating at the time of the trial but had been through several lactation periods, the last one terminating only a few weeks prior to the experiment. This animal was included with the dairy cows, group I, instead of group II, which consisted only of steers, and a heifer.

The half-life for body water in nonlactating cattle, group II, in Table 1, ranged from 2.8 to 4.1 days with an average value of 3.4 ± 0.18. This average is not significantly different from that found for the dairy cows.