

In type 1b intercrosses "pairs" were rarely observed. In the exceptional type 1a intercross that showed no pairs one break in one parental interchange was in the satellite, and hence it regularly forms chains when heterozygous.

Those with the highest percentages of "pairs" are group 2 in which both breaks in both chromosomes are in the same arm. In those with the breaks in both chromosomes in opposite arms, the percentage of pairs is intermediate. There is some overlapping between type 1a and type 2. These results indicate that high frequencies of "pairs" do not necessarily indicate that both breaks are in the same arm in both chromosomes, an assumption that has been made by Hagberg (*Der Zuchter* 28:32-36, 1958) in barley interchanges.

-- M. Tabata

5. Cytological studies of asynaptic maize.

Rhoades and Dempsey (MNL 23) reported higher than normal single crossovers and much greater than normal double crossovers in the c-sh-wx and ws-1g-gl regions in both diploid and haploid eggs of asynaptic maize. Dempsey (MNL 33) later showed that on a basis of total ovules rather than seed set single crossovers in the same two regions are reduced in haploid gametes to about half the normal value but that double crossovers still remain higher in asynaptic plants. The increase in crossing over when based on number of seed set was interpreted as due to functional gametes being derived primarily from EMC's with more frequent chiasmata and therefore more regular chromosome behavior than occurred in aborting gametes. It should be pointed out, however, that if similar increases could be demonstrated for all genetic regions of maize chromosomes, EMC's giving rise to functional gametes in asynaptic plants would have had a higher chiasma frequency per EMC than occurs in normal maize, an event which would be highly unlikely in view of the chiasma frequencies demonstrated in as plants even if the counts are limited to cells with 10 bivalents present at MI. Although diploid as eggs containing crossover chromatids at first were presumed to come from EMC's with few or no chiasmata (Rhoades, *Genetics* 32), Dempsey (MNL 32) has presented evidence that such eggs contain both sister and non-sister centromeres and suggested that diploid gametes might arise by precocious dyad centromere separation and failure of Div II following a Div I in which both bivalents and univalents had divided. The studies reported here have been confined to microsporogenesis and pollen formation but are considered pertinent with respect to crossing over and the possible origin of diploid gametes in as plants.

(1) Origin of diploid gametes. Fourteen asynaptic plants were examined from diakinesis through the quartet or 1st microspore mitosis stage. Mean number of bivalents at MI ranged from 0 to 9.95 in separate plants. Pre-meiotic fusion giving rise to polyploid PMC's occurred in six plants with 1% the highest frequency and 8N the highest ploidy observed. Although cells resulting from premeiotic fusion were observed at all meiotic stages, the infrequent occurrence in as plants and the absence of fusion in cytological studies of megasporogenesis in asynaptic mutants of certain other species would seem to rule out syncytes as a source of diploid gametes. Extremely long and curved spindles and infrequently split spindles were observed in many of the plants at MI and AI. In no instance, however, was nuclear restitution observed due to failure of spindle formation or complete failure of chromosome movement toward the poles. Precocious separation of the centromeres of chromosome dyads was noted at AI in only one plant. However, Div II spindles and quartet formation were normal although unequal distribution of monads usually occurred. Complete or partial failure of cytokinesis frequently took place after Div II and less often following Div I in most of the as plants and was independent of degree of asynapsis or year of culture. Nuclear fusion following failure of cell division occurs during the microspore interphase or at the 1st microspore mitosis. In plants showing cytokinesis failure, division figures with 10, 20 and 40 chromosomes were observed at the 1st microspore mitosis. Size of the spores was proportional to the number of chromosomes. When placed on the silks of tetraploid maize

plants, similar mature pollen produced only 4N embryos as determined by root tip counts of four germinating seed. Since failure of cytokinesis during megasporogenesis apparently occurs under genetic control (Lebedeff, *Cytologia* 10), it is probable that both male and female diploid gametes result from nuclear fusion following failure of cytokinesis during the meiotic divisions. The functioning of diploid pollen on 4N females indicates that diploid male gametes can be used in crossing over studies in asynaptic maize. Pollen from the asynaptic plants, however, should be examined for the presence of viable, diploid-sized grains before pollination.

(2) Absence of cytological exchanges in asynapsed chromosomes. Three heterochromatic knobs (K4L, K9S, K10L) were incorporated in heterozygous condition into plants with low asynapsis. PMC's were examined at early diakinesis to determine if knob disjunction was reductional or equational in univalents or rod bivalents with chiasmata in the knobless arms. The respective knobs could be critically identified in almost every cell. The number of cells in which each chromosome was present as univalents or as the proper rod bivalent was 121 for chrom 4, 78 for chrom 9, and 116 for chrom 10. The frequency of rod bivalents with a short arm chiasma was more than twice that of rods with a long arm chiasma for each of the three chromosomes. Equational disjunction of knobs never was observed although genetic data indicate that in normal stocks chiasmata would be expected to occur proximal to the knob in a high percentage of PMC's. The experimental results warrant the conclusion that no cytological crossing over followed by precocious resolution of chiasmata occurs in chromosomes which exhibit asynapsis at diakinesis or MI and supports the cytological observations that diploid gametes do not arise by restitution following failure of chiasmata formation.

(3) Extent and location of pairing during early prophase. Plants with complete, medium or low asynapsis at MI were examined during all successive stages from leptotene to MI. With the possible exception of some centromere pairing, no clearly homologous pairing was observed at any stage in plants with complete asynapsis. The amount of pairing throughout the early prophase stages in plants with medium or low asynapsis was correlated with the degree of asynapsis at MI. It was concluded that chromosome segments unpaired at late pachytene had not been previously paired. The frequency and distribution of unpaired segments in chromosomes 6-10 were ascertained at pachytene in plants with low asynapsis. Chromosome 7 showed partial asynapsis versus apparently normal pairing in 51% of the figures in which 7 was identified, chrom 8 in 35%, chrom 10 in 19%, chrom 6 in 14% and chrom 9 in 4%. With the exception of chromosome 8 which frequently exhibited terminal asynapsis in the short arm, unpaired segments usually were intercalary and were more extensive and more frequent in the long arms. Long arm to short arm ratios often were considerably higher than normal even though no asynapsis or abnormal stretching was evident. A chromatid split never was observed in the unpaired chromosome segments. No matter how extensive the partial asynapsis, the centromere always was paired and terminal regions usually paired, indicating that initial chromosome synapsis probably involves both the centromere and chromosome ends.

The two genetic regions utilized in the studies of crossing over in haploid as gametes are located in short arms. Available inversion and translocation data place the ws - lg - gl region in the distal 1/4 of the short arm of chromosome 2 and the c-sh - wx region in the distal 1/2 of the short arm of chromosome 9. Since most of the partial asynapsis observed in these studies was interstitial and proximal, it is predicted that genetic regions physically closer to the centromere than the two regions so far studied will show a large reduction in crossing over in as plants. The cause of the higher than normal crossing over in these two regions when based on the number of seed on as ears is speculated upon as follows. With the exception of chromosome 8, pairing in asynaptic plants occurs much more often in short than in long arms. When pairing occurs in the short arm the distal half pairs more often than the proximal half. When pairing occurs, tighter than normal torsion coiling (a la Darlington) takes place so that for the segments paired both single and double crossovers are produced at a higher rate than in normal material. Since functional gametes are derived from EMC's with relatively high chiasma frequencies, the distal parts of the short arms, although sometimes unpaired, will

pair and cross over often enough to show a higher frequency of crossing over than that occurring in an equivalent number of normal EMC's. Such an increase may be comparable to the increased crossing over in *Drosophila* when certain heterozygous inversions are present, and it is suggested that the introduction of one or more paracentric (to maintain fertility on female side) inversions which exhibit frequent asynapsis in pachytene figures may increase crossing over in other regions of maize chromosomes. It is hypothesized that the degree of coiling and/or crossing over is dependent on a substance which normally is limited in quantity in meiocytes and is competed for with differential success by all regions of the chromosomes. When the amount available is increased for uninvolved regions either by nonpairing within heterozygous inversions or by partial asynapsis in as plants, tighter torsion coiling and increased crossing over will result.

-- O. L. Miller, Jr.

6. Persistent nucleolus.

The nucleolus in maize is described as disappearing at late diakinesis or prometaphase (Rhoades, J. Heredity 40). Sampayo (MNL 33) reported the persistence of a nucleolar remnant throughout meiosis in PMC's of plants heterozygous for abnormal 10. During a cytological study of asynaptic maize, a similar persistent nucleolus was discovered independently and at first was assumed to be an irregularity due to the as gene. Several normal stocks with and without the abnormal 10 chromosome subsequently were examined, however, and a persistent nucleolus was found in each. The remnant is about the size of a MI univalent of one of the longer chromosomes. It appears to be a passive body and its movement essentially is that described by Sampayo. Separating from the organizer region at prometaphase, it moves through the spindle and lies at or near one of the poles at MI. Further movement along the cell periphery brings it near the cell equator outside of the spindle at AI. The position of the remnant at Telo I and interphase appears to be random. The remnant is present in the cytoplasm of some microspores but its fate after that stage is unknown. It is presumed that with proper staining the persistent nucleolus can be demonstrated in all maize stocks and, if so, must be considered a normal cell component at meiosis. The significance of the remnant in cellular metabolism is obscure. It perhaps is a relatively insoluble waste product of nucleolar activity. Persistence of a remnant in somatic mitoses has not been investigated.

-- O. L. Miller, Jr.

7. A hand scope for pollen examination.

The "Midguard" Pocket Microscope, similar to the Leitz hand scope, is available from the following source for about \$3.00 plus postage and duty. The scope is approximately 2" by 1" and has a magnification of 35X.

Nippon Microscope Works Co.
35-2 Minami Cho
Aoyama, Akasaka
Tokyo, Japan

-- O. L. Miller, Jr.