

expressed on a protein basis, mn and de*-92 activities were 13.2% and 28%, respectively, of the hybrid W64A x W182E activity.

De-branching enzyme activity was qualitatively detected in 3.75% acrylamide-agarose gels which had 0.7% phytoglycogen incorporated into them. A band which stains a deep blue color with I₂-KI was visible in the hybrid W64A x W182E endosperm and pollen and it was detectable in most of the mutants (including wx-C) examined but was absent in mn and de*-92.

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4. Identical twins of dizygotic origin.

The proportion of twins formed by cleavage polyembryony in plants has been estimated from the excess over one-half of twins concordant in phenotype for a gene heterozygous in one parent. In this form the procedure is a direct extension of the estimation in man of one-egg twins from the excess of like-sexed pairs. Caution concerning applicability of these procedures to twins induced through action of the indeterminate gametophyte (ig) mutation was indicated in an earlier report (Amer. Jour. Botany 58:1-7). Twins were invariably concordant for a gene heterozygous in the ear parent; about one-fourth were discordant when the gene was heterozygous in the pollen parent. The corresponding estimates of one-egg twins are 100% and 50%. The discrepancy was explained by a class of ig female gametophytes that differentiate more than one cell capable of functioning as egg. Two such eggs, of identical genotype, evidently can be fertilized by separate sperm.

Might the excess of twins of concordant phenotype observed when the marker gene was introduced via the pollen parent also originate by some means other than cleavage polyembryony? If the two sperm from one pollen grain were to fertilize two eggs of one embryo sac, the resulting embryos would be fully identical even though of dizygotic origin. One class of twins, comprising about one-fourth of all cases in the previous study, suggested this mode of origin. Although the seedling phenotypes were concordant for the marker gene (R:r), they derived from a kernel of noncorresponding aleurone phenotype. Clearly, sperm from two male gametophytes had participated in double (triple?) fertilization. To test

whether the paternal contribution to twins was identical in such cases, a group of twin sets was established following crosses to ig ig females of a multiple heterozygous male (W22 colored aleurone/Mangelsdorf's tester). Eight genes on as many chromosomes were followed and evaluated following self-pollination. The seedling population screened numbered 816.

Classifying 37 twins according to number of concordant gene pairs revealed two classes, as seen in the following distribution.

Concordant gene pairs	1	2	3	4	5	6	7	8
Twin sets (No.)	1	4	5	7	1	2	0	17

Excluded from the summary are ten cases: two instances of triplets, two triploid-diploid and one haploid-diploid twin sets, a case of two shoots emerging from a single coleoptile, and four instances of incomplete marker classification.

Seventeen of the 37 twin sets were concordant for all eight markers and therefore are presumed to be identical. When the R allele carried by a set was compared with the aleurone phenotype of the kernel from which they were derived, nine cases corresponded whereas eight indicated presence of the contrasting R allele. Correspondence in only one-half the cases for a single endosperm marker would be expected if two male gametophytes were involved in origin of this class. Heterofertilization, that is, approached 100%. The requirement for heterofertilization is understandable if the two sperm of a single pollen grain both fertilize eggs. On the other hand, if cleavage polyembryony were the basis for identical twinning, there would be no obvious requirement for involvement of two male gametophytes.

The current evidence favors the view that ig induced twins are regularly dizygotic. They are identical maternally because the eggs derive from a single female gametophyte. The eggs may be fertilized by sperm from different pollen grains (20 pairs in the current study) or from the same pollen grain (17 pairs). If three of the four sperm from two pollen grains were utilized at random in such cases, the proportion of fully identical twins would be one-third. The present and previous studies both show somewhat more than one-third identicals, indicating a preference for fertilization of the eggs by sperm from one male gametophyte.