The complete data show that: (1) the mutant genes are indistinguishable from standard R^r in aleurone pigmenting capacity; and (2) the Rg gametes produced by RgR^{st} heterozygotes regularly determine paramutant aleurone phenotypes.

-- Douglas Brown

6. Paramutagenic action of paramutant R^r.

Data were collected to test the possibility that paramutant R^r genes have acquired the capacity to promote paramutation in some degree.

R^rRg heterozygotes from matings of the type R^rR^{st} ♀ x $Rgrg$ ♂ were used as pollen parents in test crosses to W23 $rgrg$ parents. It is expected that paramutagenic action of the paramutant R^r allele, if such exists, would be revealed by reduced pigmentation of $RgrgRg$ testcross kernels. The controls consisted of $RgrgRg$ kernels from test matings of R^rRg heterozygotes derived from R^rR^r ♀ x $Rgrg$ ♂ crosses. Testcross kernels were scored and identified according to the procedure described in the preceding section.

The results are summarized in table 2.

Table 2. Mean scores for $RgrgRg$ kernels from testcrosses involving R^rRg plants derived from R^rR^r ♀ x $Rgrg$ ♂ and R^rR^{st} ♀ x $Rgrg$ ♂ matings

Parentage of R^rRg ♂ testcross parent	No. of testcross ears	Mean score for $RgrgRg$ kernels only
R^rR^r ♀ x $Rgrg$	4	5.45
R^rR^{st} ♀ x $Rgrg$	10	4.36
R^rR^r ♀ x $Rgrg$	4	5.70
R^rR^{st} ♀ x $Rgrg$	10	4.88
R^rR^r ♀ x $Rgrg$	4	5.53
R^rR^{st} ♀ x $Rgrg$	10	4.82
R^rR^r ♀ x $Rgrg$	4	5.09
R^rR^{st} ♀ x $Rgrg$	10	4.11
R^rR^r ♀ x $Rgrg$	4	5.45
R^rR^{st} ♀ x $Rgrg$	10	4.91
R^rR^r ♀ x $Rgrg$ ♂	4	5.53
R^rR^{st} ♀ x $Rgrg$ ♂	10	4.80
R^rR^r ♀ x $Rgrg$ ♂	4	5.69
R^rR^{st} ♀ x $Rgrg$ ♂	10	4.55
R^rR^r ♀ x $Rgrg$ ♂	4	5.38
R^rR^{st} ♀ x $Rgrg$ ♂	10	5.02

It is evident that $\underline{R}^r$ extracted from $\underline{R}^r\underline{R}^{st}$ heterozygotes regularly is paramutagenic, though weakly so when compared with $\underline{R}^{st}$.

-- Douglas Brown

7. A test for genetic influence of endosperm on embryo.

Confirmation of Pissarev and Venogradova's claim in which wheat plants of modified characteristics were produced by grafting mature wheat embryos onto rye endosperms has been reported by Muntzing. The alteration was expressed in this case by increased crossability of the "graft hybrid" to rye and was attributed to an incorporation into the embryo of substances from the endosperm upon germination. In a similar experiment with maize, Carangal (M.G.C.N.L.:32) observed no increase in the receptiveness of a dent sterile pop to dent pollen through such embryo-endosperm transplantation. He did note, however, a marked decrease in viability and seedling growth in the heterologous transplant -- pop embryo into dent endosperm -- over the corresponding homologous transplant -- pop embryo into pop endosperm. The question of whether or not there is any permanent, heritable change resulting from the association of the embryo with genetically dissimilar endosperm was the subject of these experiments.

The method of attaining embryo-endosperm dissimilarity for these trials differs from those used previously. The crosses were designed such that cases in which the egg and polar nuclei were fertilized by unlike sperm, i.e. heterofertilized, could be readily detected. In this manner the genetically deviant zygote and fusion nucleus of the endosperm can be juxtaposed at fertilization. Thus exchange of genetic material between the two tissues could occur either in the development of the caryopsis or during germination.

The first of two experiments involved recognition and subsequent study of plants arising from heterofertilized kernels produced by mating $\underline{r}^g\underline{r}\underline{g}$ females with $\underline{R}\underline{g}\underline{r}^r$ male parents. The colorless seeds from this mating were germinated and the seedlings were exposed to light. Those which failed to pigment were selected as the presumed heterofertilized class. The tassels of the resulting plants were examined for red sectors in order to ascertain whether or not the endosperm containing $\underline{r}^r$ had evoked any change in the $\underline{R}\underline{g}\underline{r}\underline{g}$ embryo. The possibility that these green plants resulted from $\underline{r}\underline{g}$ pollen contamination was excluded by verifying the presence of $\underline{R}\underline{g}$ in the backcross of the mature plants to $\underline{r}^g\underline{r}\underline{g}$. Nine heterofertilized plants ($\underline{R}\underline{g}\underline{r}\underline{g}$ embryo; $\underline{r}^r\underline{r}\underline{g}\underline{r}\underline{g}$ endosperm) were positively identified in this manner and all failed to show red tassel sectors.

In the second investigation mixed pollen from $\underline{R}^r\underline{R}^r$ and $\underline{R}^{st}\underline{R}^{st}$ plants was placed on $\underline{r}^g\underline{r}\underline{g}$ silks. The $\underline{R}^{st}$ kernels from this mating were germinated, and the heterofertilized class identified by red seedlings ($\underline{R}^{st}$ conditions green seedlings and anthers). Seven such plants were testcrossed on $\underline{r}^g\underline{r}\underline{g}$ females. The $\underline{R}^r$ expression in this case was indistinguishable from that resulting from similar testcrosses of plants grown from non-heterofertilized kernels ($\underline{R}^r\underline{r}\underline{g}$ embryo; $\underline{R}^r\underline{r}\underline{g}\underline{r}\underline{g}$ endosperm). Furthermore the darkly mottled kernels obtained in the above two sets of testcrosses are in sharp contrast to the near colorless, paramutant form of $\underline{R}^r$ derived from $\underline{R}^r\underline{R}^{st}$.

These results support the view of autonomous development of the embryo irrespective of the endosperm genotype. There was no evidence for diffusion of substances bearing genetic potentialities from the endosperm to the embryo. Due to the infrequent occurrence of heterofertilization, the numbers of individuals observed in these studies were small. The need for obtaining a larger population is particularly important if one assumes that incorporation of any single genetic factor from the endosperm (e.g. the red anther component of $\underline{r}^r$) occurs rarely if ever.