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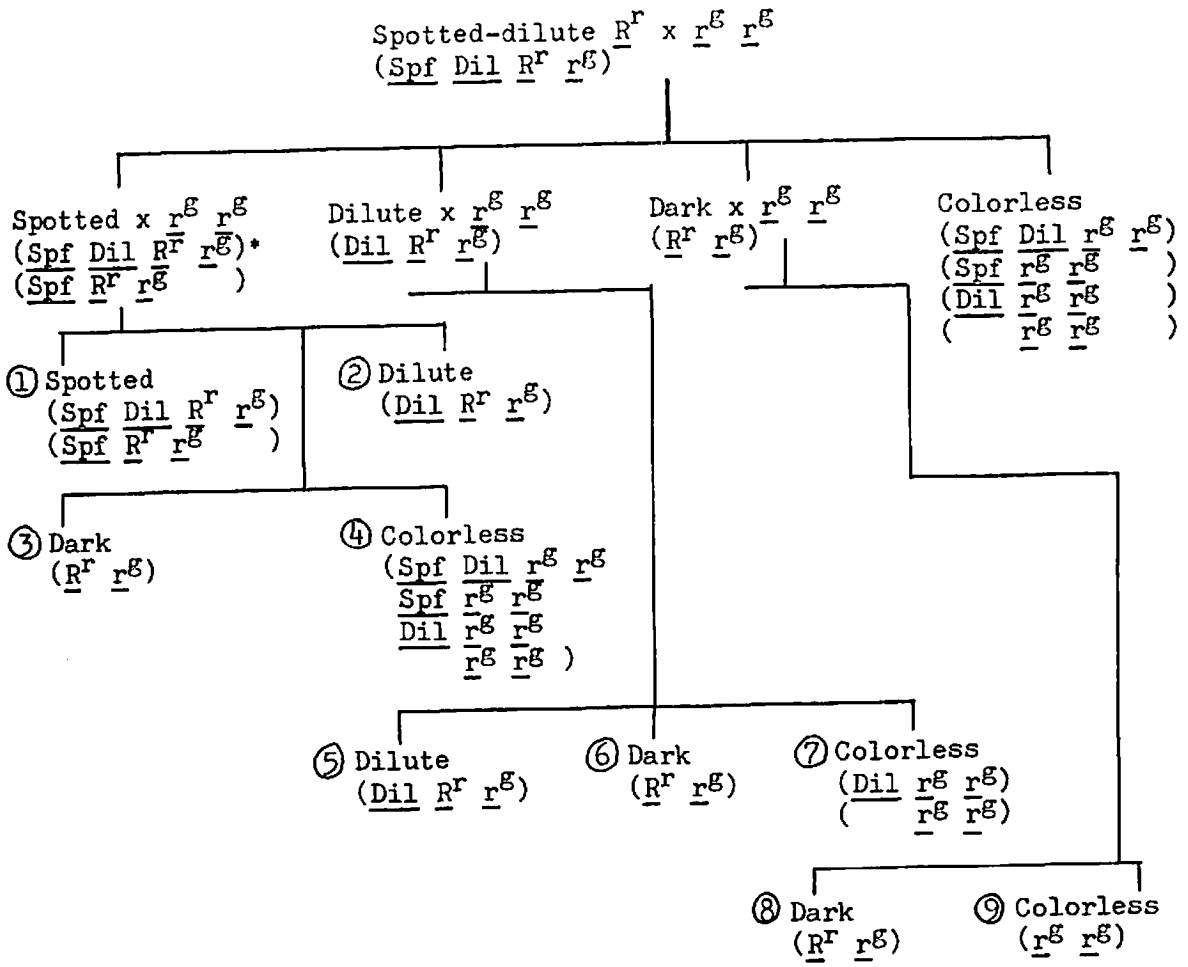
1. Spotted-dilute and the instability of R^r.

Some years ago Dr. M. Emmerling sent Dr. Brink several unstable R alleles described as Spotted-dilutes. These alleles gave dark spots on a colorless or lightly pigmented background in the aleurone. After several backcrosses to W22 (A C r^g or A C r^r), only two alleles (#2 and #4) were found to retain the original unstable pattern. The following information concerns the spotted-dilute R^r (#2).

The spotting pattern appears only in the aleurone tissue, and the anther color of the spotted-dilute stock is not distinguishable from normal A C R^r. On crossing to r^g r^g, the spotted-dilute R^r r^g segregates into spotted, uniformly dilute, dark, and colorless phenotypes. The dark phenotype is indistinguishable from that of standard R^r R^r. Plants grown from the dark kernels (R^r r^g) segregate only into dark and colorless kernels. Plants from the dilute kernels, on the other hand, show both dark and dilute kernels within the colored class. Spotted kernels never reappear in the stocks developed from dilute and dark kernels.

It is postulated that the patterns described above are affected by two dominant modifiers, one reacting with R^r to cause spotting and the other diluting the normal phenotype of R^r. These two factors will be referred to as Spf (spotting factor) and Dil (diluting factor). It is also assumed that the backcross parent W22 r^g r^g is free from the modifiers. Based on this terminology, the breeding behavior of the spotted-dilute R^r can be described in the following way:



*According to this scheme we should have two types of spotted kernels: (a) those which segregate into spotted and dilute and (b) those which give rise only to spotteds. We have both of these. Spf Dil R^r r^g and Spf R^r r^g cannot be phenotypically distinguished.

If the above hypothesis is true, then some colorless segregants (r^g r^g) from the spotted-dilute stock should carry the modifiers. Some plants from class 4, for example, should carry Spf or Dil or both or none. Similarly plants from class 7 should carry the Dil factor, while class 9 should carry no modifiers. To test these possibilities, plants were grown from all the colorless classes and each plant was selfed and crossed on the plants grown from (a) dilute kernels (Dil R^r r^g), (b) dark kernels (R^r r^g) and (c) R^{ch} R^{ch} plants. (The last mentioned cross was included in the program since the aleurone pigmenting capacity of R^{ch} was known to

be sensitive to the effect of other diluting modifiers). Results from these crosses came out according to the predictions. Some matings of the class 4 $\underline{r}^g$ $\underline{r}^g$ x Dil $\underline{R}^r$ $\underline{r}^g$ showed spotted kernels. Some crosses involving class 4 $\underline{r}^g$ $\underline{r}^g$ x $\underline{R}^{ch}$ $\underline{R}^{ch}$ showed both spotted kernels and dilute kernels, some showed dilute kernels and some showed spotteds. Half of the ears from the matings involving class 7 plants showed dilute kernels and there were no ears with the spotting pattern. Thus the data confirm the modifier hypothesis.

The fact that the spotting and dilute phenotypes are determined by modifiers of the R locus is also supported by another line of evidence. During selfing of the spotted-dilute stock we observed many ears which were homozygous for R but segregated for spotted and dilute patterns.

Further investigations showed that the standard $\underline{R}^r$ and Ecuador 1172 $\underline{R}^r$ are not sensitive to the action of Spf and Dil. Spf and Dil are independent of the R locus and independent of each other.

There was one puzzle from the beginning of the present investigations. When plants from classes 4 and 7 were crossed with the plants grown from dark kernels, the resultant ears showed only dark and colorless kernels. (Half of them should have showed spotted and dilute kernels on the modifier hypothesis.) These crosses were repeated with the $\underline{r}^g$ $\underline{r}^g$ lines known to contain the modifiers. (These lines were developed from the selfed ears mentioned above and their constitution regarding the modifiers was determined from the crosses made on the dilute and $\underline{R}^{ch}$ $\underline{R}^{ch}$ plants). Even these tests gave negative results, showing the plants obtained from the dark kernels do not respond to Spf and Dil. Although these experiments were carried out on a large scale, we were not successful in reconstituting dilute and spotted phenotypes from the dark kernels. It appears $\underline{R}^r$ in the spotted-dilute stock can maintain its sensitivity only if it is kept under control either by Spf or Dil. Once the nucleus is freed from the modifiers $\underline{R}^r$ loses the sensitivity and cannot regain it. At present we are not aware if there are any special conditions in which $\underline{R}^r$ can maintain or regain the sensitivity. $\underline{R}^{ch}$, however, is different in this respect and can maintain sensitivity even if Spf and Dil are absent.

Some tests were conducted to study the relationship between the spotted-dilute system and other well known controlling systems. The crosses $\frac{C^I\ Ds}{C^I\ Ds}\ \frac{R^r}{R^r}$ No Ac x $\frac{C}{C}\ \frac{R^r}{r^g}$ Spf Dil gave all colorless kernels indicating that Ds was not activated by any factors in the spotted-dilute stock.

Tests with McClintock's Spm system, however, gave some positive results. The functional homology between Spm and Spf has been partially established in the following way. $R^r/R^r\ a_2^{m-1}/a_2^{m-1}$ without Spm was crossed with $R^r/R^r\ A_2/A_2$ Spf Dil. The F_1 kernels ($a_2^{m-1}/A_2\ R^r/R^r$ with all combinations of Spf and Dil) showed uniform pigmentation. This demonstrates that the R^r in the a_2^{m-1} stock is not sensitive to the action of Spf and Dil. When the F_1 was backcrossed to a_2^{m-1}/a_2^{m-1} R^r/R^r (without Spm) a quarter of the total kernels in about half of the plants exhibited variegated kernels, showing that a_2^{m-1} was activated. Tests with a_1^{m-1} also gave similar results. Neither standard R^r nor $r^g\ r^g$ (all in W22 background like the spotted-dilute) carry Spm. Other preliminary tests on inheritance showed that Spm and Spf are functionally similar. The question whether R^{ch} will be sensitive to the action of McClintock's Spm (to produce spotted aleurone) is under investigation at Leeds.

If Spf and Spm are similar, what is the nature of Dil? It appears to act as a second Spm element with an ineffective component-2; also Dil functions as a weak Spm when tested against a_2^{m-1} and a_1^{m-1}. We observed frequent changes of Spf to Dil in the spotted-dilute stock.

Originally these studies were initiated to investigate paramutation of spotted-dilute R^r. In this context the following points are of interest:

1. The R^r in the spotted-dilute stock is paramutable.
2. The aleurone pigmenting capacity of R^{ch} is also paramutable and is sensitive to Spf and Dil.
3. Although the standard R^r is paramutable, it is not sensitive to the action of Spf and Dil.
4. Ecuador 1172 R^r is neither paramutable nor sensitive to the action of Spf and Dil. Thus, paramutability and sensitivity to Spf and Dil are independent features of the R locus.

With regard to the relationship between the Spm controlling system and the spotted-dilute $\underline{R}^r$ system, the following features should be emphasized.

1. The loss of sensitivity of the structural gene ($\underline{R}^r$) in the absence of controlling elements is not known in McClintock's Spm system. In this respect, $\underline{R}^{ch}$ resembles more closely $\underline{a}_2^{m-1}$ and $\underline{a}_1^{m-1}$.
2. The numerous different states of the structural genes reported by McClintock have not been observed at the R locus.

The spotted-dilute $\underline{R}^r$ (#4) resembles #2 in some respects but it does not appear to carry the Dil factor. This stock is under detailed investigation at Leeds. This work was initiated by one of us (G.R.K.S.) at the University of Wisconsin.

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1. Nucleolar number at premeiotic interkinesis.

Although the majority of interkinetic cells in the meristem of the root tip have a single nucleolus due to nucleolar fusion, cells with dual nucleoli are also apparent. Meiotic material of KYS was collected during early developmental phases to determine if dual nucleoli occur at premeiotic interkinesis. Successive early stages were not obtained in side branches of the tassels. Accordingly, to maintain orientation and sequence, leptonema was located in the main tassels and progressively younger anthers examined until the mitotic divisions preceding meiosis were encountered.