It has been suggested that confirmation of such sequences requires a study of the active synthetic stages. Twenty-day old aleurone tissues of self-fertilized colorless testers, singly recessive for $\underline{a_1}$, $\underline{a_2}$, $\underline{c}$, $\underline{r}$, or $\underline{bz_1}$, were pressed together in pairs, using pieces of distinguishable size, and placed on a complete medium or on agar at 25°C. in test tubes. Pigment was observed in one to two days and was subjected to the standard 10% hydrochloric acid test to confirm that the synthesized pigment was anthocyanin. All possible combinations with $\underline{a_1}$, $\underline{a_2}$, $\underline{c}$, $\underline{r}$, and $\underline{bz_1}$ were made and subjected to the above conditions. Complementary interaction resulting in anthocyanin synthesis was observed in all cases and it was unidirectional without a single exception. Out of every two testers (donor and receiver) combined, consistently only one (the receiver) gave pigment. This suggests that the precursors produced and/or controlled by the donor tester are subsequently used by the receiver to give anthocyanin pigment, since the receiver carries the dominant factor that is lacking in the other, as well as all subsequent factors in the sequence. With this reasoning and observations on all the combinations of the above testers it was determined that the action of $\underline{C}$ precedes $\underline{R}$ ($\underline{c}$ tester develops pigment in the pair of $\underline{c}$ with $\underline{r}$), $\underline{R}$ precedes $\underline{A_1}$, $\underline{A_1}$ precedes $\underline{A_2}$, and $\underline{A_2}$ probably precedes $\underline{Bz_1}$ in the synthesis of the pigment. Aleurone tissue of $\underline{in}$ tester, when subjected to the above conditions, was a strong donor to $\underline{c}$ and $\underline{r}$ testers, but was only a weak donor of the required substrates to $\underline{a_1}$, $\underline{a_2}$, and $\underline{bz_1}$. This quantitative criterion of intensity for $\underline{In}$ suggests its modifying action may follow the action of $\underline{R}$ in the sequence.

The behavior of homozygous $\underline{C^I}$ was interesting. When $\underline{C^I}$ aleurone tissue was combined with the others (i.e. $\underline{a_1}$, $\underline{a_2}$, $\underline{c}$, $\underline{r}$, $\underline{bz_1}$ and $\underline{in}$), $\underline{C^I}$ gave pigment, suggesting that, at the least, the inhibitory action of $\underline{C^I}$ precedes the action of $\underline{C}$. The interaction of $\underline{C^I}$ (presumably an allele of $\underline{C}$) and $\underline{c}$ tester in the production of anthocyanin draws special attention. All these observations establish the following sequence:

$$C^I,\ C,\ R,\ (In),\ A_1,\ A_2,\ (Bz_1) \text{--- anthocyanin}$$

The position of other known genes ($\underline{C_2}$, $\underline{Bz_2}$) and the modifiers is still to be determined, and attempts to identify accumulated substrates and to demonstrate catalysts are in progress.

G. M. Reddy

2. Endosperm culture.

Non-sugary (wild type) endosperm tissue, cultured last summer, has given continuous growth and pigment synthesis since that time. The medium was modified from that described in MNL 32: 103, substituting 5 gm. of Difco yeast extract for the tomato juice and using

only 20 gm. of sucrose and 8 gm. of agar. Subcultures of a_2, C^I, Pr, and pr were successful, and r tester has shown especially vigorous growth (in some cases as high as a 100-fold increase in volume in about six weeks, without transferring). This growth was not consistent throughout the cultures, of course, but was very significant in some. These observations are in conformity with the studies of Tamaoki and Ullstrup (Bull. Torrey Bot. Club, 1958), except that growth of non-sugary material so far is not limited in our cultures, even after six months. The distinctive phenotypic pigments, dark purple in Pr, dark red in pr, intense (almost black) in in and bronze in bz_1, cultures, are developed. In Pr and pr sub-cultures occasional colorless and pale-colored cell clusters are observed.

E. H. Coe, Jr.
G. M. Reddy

3. Haploid induction.

Properly-marked inducer lines have been recovered from third-generation backcrosses to stock 6 (see MNL 33:77). Although pollen of stock 6 induced as high as 2.35 ± 0.302% haploids in one gl_1 egg parent, a gl_{10}-marked parent that has a field-corn background gave only 0.98 ± 0.138%. The recovered marked lines vary in induction potential, but include individuals giving 1.18 ± 0.414% and 1.09 ± .198% in crosses to gl_{10} (8 haploids in 681 and 30 in 2755, respectively). Seed is available but quite limited in supply.

E. H. Coe, Jr.

4. Anti-inhibitor effect of bz_2: a correction.

Although the effect was attributed to bz_2, further tests show that bz_2 itself is not involved in suppression of C^I, but that the bz_2 stocks carry a special C allele and an independent modifier. Further tests are in progress.

E. H. Coe, Jr.

5. Non-homologous crossing over.

The occurrence of non-homologous crossing over is suggested by the presence of reciprocal translocations in the progeny of monoploids. However, it is possible that the crossing over occurs between duplicated segments.