4. Gene-modulating environments.

In inbred W22, the expression of the R gene can be altered by environmental conditions. The R gene, which conditions pigment in aleurone, is first sensitized by paramutation with R^{st} so that relatively small changes in phenotypic expression can be readily observed. Plants with paramutated R were grown for one month in growth chambers set for daily LD conditions of 12 hours light and 12 hours dark; other plants were given LL conditions (constant light). Plants were also given mixed treatments of LL-LD (two weeks constant light followed by two weeks of LD) and LD-LL (two weeks of LD followed by two weeks of LL). Growth chambers were held at 70°F for the month of seedling treatment. In early June, all plants were transplanted to field conditions for the remainder of the life cycle. Testcrosses of treated plants were made to colorless inbreds grown in the field; pigment in testcross ears was scored by methods outlined in our earlier reports (MGCNL 38, 39).

In Table 4 testcross pigment scores of treated plants show that the LD conditions during the first month of plant development produce paramutated R genes which condition more pigment; LL treatments show that less pigment is produced as a result of early environmental treatments. Mixed treatments LL-LD show pigment values close to those of LD-treated plants; LD-LL mixed treatments show pigment values close to the LL-treated plants. Mixed treatment scores suggest that the second two-week period of development determines the R expression of the testcrosses. Pigment differences between plants receiving LL and LD treatments during the second two-week period of development are highly significant, statistically.

Table 5 shows that plants grown in the field but which were given the above environmental treatments in 1964, still show the relative differences found between LL and LD conditions. In the report of Table 5 aleurone scores are based on phenotypes from three doses of paramutated R. Under these high R dosages, only relatively small differences can be observed but these differences made it possible to select the more sensitive level of paramutated R so that the large score-differences of Table 4 could be observed in 1965 testcrosses.

It was noted in the reports above that pigment score differences can be found in the testcrosses of different pollen samples from single tassels of the same plant. It can also be noted that while some of these differences can be quite small, the relative differences noted in one generation can be carried across into the testcrosses of the following generation. It was concluded that the phenotypic differences noted in each of the testcross kernels can be considered to represent different states of the paramutated R gene. Because of the differences in testcross scores in

Table 4

Testcross scores for R expressions from RR^{st} heterozygotes after environmental treatments during the first four weeks of seedling development. Scores represent ear means based on scores of 50 kernels per ear.

Environmental Treatments				
LD	LL	Field	LL-LD	LD-LL
12.64	8.72	8.58	13.08	7.62
11.90	6.66	10.38	12.96	5.54
16.58	10.48	12.36	13.78	8.64
16.14	4.48	7.72	12.80	7.86
12.60	7.66	11.84	13.52	7.64
14.44	10.68	14.06	13.36	10.30
14.82	10.96	11.96	13.04	8.06
14.76	8.20	13.74	12.64	8.44
pooled $\bar{X}$ 14.23	8.47	11.33	13.15	8.01

Table 5

Persistence of relative pigment differences associated with specific early plant environments.

1964 Treatment	1965 Treatment	Total Kernels Scored	% Kernels Fully Pigmented
LD	Field	*1299	77.5
LL	Field	1106	75.6
Field	Field	1072	76.7
LL-LD	Field	1571	74.9
LD-LL	Field	1430	68.1

*Scores based on self-pollinated ears. All kernels on each ear were scored according to the numbers of kernels showing full pigmentation over the crown of the kernel.

Table 4, one may conclude that the LL and LD environment can make significant contributions to the state of the paramutated R gene. One may no longer assume single gene expression to be immune from environmental influence from generation to generation.

One can only speculate about the mechanisms involved in this unusual behavior of R. One line of speculation which will offer experimental test possibilities is that R expression is internally regulated by specific growth substances. The LD and LL conditions may affect R expressions through internal balance of growth substances--at this point it may be useful to consider the model developed for insect larval development where hormonal control of chromosome puffing has been demonstrated. What appears novel in our situation with corn is the possibility that the differences observed are also carried over into the following generations.

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