Environmental Programming of Heritable Epigenetic Changes in Paramutant *r*-gene Expression Using Temperature and Light at a Specific Stage of Early Development in Maize Seedlings

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Manuscript received April 21, 1994
Accepted for publication May 12, 1995

ABSTRACT

Different heritable expression-states were programmed into *R* alleles from *R/R-lst* heterozygotes under different temperature conditions applied during a developmental period in which flowering is induced. At maturity, *R*-allele expressions in test crosses of male gametes derived from *R/R-lst* seedlings raised 15 days in 32° and continuous light conditions differed significantly from those of sib seedlings raised for 15 days in 22° and continuous light conditions and shifted to six 12-hr light-dark cycles, days 16–21. This experiment provides the first evidence in higher organisms that environmental conditions, applied at a specific stage of development cause a heritable change in a specific allele expression. My earlier evidence required a statistical analysis for demonstrating heritable change; I present photographic evidence of this environmental effect on four *R* alleles.

PARAMUTATION (BRINK 1956) provides a sensitive genetic system where heritable quantitative variation in the expression of a single gene can be followed from generation to generation. BRINK showed that when the *R* allele (dark kernel pigmentation) is made heterozygous with its allele, *R-st* (stippled pigment pattern), the *R* allele produces significantly less pigment after removal from a heterozygote with *R-st*. The change in *R*-allele expression is heritable; furthermore, all *R* alleles (100%) upon removal from this *R/R-st* heterozygous combination are changed, and the change is directed at the *R* locus only. Later studies (MIKULA 1961; MCWHIRTER and BRINK 1962; COOPER 1964; STYLES and BRINK 1966) showed that if the *R* allele was maintained with *R-st* alleles for several generations, paramutation of the *R* allele was additive. This additive effect on the *R* allele was followed in male gametes for five generations (STYLES and BRINK 1966). FEDOROFF (1989), while studying the ontogenetic expression of the transposon *Spm* (*Suppressor-Mutator*), reported evidence that an inactive *Spm*, by association over several generations with an active *Spm*, can become progressively more active. The *R* alleles and cryptic *Spm* elements in maize represent genetic systems whose phenotypes can be incrementally programmed by previous association with other genetic elements during development (FEDOROFF and BANKS 1988).

Paramutation (BRINK *et al.* 1968; DOONER *et al.* 1991) was considered to be the result of changes accumulated in the *R* gene throughout somatic development. COOPER (1964) and SASTRY *et al.* (1965) reported variation in paramutation from gametes sampled from different tassel branches reflected a mosaicism developmentally controlled before or during tassel differentiation. I reported in a previous paper (MIKULA 1967) that when *R* is exposed to a paramutagenic allele (*R-st*), the environmental conditions can promote significant heritable change in *R*-allele expression. *R* alleles from plants that as seedlings were raised at 21° and LL conditions (continuous light) during the third and fourth week of seedling development, when compared in testcross progeny, conditioned significantly less pigment in test crosses at maturity than *R* alleles of plants matured from seedlings grown at the same temperature in LD (12-hr light-dark cycles) conditions for this same period. The identification of a critical developmental period leading to changes in *R* expression suggested that experimental control of paramutation was possible. Three other observations reinforced this inference: the level of paramutation could be correlated with the number of LD cycles in the third week of seedling development; preliminary tests showed a polarity of paramutant *R* allele expression in which early pollen samples from the upper branches of single tassels showed more paramutation than samples taken from lower branches of the same tassel; and incremental changes in paramutant *R* expression from generation to generation (MIKULA 1961) showed that small changes in the level of paramutation could be detected and amplified from generation to generation.

Further, it was found that tassel initiation in the W22 inbred could be delayed under continuous light conditions. It then became possible to ask the following questions: how early in the life of the seedling could tassel determination take place; did temperature and light play a role; how many light-dark cycles were re-

quired for tassel determination; could the level of paramutation be correlated with programmed environmental conditions applied in early seedling development; could paramutation be inhibited or driven toward the completely colorless level; when during early development is the seedling most responsive to environmental variables of temperature and light; and could the level of paramutation in four new *R* accessions be correlated with environmental conditions administered to young seedlings?

MATERIALS AND METHODS

Seed stocks: Environmental programming strategy for the first 3 weeks of seedling development was based on work with the *R* and *R-st* alleles in inbred W22 background provided by JERRY KERMICLE, University of Wisconsin. The effect of the environment on paramutant *R*-allele expression, first reported in inbred W22 (MIKULA 1967), required a statistical analysis for its evaluation. In an effort to find more responsive *R* alleles, new *R* allele accessions that had not undergone a high degree of inbreeding and selection were screened for dark kernel pigment expression. The four new *R-g* alleles from New Mexico and Arizona, Z21, Z29, Z1, Z69, supplied by Native Seeds/SEARCH, Tucson, AZ, are reported as *G1, G3, G4,* and *G5,* respectively in this document. The new *R-g* accessions condition, in *r/r/R* endosperm, a uniformly dark pigmentation, comparable to that of the W22 *R-r* gene. All four *R-g* lines were crossed to a *R-lst* (light stipple with few very fine spots) known to cause intermediate levels of paramutation of the *R* allele. For convenience, the *R-g* genes will be referred to simply as *R,* eliminating the lower case designation,-*g,* signifying lack of plant color. The *R-lst* allele, in W22 background, was supplied by the Maize Genetics Coop., University of Illinois.

Growth chamber conditions: Seeds from single ears of the *R/R-lst* heterozygotes, representing the four different *R* alleles, were germinated on white, moist facial tissue pads in stainless steel, glass-covered pans. Continuous light was supplied by 14 200-W VHO cool white fluorescent tubes supplemented by 12 60-W incandescent bulbs. In 3 or 4 days, when coleoptiles were large enough to handle safely, seedlings were transplanted to soil conditions in plastic pots. Seedlings were started 50–60 cm distant from the light source and grew toward the light for the remaining 2- or 3-week periods they were held in the controlled environment conditions. Average light intensity during this early stage of seedling development was 35 W/m^2. Dark conditions were obtained by placing the seedlings into light-tight boxes. For each environmental treatment, eight seedlings were used. Under continuous light conditions, after 14 days at 22° or 7 days earlier at the higher temperature, 32°, seedings developed necrotic conditions in youngest leaf tips. The deterioration of youngest leaves progressed with time, especially at the higher temperature and was reminiscent of calcium deficiency symptoms. Most plants survived this stress and recovered after dark treatment.

Field operations: Seedlings were transferred to the field at the end of the growth chamber treatments. At maturity, plants that were subjected to different environmental conditions as seedlings were mated to *r/r* (W22 × W23) females grown under field conditions. Test-crossed ears were evaluated on the basis of their kernel pigmentation. Pollinations were performed by sampling pollen over 7 days; the test crosses from the last pollen sample (of each plant) were used for the photographs.

Scoring procedures: A sample of 50 kernels from each testcross ear was scored by matching each kernel against a set of standard kernels ranging from 0 (colorless) to 20 (completely pigmented). Pooled means and standard deviations for each environmental treatment are reported in Figure 6.

RESULTS

Extensive preliminary testing showed no tassels were initiated in inbred W22 under LL conditions. This made it possible to determine the number of LD cycles essential for tassel initiation. Tassel initiation was determined 1 week after LD treatments, however plants remained in the inhibitory LL conditions until six to eight plants could be assayed for the presence of tassel primordia. The presence of tassel primordia was determined by dissecting away the leaves surrounding the terminal meristem, under magnification. The presence of an elongated terminal meristem and the presence of branch primordia were observed when tassel initiation had taken place. Figure 1 shows that tassels could be induced with six LD cycles applied days 16–21 at 22° or a week earlier with only four LD cycles applied days 11–15 at 26°. The results in Figure 1, based on the presence or absence of tassel primordia in seedlings of the W22 inbred for each combination of environmental conditions, provided a model system that made it possible to test whether paramutation could be influenced by combinations of temperature and light variables applied at a time seedlings were undergoing the transition from vegetative to floral phase of meristematic activity.

The test-cross ears of *RR-lst* heterozygotes of alleles *G1, G3, G4,* and *G5,* in Figure 2, A and B, show that a difference in the amount of pigment for all four new *R* alleles can be observed in response to early environmental conditions. Seedlings raised in 31° LL conditions for the first 3 weeks in the left column of both photographs show kernels with less pigment than those raised in 22° LD conditions during the same period. The paramutagenic allele, *R-lst,* present in half of the kernels on each ear, has a phenotype too light to be visible in the photographs; thus, it is possible to compare, visually, the environmental contribution to the paramutation of the *R* alleles. *R/r* seeds representing median pigment values from each of the columns of testcross ears pictured in Figure 2, A and B, were planted directly in the field in 1991. The differences observed in the test crosses of the treated generation, 1990, were carried over in the test crosses of 1991, Figure 3, A and B, with no further environmental treatments.

The *R* allele designated *G4* (Figure 2B, upper ears), that showed the most reduction in *R*-allele expression in response to early environmental treatments of seedlings in 1990, was selected for shorter exposure periods at elevated temperatures in 1991. Test crosses of R_{G4}/*R-lst* from seedlings given LL conditions for 15 days at

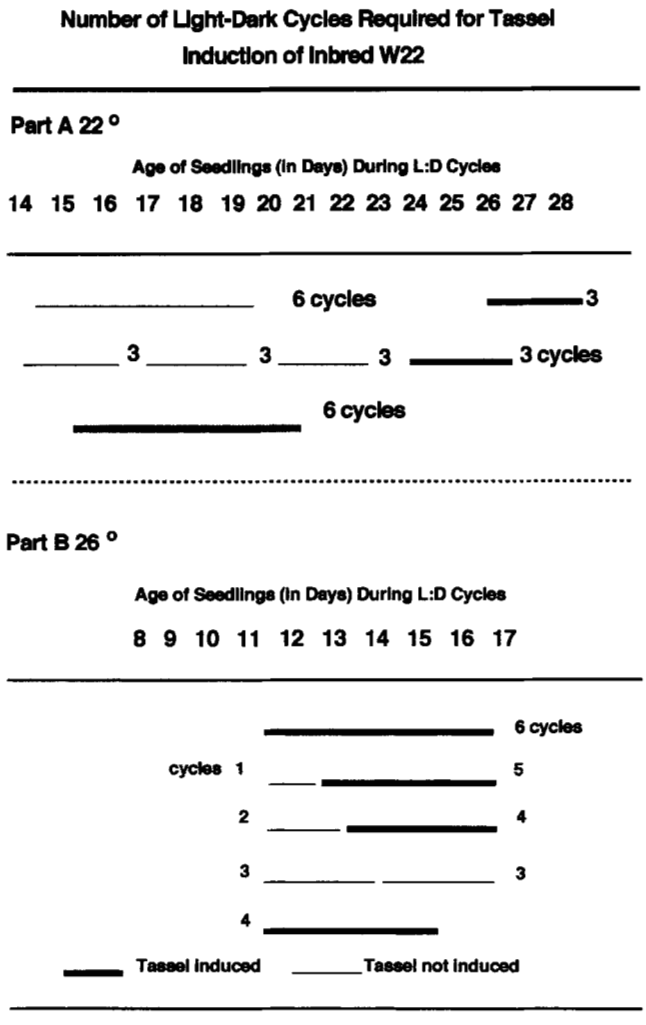

FIGURE 1.—Developmental periods, in days, where repeated LD cycles induced tassels in seedlings. (A) Summarization of seven experiments performed in 22°; (B) summarization of eight experiments performed at 26°. Numbers at the end of each line refer to the number of LD cycles applied in each experiment. Seedlings were grown in continuous light except for the periods covered by the lines. Tassel induction was confirmed a week after the last LD treatment.

32° are shown in the left column of ears of Figure 4. *R*-allele expressions in the right column of testcross ears of Figure 4 are from seedlings raised in 22° and treated with LL conditions through day 15, then subjected to LD conditions, days 16–21. Seeds (*R*/*r*) from 1991 testcross ears, representative of each of the columns in Figure 4 were planted directly in the field in 1992 with no environmental treatment of seedlings. Figure 5 shows the differences observed in 1991 were maintained in 1992. In fact, in 1992 a greater contrast can be observed between paramutated *R*-allele expressions from plants which received 32 and 22° the previous year. Reversion has been reported (KERMICLE 1963; STYLES and BRINK 1966) to take place in paramutated *R* alleles after removal from the heterozygote with paramutagenic allele *R-st*. Because less reversion takes place in the more highly paramutated *R* genes (KERMICLE 1963), a greater contrast is observed in the years after seedling treatment, Figures 3 and 5. This higher contrast in the year after the seedling treatment gives emphasis to the heritable nature of the change in *R*-allele expression.

The *G4* allele was retested in 1992 to identify more precisely when LL and LD conditions at 32 and 22° can influence paramutation. Figure 6 presents a schematic diagram showing the times LL and LD conditions were applied to program the epigenetic change in paramutant *R*-allele expression. Seedling treatments under 32° LL conditions (Figure 6, line c) were terminated a week earlier than seedlings started in the 22° LL environment (line a); both received natural LD conditions when transplanted to the field. LD cycles were applied days 16–21 at 22° (Figure 6, line b) or in days 11–15 at 32° (line d) before seedlings were transplanted to the field. The differences observed in the photographs of Figure 4 were replicated in 1992 and are presented as testcross pigment scores in Figure 6 (lines b and c); a difference of 10 scoring units was found. Significant differences in paramutation can be related to 5- or 6-day intervals in seedling development when lines b–d are compared in Figure 6. These changes in the level of paramutation presented in Figure 6 can be correlated with temperature and light conditions applied during the developmental interval sensitive to floral induction in Figure 1. Plants that were kept under LL conditions before being transplanted to field conditions have two more nodes and many more tassel branches than those given LD conditions in the last 6 days of programmed environmental conditions. This observation supports the conclusion that tassels were determined in the periodic (LD-controlled) environment.

DISCUSSION

The developmental events unfolding in the corn seedling were considered a biological clock, genetically driven internally, and regulated externally by LD cycles. Denying the plant its external regulatory, light-dark cycles in a constant temperature environment, was expected to perturb the internal developmental clock mechanism (a developmental disequilibrium) such that paramutation might be affected. Continuous light kept the seedlings vegetative with the terminal meristems periodically initiating new leaf primordia. This made it possible to show (Figure 1) when tassel determination takes place at 22 and 26° and by inference at higher temperatures. It was possible to correlate the presence of tassel primordia and number of leaf primordia in seedlings with the results observed in similarly treated seedlings that had been transferred to field conditions for maturation after environmental treatments. Seedlings from LL conditions, when compared at maturity

FIGURE 2.—A comparison of the effect of early seedling environments on testcross expressions of four different *R* alleles, *G1, G3, G4, G5*, heterozygous with *R-lst*. Seedlings were given either 31° LL or 22° LD conditions during the first 3 weeks of their development. Test-cross ears from the 31° conditions are in the left column of A and B; those from 22° are to the right. Fifty percent of the kernels on each of the ears represent the *R-lst* allele whose spots are not visible in the photographs; the remaining 50% of the kernels carry the paramutant *R* alleles. The difference between the amount of kernel pigment in the left and right columns of each photograph represents the environmental influence on paramutant *R*-allele pigment expression that occurred in the first three 3 of development.

with plants of the same age given the appropriate number of LD cycles to induce tassels, matured later, were taller and showed increased node numbers related to the number of days under continuous light.

The experimental design in my early work (MIKULA 1967) could only implicate the third and fourth week of seedling development in the control of paramutation. Figure 1A shows that at 22° tassels were determined by the end of the third week when six LD cycles were applied, days 16–21. When the temperature was raised to 26°, four LD cycles, days 11–15, tassel determination occurred a week earlier (Figure 1B). Subsequent tests showed that seedlings, though stressed, could survive continuous light and 31° for 3-week test periods. This made it possible to compare paramutation from plants raised as seedlings in 31° LL conditions compared with those from 22° LD conditions. The photographic evidence of Figure 2, A and B, with the four new *R* alleles, confirms the results previously reported (MIKULA 1967) that significant differences in paramutation could be induced in seedlings in the first 3 weeks of development and that the differences were heritable (Figure 3, A and B). An even greater level of paramutation was programmable in <3 weeks if *R*-allele expressions of seedlings held 15 days in 32° LL conditions were compared with those of sibling plants given 22° LL conditions days 1–15, followed by LD conditions, days 16–21 (Figure 4). Figure 5 shows the differences noted in Figure 4, the previous year, were maintained the following year. In Figures 1–6 only a limited number of variables involving time, temperature and light were explored. The objective in this preliminary work was to find combinations of light and temperature variables that could show conclusively that specific environmental conditions, at a specific stage of development, influence paramutation. Given the number of potential variables: age of seedling, plant to plant developmental heterogeneity, day length, light quality-intensity-duration, LD cycling combinations, temperature cycling, heat shocks, together with the constraints of limited growth chamber space and one growing season a year, many combinations of variables remain unexplored. Nevertheless, a significant increase in paramutation was achieved early in development and implicated a 5-day period of development.

To program seedlings within the 2- or 3-week period, seeds were germinated in continuous light and transplanted to soil when coleoptiles and roots were large enough to handle safely. This guaranteed that coleop-

FIGURE 3.—The carry-over, in 1991, of the influence of early temperature and light conditions on four paramutant *R*-allele expressions induced in two different environments the previous year, 1990. *R*/*r* seeds of each of the alleles in Figure 2, A and B, were planted directly in the field. The left columns of test-cross ears, in each photograph above, represent *R* alleles from plants that, for 3 weeks as seedlings, received 31° LL conditions in 1990. The right columns represent *R* alleles from plants that, for 3 weeks as seedlings, received 22° LD conditions in 1990.

tiles remained in continuous light until LD cycles were employed in the second or third week. Because in soil conditions coleoptile emergence may take 7 days, acceleration of seedling development was achieved under the continuous light of growth chamber conditions making it possible for tassel determination with four LD cycles at the end of a 14-day period at 26° (Figure 1B). All four newly introduced *R* alleles (Figures 2–5) showed a significant response to temperature and light conditions applied at the early stages of seedling devel-

FIGURE 4.—Test crosses showing environmental enhancement of paramutation (repression of pigment expression) that took place in the first 2 weeks of seedling development. The *G4* allele from Figure 2B (top), heterozygous with *R-lst*, was retested in 1991. Seedlings that received 32° LL for days 1–15 produced, at maturity, the test crosses in the left column. Sib seedlings raised in 22° LL conditions days 1–15, then subjected to LD cycles days 16–21, are represented in the test crosses in the right column.

FIGURE 5.—Test of heritability of differences observed in Figure 4. The *R/r* seeds from test-cross ears of 1991 in Figure 4 were planted the following year, 1992. The differences observed in 1991 persist in the test crosses of 1992 without further temperature treatment and in the absence of the paramutagenic *R-lst* allele.

opment. The *G4* allele (Figures 4 and 5) was the most responsive to the two different temperatures; seedlings held in LL conditions for 15 days at 32° when compared with those from LL conditions for 21 days at 22°, at maturity, produced test crosses whose scores were significantly different (Figure 6, lines c and a, respectively). Significantly different levels of paramutation were also achieved within the last 5 days of the first 2 weeks of development with the same temperature (Figure 6, lines c and d). Thus, the period of seedling development most sensitive for increasing the level paramutation was found in the last 5 days of the first 2 weeks of seedling development at 32°, under LL conditions. At 32°, the developmental interval in which LD conditions were applied (Figure 6, line d), corresponds with the time tassels were determined in 26° LD conditions (Figure 1).

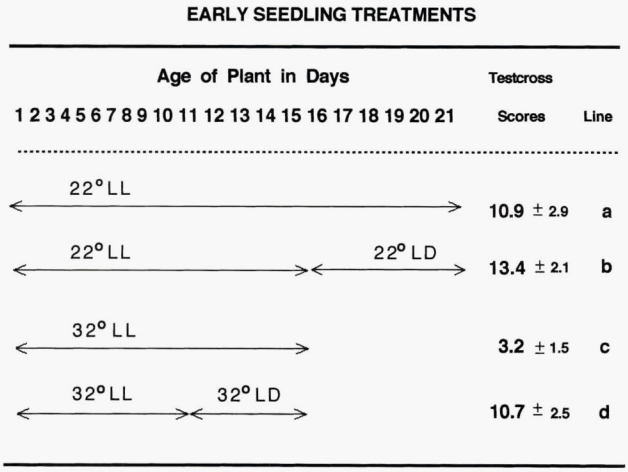

FIGURE 6.—The environmental programming schedule for 2- and 3-week-old seedlings which were started in LL conditions. LD treatments that induce tassel formation were applied the last 5 days in 32° (days 11–15) and in the last 6 days in 22° (days 16–21). Seedlings were removed to field conditions after each treatment. Test-cross scores of matured plants reported on the right show the magnitude of change in *R*-allele expression. Each of the four scores are pooled means and standard deviations from six different plants. All seeds used came from the same *R/R-lst* ear carrying the R_{G4} accession.

When the scores 3.2 and 10.7 in Figure 6, lines c and d, are compared, light appears to be the variable that contributed the higher level of paramutations (least pigmentation) in test crosses at maturity. However, when the lights were turned off for dark cycles it was inferred the absence of radiant energy from the growth chamber lights lowered plant temperatures; therefore, temperature cannot be excluded from comparisons based solely on lines c and d in Figure 6. Unpublished experiments show that, given the same continuous light conditions, more paramutation was found from plants which as seedlings were held 2 weeks in 22° then shifted to 32° for the third week compared with those that were continued in 22° for the third week. It is concluded, therefore, temperature is the more important variable at this stage of development. However, the effect of light cannot be entirely discounted because continuous light serves to delay tassel determination and could thereby enhance the effect of temperature through some developmental disequilibrium.

In the early literature on paramutation, MCWHIRTER and BRINK (1962) reported that 67 *R-sc* mutants from *R-st* were found to produce an allelic continuum of strong to weak paramutagenic action on the *R-r* allele. When the effects of 32 and 22° conditions on paramu-

tant *R*-allele expression are compared, the same *R-lst* allele can be both strongly and weakly paramutagenic depending on the seedlings' early environment. Test-cross pigment scores in Figure 6, lines b and c, show a separation of 10 scoring units. It may be inferred that the strongly and weakly paramutagenic alleles reported by McWHIRTER and BRINK (1962) represent mutants whose paramutagenicity differ in temperature sensitivity.

To determine precisely when paramutation takes place, more work needs to be done exploring combinations of variables as well as the timing of their application on developing meristems. BRINK *et al.* (1968) suggested, on the basis of a survey of various paramutation-like phenomena in Pisum, Malva and Oenothera, that paramutation could take place gradually throughout somatic development. My experiments cannot rule out that some paramutation takes place during the early vegetative phase of development; however, the results in Figure 6, lines c and d, show that a significant amount of paramutation was achieved within the 5 days in which tassels were determined, Figure 1B. The most economical hypothesis for paramutation of the *R* allele, a transcriptional activator, would be to assume the allele is turned on throughout seedling development and can be turned off late in development under paramutagenic conditions, during the developmental transition from vegetative to floral tissue in the meristem.

It is clear paramutation in the new *R* accessions responds to environmental conditions applied early in seedling development as was first reported for inbred W22. Whether the new *R* alleles respond more or less than the highly inbred *R-r* allele first reported (MIKULA 1967) no longer seems relevant because the level of paramutation shows a sensitive dependence on early developmental conditions. The conditions that influence paramutation of the *R* allele take place many cell divisions before gametogenesis. The result is a clonal mosaic of cells observed in the test crosses of treated plants. The size of the clones of cells can be associated in Figures 2–5 with the initial conditions that determined the switching of the meristem from the vegetative to the reproductive state. The smallest clonal patches of cells are found in those test-cross ears of plants that as seedlings were given the stress conditions of 32° and continuous light before removal to natural LD conditions of the field.

Where transposable elements *Mu*, *Spm* and *Ac/Ds* were involved, small clones of cells expressing pigment were interpreted as resulting from a transposition event involving the last few of the 17 cell divisions of the aleurone layer (LEVY and WALBOT 1990). If the analysis of LEVY and WALBOT is applied to clonal patterns resulting from paramutation of the *R* allele, it is possible to infer that events controlling the timing of pigment expression in the last few cell divisions of the aleurone were determined many cell divisions earlier during developmental conditions at the time of tassel determination. Proposed models for paramutation-like events must account for the signal receptor, the cellular events in the time interval to gametogenesis as well as the lag-time involving *R*-allele expression in the earliest of the 17 cell divisions of the aleurone of test crosses where the paramutated *R* allele is normally expressed. A corollary of this line of reasoning requires a consideration that clonal patterns resulting from transposable elements leaving a locus might also be determined within this same developmental interval. A transposable element has been associated with *Rst* (ASHMAN 1970; WILLIAMS *et al.* 1984) though as yet none has been reported to be associated with the *R* allele, a transcriptional activator (LUDWIG *et al.* 1989; ROBBINS *et al.* 1991).

The paramutation behavior of the *R* allele reported above and the programmable *Spm* (FEDOROFF 1989) share common characteristics. Both show: expressions correlated with methylation (M. ALLEMAN and J. L. KERMICLE, unpublished data, reported in PATTERSON *et al.* 1993); dependence for changes in expression on the presence of a *trans*-acting element, *R-st*, in the nucleus (ASHMAN 1970; WILLIAMS *et al.* 1984); different expression states which can be correlated with developmental regulation in different regions of the main stem and different tassels of the same plant (COOPER 1964; SASTRY *et al.* 1965); and incremental change from generation to generation (MIKULA 1961; McWHIRTER and BRINK 1962). The ability to program and monitor high-frequency incremental change in a gene expression, from generation to generation, is unique to the paramutated *R* allele. FEDOROFF and BANKS (1988) inferred from their data that developmental events in the plant, together with a transacting *Spm* could incrementally program increased responsiveness of an inactive *Spm*, ultimately resulting in changes in the methylation of the promoter of *Spm* in subsequent generations (FEDOROFF 1989). Because, as reported above, light and temperature conditions, applied at a specific stage of development, can be implicated in the control of paramutation at the *r* locus, and, because the similarity of paramutation at the *r* locus and transactivation of *Spm* has been noted (JORGENSEN 1993; MATZKE and MATZKE 1993), the role of environment for incrementally programming epigenetic change at a specific stage of development in other genetic systems can now be addressed.

The environmental effects of temperature on somatic mutability in the earlier literature presaged the more recent findings of temperature and light influencing mutable (unstable) gene expression. Somatic mutability (variegation) (RHOADES 1941; FABERGE and BEALE 1942; PETERSON 1958; in Antirrhinum, BONAS *et al.* 1984; HARRISON and CARPENTER 1973) was reported to be increased with temperature changes. Differences in light intensity increased somatic mutability at the *Gl 1* locus in maize (MADDALONI *et al.* 1990). Reduction of

floral pigment in transgenic petunias (VAN DER KROL *et al.* 1990) was correlated with changes in temperature, light duration and intensity; however, suppression of pigment did not persist after segregation of the transgene (NAPOLI *et al.* 1990).

Heritable change in floral pigment expression in transgenic petunias (MEYER *et al.* 1992) was correlated with changes in environmental conditions during development. The number of weakly pigmented flowers increased with the age of the plant and with field conditions of higher temperature and light intensity. Because reduced floral pigment could be monitored in the many flowers of a single plant from May through September, it was concluded methylation associated with reduce pigmentation could take place at any time in the petunia life cycle. The reduced pigment expression noted in the field showed up with high frequency in progeny of affected flowers.

In maize, paramutation of the *R* allele is observed only in aleurone tissues of test crosses. It can be inferred that if the environment plays a role in paramutation during development, then it would be expected that on a single plant showing paramutation-like behavior one could find that, as in petunia, gametogenic tissues from the main stem and tillers and ears in maize might differ because they are determined at different times. In the work I have reported above, only the terminal meristem of the main stalk was determined under controlled environmental conditions. When treated seedlings were moved from LL to field conditions, induction of ears and tassels on tillers of the same plant were subjected to different light and temperature environments. Differences between the gametes of the main stalk, tillers and ears have been reported (COOPER 1964; SASTRY *et al.* 1965; FEDOROFF 1989).

This report on the *r* locus may be considered the first where environmental variables, applied at a specific developmental stage elicit high-frequency heritable changes in a specific allele expression. For reasons still not understood, the heterozygous condition (paramutation) with the *R-lst* allele is a requirement for the environmental effect on the *R* allele. Homozygous *R* alleles as well as *R* alleles heterozygous with a paramutated *R*, tested under the same conditions reported above, have not shown enhancement of paramutation. However, as pointed out earlier, many combinations of environmental conditions remain untested. In a recent review of heritable changes induced by environmental stress conditions, CULLIS (1990) reported no examples in which the effect of the environment could be directed at a specific gene. The *R* allele is known to respond to light and temperature and therefore could provide, as a transcriptional activator, a mediator function for the two most important external variables that control timing in biological systems, light and temperature.

Paramutation at the *r* locus represents a model system to monitor heritable effects of environment on epigenetic change. It is quite possible, however, that those systems that have been described as unstable genes, transgenes, mosaicism, variegation, transactivation, transvection, transdetermination, TRANS-sensing, expression states, cosuppressions, phase changes, position effect variegation, chromatin spreading, or "sick genes" may represent facets of common mechanisms shared by many genetic systems designed to help genetic programs to "learn from experience" (JACOB 1982).

I wish to thank BETH BESAW and MARGARET NOBLE for dedicated technical assistance. Grateful acknowledgement is made to The Defiance College for making growth chambers, field facilities and equipment available for the past 30 years. Without the provision of growth chambers by The Charles F. Kettering foundation, Dayton, OH, this work would not have been possible.

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Communicating editor: B. BURR