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Using the *R* paramutation system, over the past 50 years our reports to the CNL aimed to develop the evidence for photo thermal (PT) control of heritable changes in a Mendelian gene. The challenge of the 20th Century was to find a gene whose epigenetic variation could be induced and the variation was heritable. Since the environment was dismissed as Lamarckian in 20th Century efforts needed to be directed at a specific Mendelian gene.
In 1956 *R* paramutation provided an example in which every *R* allele from the *R/Rst* heterozygote was heritably silenced (reduced in level of pigmentation) thus violating the first law of Mendelian genetics. Because *R* silencing occurred in 100% of the *R* alleles segregating from the heterozygote, there was a high probability that if the environment could perturb the paramutation process, it was likely that a phenotypic change could be detected. A further advantage of *R* paramutation was that a continuum of expression-states was available for monitoring changes in *R* on a test cross ear where as many as 200 kernels can exhibit changes in expression-states. Another advantage was paramutated *R* alleles showed incremental change ("memory") from year to year which meant small changes in expression one year could be amplified the following year. The continuum of expression-states in testcrosses of paramutated *R* alleles suggested that progression of epigenetic variation in pollen from different tassel branches needed to be examined carefully.
Thus conditions were available to test whether environmental conditions (photo therma, PT) could influence paramutation, that is, cause heritable change in a Mendelian gene expression.

Plants grown under field conditions showed no clear variation between pollen tested from the upper and lower tassel branches so effort was directed at timing how and when tassels formed in the W22 inbred. The Kettering Foundation provided controlled environment chambers where the maize seedling was germinated under defined conditions of light (LD) and temperature.
Seedlings germinated and maintained under continuous light (LL) developed quickly during the first week after which necrotic lesions were observed at the leaf tips. When light/dark (LD) conditions were then applied, seedlings continued developing and necrotic tissues were reduced. With the appearance of necrotic lesions, following LL conditions, the seedling was ready for tassel induction with the application of the LD conditions. Under LL seedling continued to initiate leaf nodes until LD conditions are applied.
At maturity, pollen from upper tassel branches of plants that as seedlings were subjected to controlled conditions showed more silencing than pollen test crossed from lower branches of the same plant. Results shown in Fig.1 and were reported to the Genetics Congress in Australia in 2003. The pooled means of columns three and four, each representing several plants, obscured the epigenetic changes in individual plants. Early pollen from upper branches showed less pigment (more silencing) than later pollen from lower branches among seedlings started at LL 22°. The epigenetic dynamics that was taking place in the tassel pollen of individual plants was obscured in the pooled mean scores.
A better graphic was needed to show the epigenetic changes taking place in each tassel. Figures 1-3 show the changes in 75 individual plants as pigment expression profiles for the 75 plants whose pigment expression was presented as pooled means in Table 1.
The mean values of 50 kernel-pigment scores from the early and 50 from late pollen collections determined the linear profiles of the 75 plants in the three figures.
Pigment scores were determined by visually matching individual kernels against a set o standard kernels that ranged from 1 (near colorless) to 20 (full pigment). Individual plant pigment-score profiles are distributed across the spread sheet in the three figures. In all 75 plants the earliest pollen sampled from

the upper tassel branches showed the greatest silencing expressions when compared with late pollen from the same plant.
Fig. 1 represents plants that as seedlings received mostly $32^{o}$ conditions with pigment scores falling largely in the lower half of the pigment scoring range (most silencing).
Fig. 3. Shows plants that received $22^{o}$ conditions as seedlings gave pigment scores represented in the lower half of the scoring range (least silencing).
Fig. 2. Plants that received combinations of $32^{o}$ and $22^{o}$ for various cycles over a six-day period show scores that range across the upper and lower half of the pigment range.
The ovals in the three figures show how the pigment scores were biased by the early conditions experienced by the seedlings.
The dynamics of paramutation responses, in single plants, to PT conditions becomes more apparent in the spread sheet display of pigment profiles figures 1-3. This dynamic was lost in the pooled means of Table 1,.
Within the second and third week a critical period of development was identified by pigment responses from pollen test crosses of the mature plants exposed to earlier temperature and LD cycles. In Fig. 2 temperature shifts for as few as two cycles into and out of $22^{o}$ and $32^{o}$ can cause differences in the level of *R* silencing. The variation in the profiles of the three figures helps to understand why the continuum of pigment expression was difficult to interpret from field grown material when, for 50 years, only single pollinations from single plants were examined. The variation in a single tassel was interpreted as stochastic when reported as pooled means from single pollen samples from single plants.
The superscript numbers over each profile indicates the interval in days between the early and late pollen samples from the same plant. The number indicates that the expression gradient between upper and lower tassel branches from plant to plant could be quite variable suggesting that the rate of silencing in the tassel was determined by early conditions but at the same time variable from plant to plant.
The three figures provide 7500 epialleles, 100 from each plant, 50 from early pollen and 50 from late. Only two days of pollen are represented in each profile. Since pollen is shed over a period of eight days there is at least six times more variation available from the 75 plants sampled. Thus under the conditions of paramutation an immense amount and range of ***R*** variation is available for natural selection for a single generation, variation that can be incremented the following generation.
The epigenetic nature of silencing means that the expression-states can be incremented each generation at the lower temperature or achieved within one or two LD cycles under the higher temperature. This means that temperature variation early in plant development can have a significant role in determining variable heritable phenotypes. What is remarkable is that the variation is PT regulated. This means that pigment will be more intense at the lower temperature and more UV will be filtered by the red pigment. 75 profiles of epigenetic *R* expression-states (epialleles) are determined by PT conditions early in seedling development but expressed at maturity in pollen testcrosses following gametogenesis. It is remarkable that the silencing is orchestrated by a TE fragment in the promoter of an *R* inverted repeat and amplified by R duplications on the homologous chromosome in the heterozygote *R/Rst*.
The TE silencing mechanism has taken on the role of modulating gene expression in response to PT (environmental conditions) at a critical stage of development. The heritable epigenetic nature of the change in expression permits the inference that TEs can have a role providing diverse expression-state for natural selection.

References in support of our interpretation (see also the accompanying note):

Kermicle, in a review of paramutation, suspected involvement of *doppia* in paramutation. The reports on tandem ***r*** repeats in the *Rst* haplotype made it possible to consider that the role of *doppia* on silencing was amplified by duplications on the homologous chromosome. Kermicle, J., 1996 Paragenetic Modifications in Maize. In Epigenetic Mechanisms of Gene Regulation (ed. V. E. A. Russo, R. A. Martiensses, and A. D. Riggs.) *Cold Spr. Hbr. Lab. Pr*

That *R* paramutation silencing involved the silencing machinery was inferred from:
Sidorenko, Lyudmila and Vicki Chandler. 2008 RNA-Dependent RNA Polymerase Is Required for Enahancer-Mediated Transcriptional Silencing Associated With Paramutation. Genetics . 180: 1983-1993.
Chandler, V. and Maike Stam, 2004 Chromatin Conversations: Mechanisms and Implications of Paramutation. Nature Reviews, GENETICS. **5:** 534-543.

The interpretation of paramutation/silencing has been based on three extensive reviews of epigenetic silencing associated with transposable elements (TEs).
Fedoroff, Nina. 2000 Transposons and genome evolution in plants. PNAS Vol. 97, No. 13,7002-7007.
Feschotte, C. and Ellen J. Pritham, 2007 DNA Transposons and the Evolution of the Eukaryotic Genomes. Ann. Rev. Genet. 41: 331-368.
Slotkin, R. Keith, Robert Martienssen, 2007. Transposable elements and the epigenetic regulation of the genome. Nature Reviews GENETICS Vol. 8, 272-285.

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**Comparison of Pigment Scores of Earliest and Latest Pollen Samples**

| | | Early | Late | Growth | |
|---|---|---|---|---|---|
| **Part A** | | **Pollinations** | **Pollinations** | **Chamber Environment** | |
| **Line** | **n** | **Pooled Means** | | **Day 1-15** | **Day 16-21** |
| **48** | **8** | **8.0 ±3.4** | **9.3 ±5.0** | **LL 22$^o$** | **2 LD 22$^o$-4 LL32$^o$** |
| **47** | **7** | **10.3 ±2.0** | **13.5 ±3.2** | **LL 22$^o$** | **2 LD 22$^o$-4 LL 22$^o$** |
| **46** | **6** | **8.7 ±3.1** | **10.9 ±2.9** | **LL 22$^o$** | **6 LL 22$^o$** |
| **49** | **9** | **9.3 ±1.7** | **12.5 ±1.1** | **LL 22$^o$** | **4 LD 22$^o$-2 LL 22$^o$** |
| **50** | **6** | **9.9 ±4.0** | **14.4 ±3.3** | **LL 22$^o$** | **4 LD 22$^o$-2 LL 32$^o$** |
| **45** | **8** | **9.8 ±2.6** | **13.4 ±2.1** | **LL 22$^o$** | **6 LD 22$^o$** |
| **Part B** | | | | **Day 1-10** | **Day 11-15** |
| **30** | **6** | **3.3 ±.6** | **3.2 ±1.5** | **LL 32$^o$** | **5 LL 32$^o$** |
| **31** | **7** | **8.2 ±2.3** | **9.6 ±3.8** | **LL 32$^o$** | **5 LL 22$^o$** |

| | | | | | |
|---|---|---|---|---|---|
| **26** | **5** | **8.7 ±3.5** | **9.2 ±3.2** | **LL 32°** | **5 LD 32°** |
| **27** | **7** | **9.3 ±3.2** | **10.7 ±2.5** | **LL 32°** | **5 LD 22°** |
| **28** | **7** | **9.2 ±3.8** | **9.2 ±3.6** | **LL 32°** | **2 LD 32°-3 LL 22°** |
| **29** | **4** | **8.8 ±1.2** | **10.8 ±3.2** | **LL 32°** | **2 LD 22°-3 LL 22°** |

**Table 1** correlates the effect of seedling photo thermal environments with phenotypic *R* paramutation pigment scores from pollen test crosses made from upper (early) and lower (later) tassel branches at maturity. Multiple pollen test crosses were made from the same plant within a period of eight days.
**Part A**. Seedlings were started in continuous light (LL) at 22°, Days 1-15, as indicated in column five. On days 16-21, seedlings were treated to a variety of LD (Light/Dark) LL (continuous light) and temperature cycles as indicated in column six.
**Part B.** Seedlings were started in continuous light (LL) at 32°, days 1-10, as indicated in column five. On Days 11-15 seedlings were treated to a variety of LD, LL and temperature cycles as indicated in column six. The lines of column one represent siblings whose treatments differed as indicated in column siz. The number of plants tested in each line is given in the second column. The range of pigment scores from single plants is presented in Figures 1-3 as pigment score profiles for each of the 75 plants sampled.

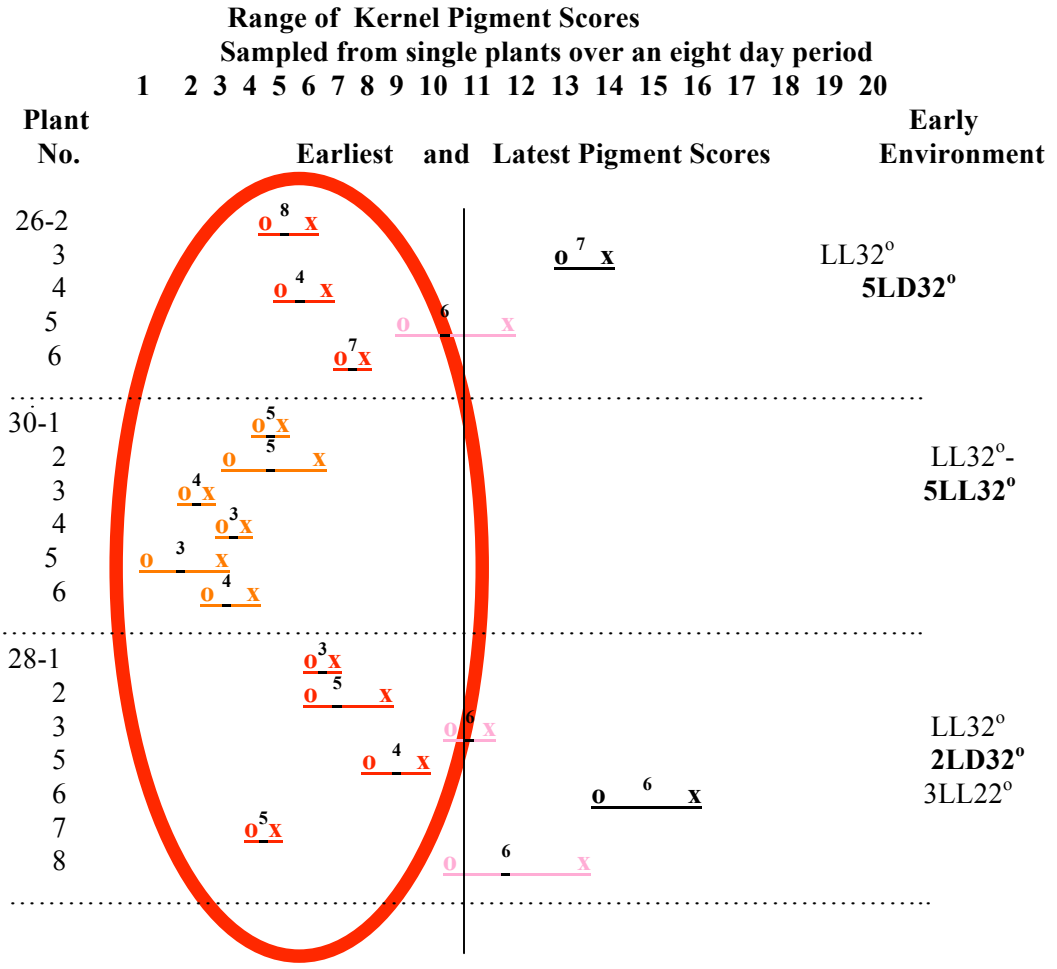

**Figure 1.** Three lines of sibling plants, 26, 30 and 28, were started in 32° LL conditions, days 1-10, then shifted to conditions, in bold type, days11-15, the critical period. Symbols o and x represent means of 50 kernels from early and late pollen test cross scores, respectively. The superscript over each profiele indicates the interval in days between early and later pollen collections

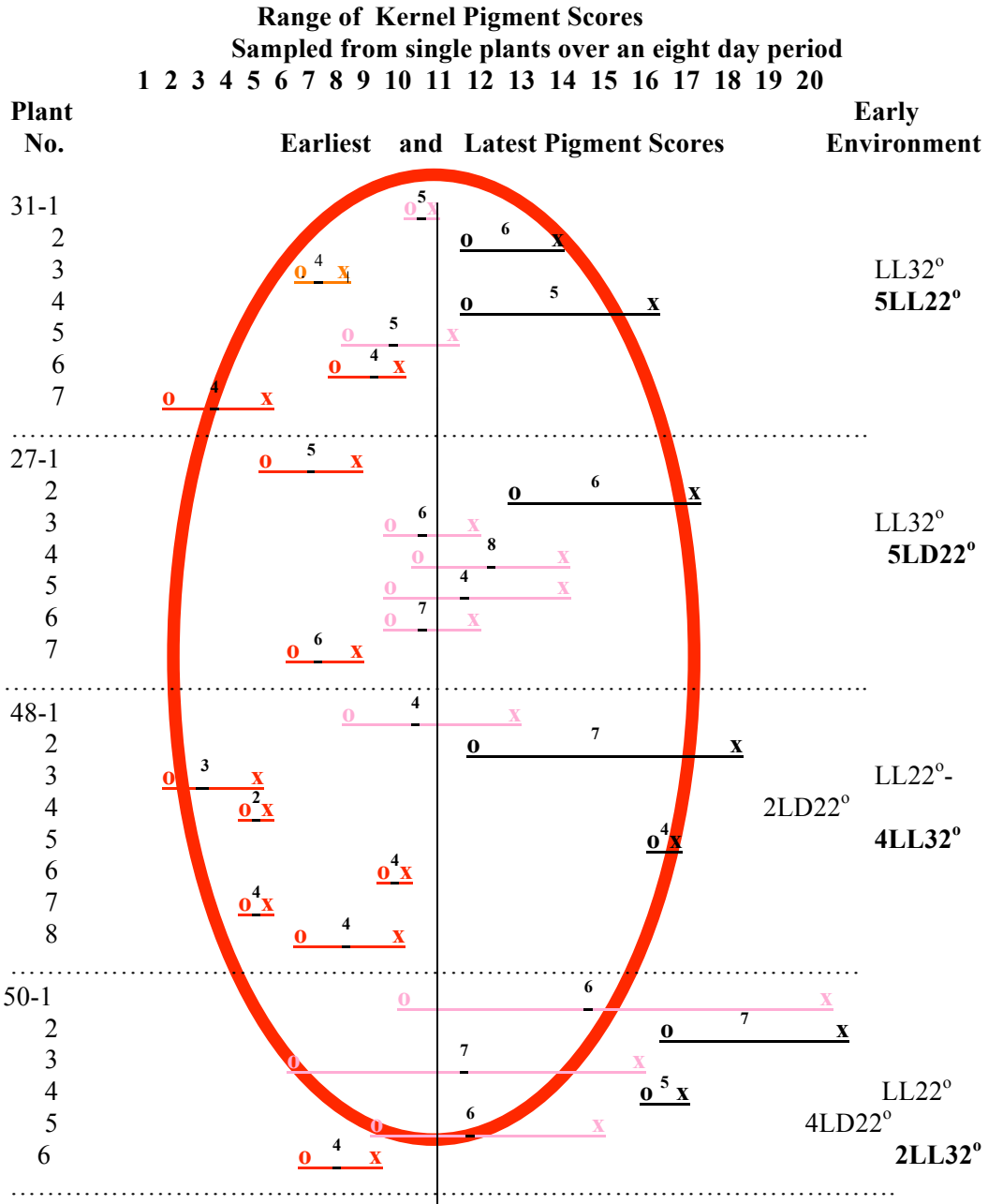


**Figure 2.** Lines 31 and 27 were started in 32°LL conditions, days 1-10, then transferred to 5LL22° and 5LD,respectively, days 11-15. Lines 48 and 50 were started in 22°LL conditions, days 16-17, line 48 received two LD22° cycles, then was transferred to 4LL32° cycles. Days 16-19 line 50 received 4LD22o cycles then was transferred to 2LL32° cycles, days 20-21. Symbols o and x represent means of 50 kernels from early and later pollinations, respectively. The superscript indicates the interval in days between early and late pollen collections.

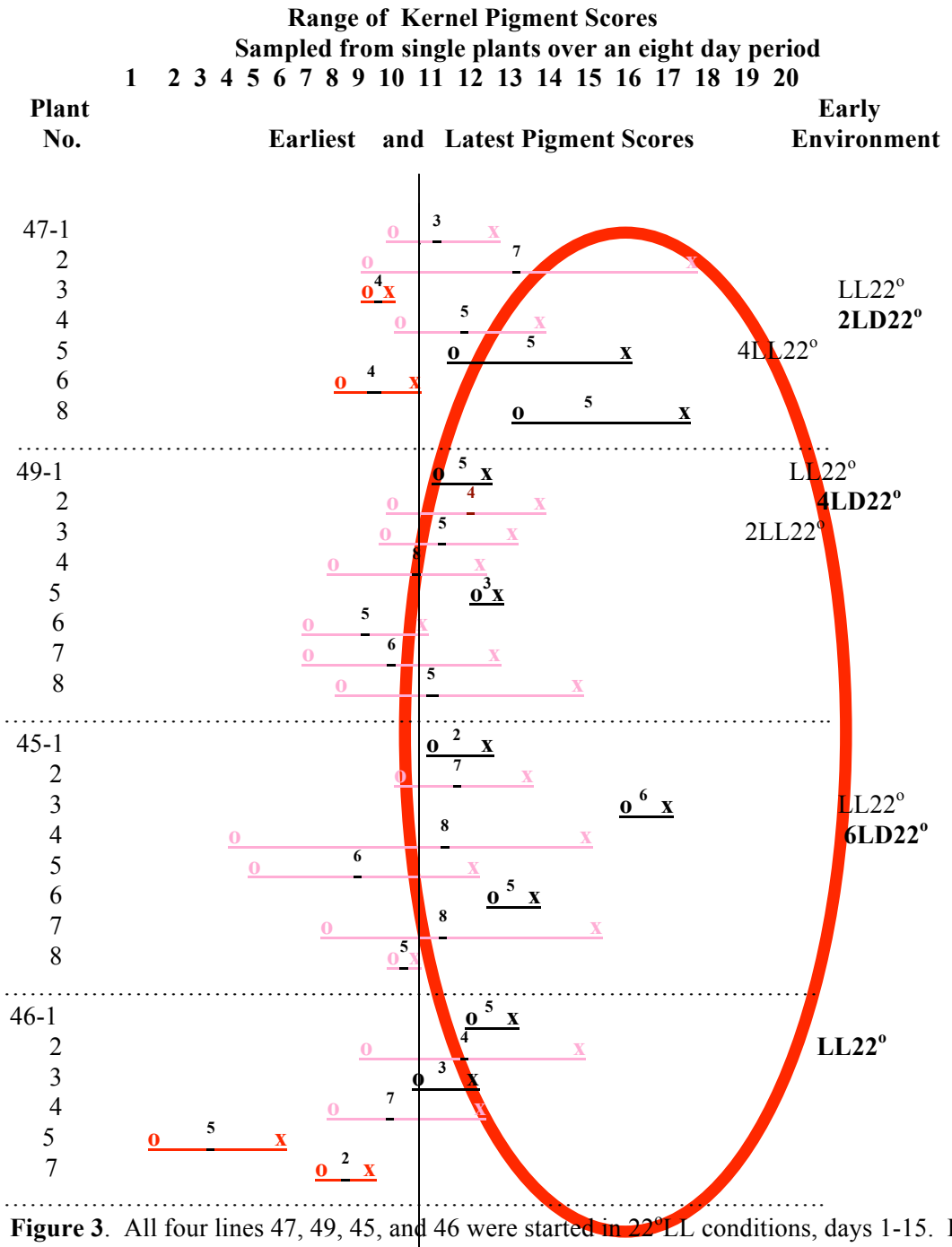


**Figure 3**. All four lines 47, 49, 45, and 46 were started in 22°LL conditions, days 1-15. During the critical period, days 16-21 seedlings were shifted to the conditions and numbers of cycles listed under column Early Environment. Symbols o and x represent means of 50 kernels from early and late pollinations, respectively. The superscript indicates the interval in days between early and late pollen collections.