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1. En at the mutable locus, a_1^m .

The identification of the regulatory element En at a mutable locus can be facilitated by the diversity of the forms of mutability that exist. Among the diverse mutable alleles, one that mutates to a readily identifiable mixture of pale and deep purple dots a_1^m (p and p) is available. Colorless forms, noted as $a_1^m(r)$, also exist and these respond to the presence of En in a predictably recognizable manner (Peterson, Genetics 1961). When a heterozygote is made between a_1^m (p and p) and $a_1^m(r)$ the resulting expression is a very heavily mutable form showing the effect of En on $a_1^m(r)$. Proof that it is the En of the a_1^m (p and p) allele that is causing the mutability is obtained by testcrossing the heterozygote (by a_1^{sh}/a_1^{sh}). The resulting progeny shows the separation of kernels -- 1/2 of which are pale and purple dotting and 1/2 are colorless, $a_1^m(r)$, since the En is coupled with the a_1^m (p and p) allele. A small percentage of exceptions appear and these will be discussed in the next section.

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2. Changes at the a_1^m (p and p) allele: The status of En.

Among the progeny arising from testcrosses (by a_1^{sh}/a_1^{sh}) of the a_1^m (p and p) allele, stable non-dotting pale types ($a_1^p(nr)$) and colorless types ($a_1^m(nr)$)* are observed. It has previously been reported (Peterson, 1961 Genetics) that the colorless types do not respond to the presence of En and are designated $a_1^m(nr)$. Similarly, the pales do not respond to the presence of En and are therefore nr (non-responding) types. If these derivatives are canvassed for the presence of En, it is found that they invariably do possess En. In crosses of $a_1^p(nr)$ and $a_1^m(nr)$ by $a_1^m(r)$ (Cross #1), mutability is observed in the heterozygote. In testcrossing these heterozygotes -- $a_1^p(nr)*/a_1^m(r)* \times a_1^{sh}/a_1^{sh}$ (Cross #2) -- a variable percentage of mutable kernels results. These mutable kernels represent the effect of En on the $a_1^m(r)$ allele. This would indicate that the nr kernels possess En in coupling $a_1^p(nr)$ En and the distance between $a_1^p(nr)$ and En is proportional to the frequency of mutable kernels that arose from Cross #2**.