A STABLE SOMATIC MUTATION TO COLORLESS FROM VARIEGATED PERICARP IN MAIZE

R. ALEXANDER BRINK¹

*Department of Genetics, University of Wisconsin
Madison, Wisconsin*

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COLORLESS pericarp and cob is one of the least frequent mutant phenotypes among the offspring of maize plants carrying the somatically unstable variegated pericarp allele, P^{vv} . BRAWN (1956), for example, found only eight mutations to colorless pericarp and cob in contrast to 125 to red pericarp and cob (P^{rr}) among 4575 offspring from the mating homozygous variegated times standard colorless ($P^{vv} P^{vv} \times P^{ww}$) in the W22 inbred line with which we are here concerned.

Tests show that some apparently colorless mutants actually are very low grade variegateds, in that they occasionally show one or more, usually very small, colored stripes in the pericarp. Evidently others are highly stable for colorless. The present study is concerned with a mutant of this latter type. The case is of interest in demonstrating that Modulator, which ordinarily is a readily transposable factor, may become firmly bonded at the P locus, resulting in the formation of an isoallele of P^{ww} , the commonly occurring highly stable colorless form.

ORIGIN OF THE COLORLESS MUTANT

The colorless mutant, termed P_{m-1}^{ww} , appeared originally as a 43-kernel patch on an otherwise typical medium variegated pericarp ear bearing about 320 seeds. The plant in question was heterozygous for colorless pericarp, red cob (P^{wr}) and belonged to the first backcross generation of a variegated stock being graded to a commercial-type P^{wr} inbred line, known as W22. The mutant ear, in turn, was pollinated by W22.

The original mutant kernels were weakly colored in the silk attachment region, rather than entirely colorless. This pigmentation is attributable to "dark crown," a rather common, nonheritable manifestation of P^{vv} in the pericarp epidermis (EMERSON 1917, 1929). It did not recur among the descendants of P_{m-1}^{ww} . Seventeen plants were reared from mutant seeds, all of which had entirely colorless pericarp. Pollen from 16 of these individuals was placed on W22 ear shoots, and the plants themselves were pollinated with a homozygous P^{vv} stock. The resulting progenies showed that two equally frequent genotypes were represented in the original mutant patch of kernels. Seven of the individuals gave about equal numbers of

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offspring with variegated or red cob in the testcross with $P^{vv}P^{vv}$, and the same proportion with light red ($P^{wv}P^{wr}$) or red ($P^{wr}P^{wr}$) cob in the mating with W22. Evidently these seven plants were heterozygous $P_{m-1}^{wv}P^{wr}$. The remaining nine sibs proved to be $P^{wr}P^{wr}$ by the same tests. Twenty-two plants grown from seed on the nonmutant portion of the foundation ear were distributed as follows: 14 medium variegated, one light variegated, two self color, and five colorless pericarp, red cob. This is a result expected from a heterozygous $P^{vv}P^{wr}$ plant. One may conclude from these data that the aberrant patch of kernels was due to a somatic mutation of the P^{vv} allele to colorless, occurring fairly early in the ontogeny of the ear. The epidermal layer of the pericarp was not involved in the change of P^{vv} to P_{m-1}^{wv} , as would be expected in view of the separate origin of this structure; it happened to show the so-called dark crown pattern as a result of an independent mutation in this superficial tissue.

PROBLEMS AND PROCEDURES

The first question to which an answer was sought was whether the exceptional stability of the mutant rested upon a deficiency at the P locus, giving a null effect for pigmentation. Since all known deficiencies in maize either are not normally transmitted through the pollen or are lethal when homozygous, these criteria were applied in the present case.

The second point to which attention was directed was the degree of stability of the colorless mutant. This led to a search among some 4300 descendants of the mutant for even very small colored sectors on the ears and for plants arising from germinal mutations. Such mutations were looked for in both P_{m-1}^{wv} homozygotes and heterozygotes, and in families varying in genetic background.

The third main problem was concerned with the relation of Modulator (Mp) to the colorless mutation. Earlier evidence has led to the view that the variegated pericarp allele is a dual structure comprising the P^{rr} gene and Modulator, the latter suppressing the pigment-producing action of the former when the two elements are in conjunction at the P locus. Mutations of variegated to self color (P^{rr}), which occur with frequencies of 3–10 percent in different $P^{vv}P^{wr}$ strains, were shown to involve loss of Mp from the P locus by selective transposition of the element, usually to another chromosome site (BRINK and NILAN 1952). What is the colorless mutant in terms of P^{rr} and Mp ? Is P_{m-1}^{wv} a counterpart of ordinary P^{vv} in which Modulator merely has become fixed at the P locus, or has Mp also undergone a qualitative change? Another possibility was that the colorless phenotype was due to mutation of P^{rr} to the stable P^{wv} colorless allele. This is not a frequent event, but it has been observed in an experimental culture (BRINK 1929). Had it been found that the mutation was due to a change in P^{rr} , then the observed persistence of Modulator at the P locus would have had to be accounted for.

Advantage was taken in this part of the study of the fact that Modulator promotes the Dissociation (Ds) type of chromosome breakage (BARCLAY and BRINK 1954). Populations may be scored by the Ds test for distribution of Modulator in

the absence of an overt effect of the latter element at the P locus. Also the Ds chromosome breakage pattern varies both with the dosage and qualitative nature of the co-factor present in the genome, Activator, as McCLINTOCK (1951) first showed, or Modulator, as BARCLAY and BRINK (1954) subsequently observed. It was thus possible to characterize Modulator in the P_{m-1}^{ww} mutant by a criterion independent of the phenotypic effect of Mp at the P locus. The procedure followed in the Ds tests will be described later in the report.

Additional evidence on the relation of Modulator to the colorless mutant was afforded by observing the effect of the P_{m-1}^{ww} allele in heterozygotes with ordinary variegated pericarp. WOOD and BRINK (1956) recently have shown that the pronounced decrease in mutability of the P^{vv} allele in P^{vv} homozygotes, as compared with $P^{vv}P^{wr}$ heterozygotes which EMERSON (1929) first noted, can be explained in terms of dosage action of Modulator. A comparison was made in the present study of the variegation grades of $P_{m-1}^{ww}P^{vv}$ and $P^{wr}P^{vv}$ sib ears resulting from $P_{m-1}^{ww}P^{wr} \times P^{vv}P^{vv}$ matings to determine whether the Mp component of P_{m-1}^{ww} retained the capacity to reduce mutability of P^{vv} .

EXPERIMENTAL RESULTS

Transmission of P_{m-1}^{ww}

The mutant colorless allele is transmitted normally through both the female and male gametophytes. Among 1278 offspring from $P_{m-1}^{ww}P^{wr} \text{ } \text{f} \times P_{m-1}^{ww} \text{ } \text{m}$ matings, for example, 658 had red cobs ($P^{wr}P^{ww}$) and 620 had white cobs ($P_{m-1}^{ww}P^{ww}$). The reciprocal type of cross gave 1693 plants with light red cobs and 1695 with colorless cobs, plus one exceptional individual (see later). The deviations from expectation on a monohybrid basis are well within sampling limits in both these distributions.

Homozygous P_{m-1}^{ww} plants are indistinguishable in plant and ear size from P^{wr} individuals of the same genetic background.

There is no evidence, therefore, that P_{m-1}^{ww} adversely affects development either of the gametophytes or of the sporophyte, as would be expected if it represented a deficiency for the P locus.

Stability

The uniformly colorless pericarp resulting from the first few testcrosses with the P_{m-1}^{ww} mutant showed that the latter was remarkably stable, as compared with ordinary variegated. Additional data on stability then were sought from larger scale experiments.

A total of 4330 plants were reared which were heterozygous for P_{m-1}^{ww} and one or the other of the standard colorless alleles, P^{wr} or P^{vw} . Among these plants, 553 occurred in families from recurrent backcrosses to the W22 inbred line. The remainder were derived from outcrosses of W22-type, $P_{m-1}^{ww}P^{wr}$, individuals to one or another of three uniform, but unrelated P^{vw} stocks, one of which was the

4Co63 inbred line. The latter was known to give particularly strong expression (high somatic mutation rate) of variegated pericarp when an ordinary P^{vv} allele was introduced into it. In addition to these heterozygotes, 206 plants homozygous for P_{m-1}^{ww} also were scored. The ears from all these plants were examined minutely under $2\times$ magnification for red sectors, the scoring usually being checked by a second person.

The pericarp of all 4330 plants carrying the P_{m-1}^{ww} allele, with one exception, was colorless. One ear occurred in which there were numerous, very fine, red stripes. The pattern in this case was not typical of any of the commonly encountered phases of variegated pericarp in that all the colored sectors were narrow, rather than of variable widths.

The exceptional, lightly striped, ear occurred in a highly uniform family, 38-101C, resulting from a $P^{ww} \text{♀} \times P_{m-1}^{ww} P^{wr} \text{♂}$ mating. Since the staminate parent was the heterozygote in the testcross, it is possible that the aberrant individual was a contaminant. This explanation cannot be excluded, because distinctive marker genes were not present and there were other variegated plants in the vicinity from which pollen might have been derived at the time the mating in question was made. It is quite unlikely, however, that the variegated individual resulted from stray pollination. The ear was typical in size and form for the uniform family in which it occurred. Secondly, the variegation pattern was unusual, and not of the sort expected from an ordinary P^{vv} allele, even if accompanied by one or more transposed Modulators. A regular effect of the latter is to suppress most late occurring mutations of P^{vv} to P^{rr} , which are expressed as narrow sectors in the pericarp. Uniqueness of the color pattern and conformity of the ear to family type point to the conclusion that the exceptional plant carried a mutation of the P_{m-1}^{ww} allele to an uncommon form of variegated pericarp. Further tests could not be made because the mutant plant, like its sibs, was open pollinated in a section of the nursery in which heavy contamination from other stocks would be expected.

Modulator as a component of the P_{m-1}^{ww} allele

Tests will now be described the results of which show that Modulator is a component of P_{m-1}^{ww} , and that the latter regularly assorts as a P allele.

Plants with a W22 genotypic background and heterozygous $P_{m-1}^{ww} P^{wr}$, were pollinated by a standard colorless (P^{ww}) inbred stock carrying the marker genes wx (waxy endosperm), γ (white endosperm), and W^c (dominant white capping when Y is present). An occasional deep yellow (non- W^c) kernel, probably the result of contamination, was removed from the resulting ears. The F_1 kernels were homozygous for the recessive genes, c and r , for colorless aleurone. They were planted in an isolated detasseling plot in which the pollen source was $wx \gamma P^{ww} R C$ plants, either homozygous or heterozygous for Dissociation (Ds). Ds in this stock was located in the standard position (McCLINTOCK 1953), proximal to the C locus on the short arm of chromosome 9. Had Ds not been present the kernels

formed on the detasseled plants all would have been self-colored (or dark mottled) as a result of fertilization of the duplex *cc rr* fusion nuclei of the female gametophytes by *C R* sperm. The *C* chromosome in a large proportion of the male gametes, however, carried *Ds* in a proximal position. This factor, in the form here used, promotes chromosome breakage with high frequency at the *Ds* locus if either Activator (McCLINTOCK 1951) or Modulator (BARCLAY and BRINK 1954) is present in the genome. Such breakage in the present case would give rise to acentric chromosome fragments bearing the *C* gene, which would then be lost to the nucleus. The result of a *C*-loss, in mitotically active endosperm tissue, is a lineage of cells in which the aleurone phenotype is colorless rather than colored. Since such *C* to *c* mottling would occur only in kernels formed by plants carrying Modulator, the procedure serves as a test for distribution of plants carrying *Mp* in the population. *R*-mottling did not interfere significantly in the present material with identification of the two expected classes of plants, those forming kernels with and those without *C* to *c* changes.

A further check on contamination was afforded in this generation by the presence of the *wx* and *γ* markers. Any ear which did not segregate for both these genes could not have been of the designated parentage. No such contaminants were found, the prior screening based on dominant white capping (*W^c*) apparently having removed all of them.

The mature ears borne by the pistillate plants in the detasseling plot were harvested and separated into two groups, red cob and colorless cob. Each ear then was examined for the presence of kernels showing *C* to *c* mottling in the aleurone, the specific reaction denoting presence in the genome of Modulator. The distributions for cob color and *Mp* are summarized in Table 1.

TABLE 1

The distribution of cob color and C to c aleurone mottling resulting from Ds action among the

offspring from $P_{m-1}^{ww}P^{wr} \times P^{ww}$ matings

Family	Red cob		Colorless cob	
	Mottled	Nonmottled	Mottled	Nonmottled
38-148	0	175	161	0
-149	0	95	107	0
-150	0	81	87	0
-151	0	106	99	0
-152	0	95	105	0
-153	0	60	59	0
-154	0	59	69	0
-155	0	61	50	0
-156	0	115	88	0
-157	0	93	116	0
-158	0	116	101	0
-159	0	107	94	0
-161	0	57	63	0
Totals	0	1220	1199	0

The numerical results show that the colorless mutant behaves as an allele of P^{wr} , as expected from the outcome of earlier tests. Secondly, the 1220 red cob (P^{wr}) segregates, without exception, were negative for C to c mottling, whereas all 1199 colorless cob offspring were positive for this character. Thus, there was no recombination in the 2419 plants tested between the colorless pericarp mutant phenotype and the capacity to induce Ds chromosome breakage. The regular assortment of Modulator with colorless shows that Mp is "fixed" at the P locus, and may be assumed to be a component of the P_{m-1}^{ww} allele.

The C to c aleurone mottling pattern associated with the P_{m-1}^{ww} allele

It is a question of interest whether the Modulator components of P_{m-1}^{ww} and the P^{vv} parent allele are alike in number and kind. The Ds chromosome breakage patterns which they incite provide a basis for comparing them which is independent, presumably, of their local action on the P phenotype.

Plants homozygous for P^{ww} and $C Ds$, the latter element again being in standard position proximal to C , were pollinated by (1) $P^{vv}P^{vv}$ and (2) $P_{m-1}^{ww}P^{wr}$ individuals which conformed closely in residual genotype to the W22 inbred line. The P^{vv} allele was from the stock in which the colorless mutant occurred. The triploid endosperm genotypes expected from these matings are as follows:

Mating 1. $C Ds, P^{ww} \varnothing \times c ds P^{vv} \delta = C Ds/C Ds/c ds, P^{ww}P^{ww}P^{vv}$

Mating 2. $C Ds, P^{ww} \varnothing \times c ds P_{m-1}^{ww}P^{wr} =$ (a) $C Ds/C Ds/c ds, P^{ww}P^{ww}P_{m-1}^{ww}$
(b) $C Ds/C Ds/c ds, P^{ww}P^{ww}P^{wr}$

The (b) kernels from mating 2 lack Modulator, and so would be expected to have self-colored aleurone. Mating 1 and the (a) kernels from mating 2, on the other hand, should show C to c mottling according to the respective effects on Ds chromosome breakage of the Modulator components of the P^{vv} and P_{m-1}^{ww} alleles. The mottling in these cases would ensue from "double breaks," simultaneous or tandem, at Ds , whereby both the C genes initially present in the nucleus would be lost on acentric fragments.

The results of the two sets of testcrosses are presented in Figure 1. The middle column of self-colored kernels is the 2(b) class mentioned above. The two columns at the left show the pattern of C to c aleurone mottling resulting from action on Ds of the Modulator present in the parent P^{vv} allele. The two right hand columns illustrate the effect of Mp in the P_{m-1}^{ww} mutant. It is evident from the photograph that there is no discernible difference in the mottling patterns in the two sets of kernels.

Two conclusions follow from these facts: (1) The dosage level of Modulator is the same in the P^{vv} and P_{m-1}^{ww} alleles and (2) The Mp units in the two cases are indistinguishable by this test.

FIGURE 1.—The C to c aleurone mottling patterns resulting from Ds chromosome breaks in response to the presence of a standard medium variegated pericarp allele, P^{vv} , (left), and the colorless pericarp mutant, P_{m-1}^{wv} , (right). The kernels in the middle column lack Modulator, and thus show no aleurone mottling associated with Ds chromosome breakage.

*Effect of P_{m-1}^{ww} on expression of variegated in heterozygotes with
an ordinary P^{vv} allele*

Each of the P^{vv} alleles in a homozygous $P^{vv}P^{vv}$ plant tends to reduce mutability to self-color of the other. This usually results in a smaller total of such mutations in homozygotes than in plants heterozygous for variegated and a standard stable allele, such as P^{wer} . WOOD and BRINK (1956) showed that Modulator, when transposed from the P locus to one or another chromosome site, has the same effect on mutation of P^{vv} in $P^{vv}P^{wer}$ plants as each of the P^{vv} alleles in a $P^{vv}P^{vv}$ individual has on mutation of its homologue. Thus the mutation rate of P^{vv} to P^{rr} is a function of Mp dosage.

A test was made in the present study for an effect of P_{m-1}^{icw} on the mutation frequency of an ordinary P^{vv} allele in $P_{m-1}^{icw}P^{vv}$ heterozygotes comparable to the reciprocal transallelic action previously observed in $P^{vv}P^{vv}$ homozygotes. $P_{m-1}^{icw}P^{wr}$ plants in an inbred W22 stock were pollinated by a $P^{vv}P^{vv}$ individual carrying a P^{vv} allele known to give the conventional medium variegated pattern in heterozygotes with P^{icr} . The two classes of offspring from this mating, $P^{vv}P^{icr}$ and $P^{vv}P_{m-1}^{icw}$, are readily separable in that cob color is red in the first and very lightly variegated in the second (cf. middle ears in Figure 2).

Photographs of the two classes of testcross ears are shown in Figure 2. The pair of $P^{vv}P^{icr}$ ears with red cobs, at the left, are typical medium variegateds, as expected. The two ears at the right, with weakly colored cobs, and carrying the same P^{vv} allele but, in this case, heterozygous for the P_{m-1}^{icw} mutant rather than P^{icr} , show markedly fewer mutations to P^{rr} . Presumably the reduction is a result of the stabilizing action of the Modulator component of P_{m-1}^{icw} on the P^{vv} allele. Al-

FIGURE 2.—The depressing effect of the colorless pericarp mutant allele, P_{m-1}^{icw} , on mutation of a standard medium variegated pericarp allele in $P_{m-1}^{icw}P^{vv}$ heterozygotes (right) as compared with $P^{icr}P^{vv}$ heterozygotes (left).

though a quantitative comparison was not made in this stock, it is evident that the amount of stabilization of P^{vv} induced by P_{m-1}^{wv} is of the same order of magnitude as that observed in light variegateds (cf. WOOD and BRINK 1956).

The ears in six families from $P^{wv}P_{m-1}^{wv} \times P^{vv}P^{vv}$ matings were divided according to cob color and then scored for variegation grade by matching with a standard set of P^{vv} ears, in which class 1 represented the least, and class 4 the largest degree of pigmentation. The results are summarized in Table 2.

TABLE 2
Grade of pericarp variegation in $P_{m-1}^{wv} P^{vv}$ and $P^{wv} P^{vv}$
individuals from $P_{m-1}^{wv} P^{wv} \times P^{vv} P^{vv}$ matings

Family	Parents	Cob color	No. individuals in variegation class				Av. variegation grade
			1	2	3	4	
63-159	Inbred W22 $\times$ 63-7-10	red	1	34	17	6	2.48
63-160	Inbred W22 $\times$ 63-7-10	red	1	22	28	5	2.66
63-163	63-27-4 $\times$ 63-7-10	red	0	14	12	2	2.57
	63-27-4 $\times$ 63-7-10	var.	1	38	4	0	2.07
63-164	63-27-7 $\times$ 63-7-10	red	2	17	20	0	2.46
	63-27-7 $\times$ 63-7-10	var.	3	27	2	0	1.97
63-333	63-27-5 $\times$ 63-7-6	red	1	29	5	0	2.11
	63-27-5 $\times$ 63-7-6	var.	1	33	0	0	1.97
63-335	63-27-8 $\times$ 63-7-6	red	7	27	12	0	2.11
	63-27-8 $\times$ 63-7-6	var.	3	40	0	0	1.93
63-336	63-27-11 $\times$ 63-7-6	red	1	29	14	0	2.30
	63-27-11 $\times$ 63-7-6	var.	6	34	2	0	1.90
63-340	63-27-16 $\times$ 63-7-6	red	3	15	10	3	2.42
	63-27-16 $\times$ 63-7-6	var.	1	31	2	0	2.03

Averages: red cob — 2.33
variegated cob — 1.98

The first two entries in the Table (families 63-159 and 63-160) represent control matings in which pollen from $P^{vv}P^{vv}$ plant 63-7-10 was applied to the original W22 P^{wv} inbred line itself. All these individuals, therefore, are of the $P^{vv}P^{wv}$ genotype. The two following families, 63-163 and 63-164, had the same pollen parent, but the pistillate parents were $P_{m-1}^{wv}P^{wv}$ individuals of W22 type. A sib $P^{vv}P^{vv}$ plant, 63-7-6, was used as the pollen parent on the inbred $P_{m-1}^{wv}P^{wv}$ individuals in the remaining families.

The scores for average variegation grade show that for all six segregating families fewer somatic mutations of P^{vv} to P^{rr} occurred in the $P_{m-1}^{wv}P^{vv}$ individuals than in their $P^{vv}P^{wv}$ sibs. The mean variegation grades of the two classes of ears are 1.98 and 2.33, respectively. These data show that the P_{m-1}^{wv} mutant regularly increases the stability of P^{vv} in heterozygotes. The similarity of the effect to that of two P^{vv} alleles on each other in $P^{vv}P^{vv}$ plants as previously analyzed by WOOD and

BRINK (1956), suggests that it is Modulator action which is involved, and that Mp has the same potency in this respect in the P_{m-1}^{ww} mutant as in P^{vv} .

DISCUSSION

The simplest view which accords with the experimental facts presented is that P_{m-1}^{ww} differs from the variegated pericarp allele from which it arose only in the physical association of Modulator and the P^{rr} gene. Both alleles are combinations of Mp and P^{rr} , and it may be assumed that the large difference in stability between them is a reflection of the firmness with which Modulator is bonded at the P locus in the two cases.

The Modulator components of P_{m-1}^{ww} and P^{vv} were found to correspond closely in three respects. Both fully suppress the pigment-producing action of the P^{rr} gene, and both incite the same pattern of Ds chromosome breakage. Likewise, in $P_{m-1}^{ww}P^{vv}$ heterozygotes, P_{m-1}^{ww} markedly depresses the frequency of somatic mutation of the P^{vv} homologue to P^{rr} , just as P^{vv} has been observed to do, reciprocally, in $P^{vv}P^{vv}$ homozygotes (WOOD and BRINK 1956).

P_{m-1}^{ww} and P^{vv} , on the other hand, are quite unlike in stability. No mutations to self-color were observed among 4742 P_{m-1}^{ww} gametes tested. It is estimated from unpublished data (BRAWN 1956) that an equal number of germ cells from medium variegated pericarp plants of similar breeding would have included about 90 such mutants. The single germinal mutation observed in the P_{m-1}^{ww} stocks involved a change from colorless, not to self-red, but to a fine grade of variegation.

Estimates of stability in somatic tissue likewise disclose a wide difference between P_{m-1}^{ww} and P^{vv} . Medium variegated ears ($P^{vv}P^{wr}$) in the W22 inbred line, carrying about 250 kernels each, average approximately five mutations of P^{vv} to P^{rr} per ear exceeding one eighth of the kernel circumference, the minimum size conveniently recorded. Among the 4330 heterozygous P_{m-1}^{ww} ears examined, 4229 showed no mutations to P^{rr} . The remaining ear, as mentioned earlier, showed only very fine red stripes, and probably represented a germinal mutation in the preceding generation of P_{m-1}^{ww} to an uncommon form of variegated pericarp.

A well recognized peculiarity of Modulator as a genetic unit is the capacity to undergo transposition from one location to another in the genome. Apparently the element can occupy a wide variety of sites (BRINK and NILAN 1952; BRINK 1954; N. W. VAN SCHAIK 1957). The present study shows that the firmness with which the element is held at or near a given locus varies greatly also. What this latter variation means in terms of physical relation of Mp to the genonema is quite unknown. It is significant that the stability in position of Modulator in the case of P_{m-1}^{ww} gives the latter the semblance of a stable allele. In fact, were it not for the fact that special tests, such as capacity to promote Ds chromosome breakage, for the presence of Mp at the P locus are available, P_{m-1}^{ww} ordinarily would be indistinguishable from the widely occurring stable P^{ww} gene for colorless pericarp and cob.

The part which factors of the Modulator type play in chromosome organization is the central question on which this study bears. The concept that the *genonema* comprises a single category of severally specific determinants, physiologically independent of each other at this level and constituting the whole of the germinal substance, was brought into question by the cytological and genetic observations on heterochromatin in *Drosophila* (reviews by LEWIS 1950; HANNAH 1951). McCLINTOCK has shown conclusively that a distinctive class of chromosomal elements occurs in maize which, by virtue of some kind of interrelationship with the conventional genes, likewise has pervasive effects on the genotype. These novel factors are pictured by McCLINTOCK (1956) as specific controllers of gene action. Controllers are thought to fall into groups, the members of each group functioning as a system in modulating gene action within the nucleus. This view of the germinal substance calls for primary genetic variations of two classes, controller mutations and gene mutations. Gene expression in a given case is considered to be a function of the associated controller system. Furthermore, McCLINTOCK assumes that the type of control over gene action exerted by the accessory elements conforms to the orderly requirements for cellular and tissue differentiation. It is implied also that mutation involving controllers is basic in regulating development of the individual.

The provisional nature of McCLINTOCK's conclusions is evident when it is considered that none of the recent data on mutable alleles in maize excludes a simpler, albeit less well defined interpretation which has been associated with the concept of heterochromatin, particularly in *Drosophila*, since it was shown that this substance is not genetically inert as was first supposed. According to this view, which has been adverted to by various investigators (cf. HANNAH 1951) the heterochromatin is relatively homogeneous and serves some kind of residual genetic function essential to nuclear metabolism. Adequate criteria for regularly distinguishing heterochromatin from euchromatin have not been established, and there is a dearth of evidence concerning action of the substance. Consequently the problems in this area usually have been treated in pragmatic fashion, pending discovery of the facts whereby they may be more clearly defined and attacked.

The suggestion that heterochromatin embodies genetic elements having a generalized nuclear function has been given fairly explicit form by CASPERSSON (see CASPERSSON 1956, for a recent statement of his views). CASPERSSON postulates that the immediate products of a portion of the chromatin are massive amounts of unspecialized proteins and possibly other substances, which are not directly functional in differentiation but constitute a pool, so to speak, which is drawn upon in the formation of developmentally active substances. The latter comprise enzymes, constituted in part of material from the pool, but whose specificities, presumably, are attributable to genes each of which forms a limited quantity of a unique substance. Heterochromatin is suspected as the site of the determinants conditioning production of the substrate materials entering the nuclear pool, by way of the nucleolus or otherwise. This chromosomal component is considered to

be adapted to rapid production of large amounts of the required ground substances.

The assumed differentiation of the genetic material into what may be termed "kinetoplasm," comprising determinants of generalized function and genes in the conventional sense, is not at variance with any of the data on mutable alleles in plants or variegating position effects and heterochromatin in *Drosophila*. Such a postulate, on the other hand, does little more than connect in a provisional way facts which are still too isolated to permit of any firm interpretation.

If the chromosome includes kinetoplasm as a primary constituent with whose mass products genes with highly definitive effects interact to form the primary agents of morphogenesis, then mutable alleles may represent unconformities between the two classes of determinants. Various kinds of disarrangements of these factors may be visualized. One might picture Modulator, for example, as a "kinetomere," or unit of kinetoplasm, normal to the maize genotype in some particular, although unknown relation in most genotypes, but as adventive to the *P* locus quite by accident in the case of variegated pericarp. Conceivably the inhibiting effect of *Mp* at the *P* locus on pigment production could be brought about by (1) distorting the molecular configuration of the *P^{rr}* gene itself, (2) sequestering the immediate product of *P^{rr}* through formation of a physiologically inactive compound, (3) interference with fibrogenesis, coiling, or other processes whereby this region of the chromosome becomes functionally differentiated. A certain versatility in the character of the physical associations between *Mp* and *P^{rr}* is indicated by the fact that *Mp* is readily transposable in the case of ordinary variegated pericarp but fixed at the *P* locus in the stable colorless allele, *P^w_{m-1}*, here discussed.

McCLINTOCK (1951, 1956) has shown and others have confirmed the facts, that instability at a locus is a function not of the resident gene but of accessory chromosome material, and also that any locus, presumably, may become unstable, and in diverse ways. The evidence points definitively to primary chromosome elements as causal factors, essentially different from the conventional genes, and complementary to the latter in some, as yet, unknown way. But the data currently available are inadequate to establish where, in the hierarchy of chromosome organization, these components fit. The evidence can be construed as readily in terms of a kinetoplasm, comprising nuclear substrate factors, as of highly ordered controllers of gene action. These two views are antithetical, and the fact that either is tenable is witness to the indecisiveness of the evidence which suggests them.

SUMMARY

Colorless pericarp and cob is an infrequent mutation from the highly unstable variegated pericarp and cob allele, *P^{vv}*, in maize. The particular mutant here discussed, *P^w_{m-1}*, originated as a 43-kernel patch on a medium variegated ear. It is normally transmitted through the pollen and gives a plant of normal vigor when homozygous. Evidently, there, *P^w_{m-1}* does not represent a deficiency at the *P*

locus. Examination of 4536 plants carrying P_{m-1}^{ww} disclosed only one mutant. This individual bore an ear with very fine, red stripes not typical of any of the commonly encountered forms of variegated pericarp. Modulator was shown to be a regular component of the P_{m-1}^{ww} allele. It could not be distinguished from that characteristic of the variegated pericarp stock from which it was derived in terms of the pattern of *Ds* chromosome breakage incited and reduction in frequency of P^{vv} to P^{rr} mutations in $P^{vv}P_{m-1}^{ww}$ and $P^{vv}P^{vv}$ plants. The evidence thus suggests that *Mp* is qualitatively the same in the P_{m-1}^{ww} and P^{vv} alleles, and that the latter differ from each other essentially in the strength of bonding of *Mp* at the *P* locus. The indecisiveness of the evidence concerning the role in chromosome organization of elements of the Modulator type is briefly discussed.

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