EFFECT OF THE *Dt* GENE ON THE MUTABILITY OF THE a_1 ALLELE IN MAIZE[1]

M. M. RHOADES

Geneticist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Received April 8, 1938

INTRODUCTION

THE mechanisms through which sudden changes in genes, that is, mutations, are produced have long been of interest to the geneticist. In attempting to obtain some insight into the nature of the mutation process, two methods of approach have been most commonly employed. One of these is the study and analysis of mutations induced by irradiation and the other the study of the so-called mutable genes or genes with high rates of spontaneous mutation. While there is no factual basis for believing that mutable genes differ from more stable ones save in their mutation rate, nevertheless there is the possibility that they constitute a distinct class in themselves and any conclusions reached about them might not be applicable to more stable genes.

In 1936, the writer described a dotted aleurone character in maize. Since that time the character has been studied in further detail and additional data obtained which have some bearing on the nature of gene mutation. These data show that the a_1 allele, which has a low spontaneous mutation rate in the presence of recessive *dt*, becomes highly mutable with dominant *Dt*.

The character dotted aleurone appeared in a selfed ear of Black Mexican sweet corn obtained from Dr. L. F. RANDOLPH. The seeds on this ear occurred in the ratio of 12 self-colored:3 dotted:1 colorless seed. The dotted seeds had small dots or spots of aleurone color which were fairly uniform in size and distributed at random over the aleurone layer (fig. 1). Aleurone color is normally formed only when at least one dominant allele of each of the four primary factors, A_1, A_2, C and R, is present. Seeds homozygous for recessive a_1 and possessing the dominant alleles of the other three primary genes have colorless aleurone. Strains of this constitution have been widely used in genetical experiments and are known as a_1-testers. As far as the writer is aware colored areas in the aleurone have never been found in these a_1-tester stocks. An examination of two thousand a_1-tester kernels with a low power binocular failed to disclose the presence of any colored cells. An analysis of the ear on which the dotted seeds

[1] Contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

appeared showed that the 12:3:1 ratio came through the segregation of A_1 a_1 and of a new dominant gene *Dt* which interacted with the recessive a_1 to give the dotted character in three-fourths of the a_1 class. The colorless seeds were homozygous for both a_1 and *dt*. As the dotted class was homozygous for a_1, it would, therefore, normally have colorless aleurone.

The *Dt* gene was specific in its effect with a_1 in that a_2, *c* or *r* testers had colorless aleurone in the presence of *Dt*. The dominant *Dt* has been found only in the Black Mexican strain. All other strains of maize which have

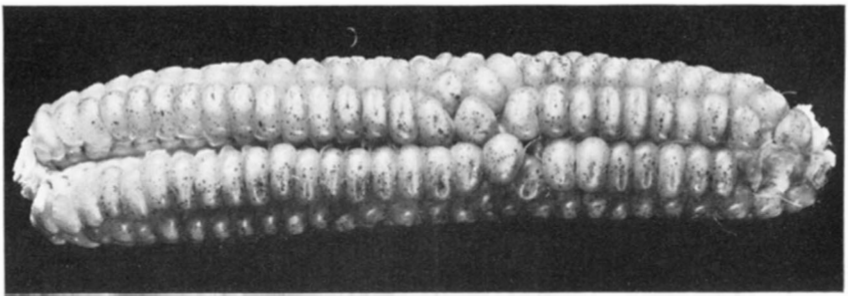

FIGURE 1.—The dotted aleurone character is shown by all seeds on this ear which came from self pollinating a plant of a_1 a_1 *Dt Dt* constitution. Each colored area or dot represents a mutation of a_1 to A_1. The small range in size indicates that mutations of a_1 occur late in the development of the aleurone.

been tested have carried the recessive allele *dt*. Black Mexican sweet corn is normally homozygous for all the dominant alleles of the four aleurone factors and the detection of *Dt* in this line came through a mutation of an A_1 allele to a_1. This a_1 allele is apparently identical with the a_1 allele found by EMERSON (1918) which is the source of all the a_1 genes found in genetic stocks. It has not been possible to differentiate between these two a_1 alleles on the basis of their interaction with *Dt*. Both of them produce colorless aleurone with *dt*.

EMERSON and ANDERSON (1932) found four alleles at the A_1 locus. Two of them, A_1 and $A_1{}^b$, give deep or strong colored aleurone, $a_1{}^p$ produces a pale colored aleurone while a_1 gives colorless aleurone. It was found that seeds homozygous for $a_1{}^p$ occasionally, though rarely, had areas or dots of deep color in the pale colored aleurone. However, the frequency of the deep colored dots was no greater on seeds with *Dt* than on seeds with *dt*. It may be concluded, therefore, that the mutability of the $a_1{}^p$ allele is not affected by *Dt*. The occurrence of the deep colored dots suggests that $a_1{}^p$ may mutate to A_1 but the frequency of such mutation is so low that it does not affect the data or the conclusions reached in this paper. However, seeds heterozygous for $a_1{}^p$ and a_1 show numerous deep colored dots on the pale background if *Dt* is present. The pale color is obviously produced by $a_1{}^p$ and the dots of color by the interaction of a_1 and *Dt*.

As the aleurone is triploid tissue, seeds possessing one, two, or three a_1 alleles may be obtained by manipulating the dosage of the a_1 gene. (See RHOADES 1936.) The mean number of dots per seed for each of the three classes was determined. As reported previously, the mean number of dots showed a linear relationship with the number of a_1 alleles present. Additional data from several ears are given in table 1. As the pale-dotted and dotted seeds are borne at random on the same ear, they possess on the average identical genetic residua except for possible segregating genes linked with the a_1 locus. The data in table 1 show that seeds of a_1^p a_1^p a_1 *Dt Dt dt* constitution which have one a_1 gene have one-third as many dots as do sister seeds of a_1 a_1 a_1 *Dt Dt dt* heredity which have three a_1 genes. Similarly a_1^p a_1 a_1 *Dt dt dt* seeds with two a_1 genes have two-thirds as many dots as sister a_1 a_1 a_1 *Dt dt dt* seeds with three a_1 genes. These data indicate that increasing the a_1 dosage results in a proportional increase in the number of dots.

The results obtained when the a_1 dosage is held constant while that of *Dt* is varied have previously been shown to be non-linear. This conclusion is supported by further data but this relationship will be discussed in a later section of the paper.

When the data showing the effect on the number of aleurone dots of varying the dosage of a_1 and *Dt* were published (RHOADES 1936) nothing was known of the mechanism through which the colored cells were produced. One possible explanation was that the a_1 allele, normally stable in the presence of *dt*, became unstable in the presence of *Dt* and mutated to dominant A_1. Each dot of color then would represent one mutation and the number of dots would represent the frequency of mutation while the size of the dot would indicate the relative time in ontogeny at which the mutation occurred. This hypothesis would account also for the dosage effect of a_1 if it is assumed that the mutations to A_1 of the several a_1 genes occur independently of one another. As the A_1 gene is known to affect anthocyanin formation in other tissues than the aleurone, experiments were planned which would determine the validity of this hypothesis and the present paper presents the results of these investigations.

EFFECT OF THE *Dt* GENE ON PLANT AND PERICARP COLOR

In his classical study of plant colors in maize EMERSON (1921)[2] showed that purple plant color is conditioned basically by the interaction of three dominant factors, A_1, *B*, and *Pl*. When recessive a_1 is substituted for domi-

[2] EMERSON defines plant colors as "colors other than those related to chlorophyll, commonly in, but not limited to, such external plant parts of maize as the culm, the staminate inflorescence, the husks, the leaf sheaths, and to some extent the leaf blades. In contrast to this group are colors and color patterns related to chlorophyll or associated with the pericarp and the cob, the silks, the endosperm, the aleurone."

nant A_1, a brown color is produced. (SANDO and BARTLETT (1922) and SANDO, MILNER, and SHERMAN (1935) have shown that the purple pigment is the anthocyanidin, chrysanthemin, while the brown pigment is the corresponding flavonol, isoquercitrin.) If the occurrence of colored areas in the aleurone of a_1 *Dt* seeds is caused by the mutation of a_1 to A_1,

TABLE 1

*Mean number of aleurone dots on seeds with three a_1 genes and seeds with two a_1 genes from eight ears of crosses $a_1\,a_1$ *Dt Dt* $\times a_1\,a_1{}^p$ *dt dt* or $a_1\,a_1$ *dt dt* $\times a_1\,a_1{}^p$ *Dt Dt*.*

PEDIGREE	MEAN NO. DOTS ON $a_1\,a_1\,a_1$ *Dt* CLASS	NO. OF SEEDS IN CLASS	MEAN NO. DOTS ON $a_1\,a_1\,a_1{}^p$ *Dt* CLASS	NO. OF SEEDS IN CLASS
2676(1) ×2511a	15.2	46	10.0	48
2500 ×2511b	9.0	32	6.2	49
4345(4) ×4344(1)	10.2	152	6.1	172
4345(6) ×4344(7)	7.4	61	5.0	62
4345(8) ×4344(1)	5.6	70	4.2	76
4345(9) ×4344(2)	11.5	16	9.6	19
4345(11)×4344(2)	3.5	39	2.6	42
4345(12)×4344(8)	2.9	20	1.4	27
	8|65.3	436	8|45.1	495
Observed =	8.16		5.64	
Theoretical = on 3:2 ratio	8.28		5.52	

*Mean number of aleurone dots on seeds with three a_1 genes and seeds with one a_1 gene from nine ears of crosses $a_1\,a_1{}^p$ *Dt Dt* $\times a_1\,a_1$ *dt dt* or $a_1\,a_1{}^p$ *dt dt* $\times a_1\,a_1$ *Dt Dt*.*

PEDIGREE	MEAN NO DOTS ON $a_1\,a_1\,a_1$ *Dt* CLASS	NO. OF SEEDS IN CLASS	MEAN NO. DOTS ON $a_1\,a^p\,a^p$ *Dt* CLASS	NO. OF SEEDS IN CLASS
2511 ×2500	11.6	50	3.7	63
2511a ×2500	6.6	43	2.4	40
4344(3)×4345(5)	23.6	66	7.0	62
4344(4)×4345(6)	13.3	59	3.9	62
4344(5)×4345(6)	29.5	23	12.0	26
4344(6)×4345(3)	20.8	38	8.4	35
4344(8)×4345(12)	27.5	26	9.2	28
4344(1)×4345(4)	27.3	79	8.3	83
4346(7)×4345(4)	20.3	55	6.5	60
	9|180.5	439	9|61.4	459
Observed =	20.06		6.82	
Theoretical = on 3:1 ratio	20.16		6.72	

we would expect to find on brown (a_1 *B Pl Dt*) plants areas of purple representing tissue descended from cells in which a mutation from a_1 to A_1 had occurred. Therefore, a strain of maize of a_1 a_1 *B B Pl Pl Dt dt* constitution was synthesized. When plants of this strain were self-pollinated there resulted a ratio of 3 *Dt*: 1 *dt* seed. Four hundred twenty-six plants were grown from seed with dotted aleurone and hence carrying *Dt*. They had the brown pigment characteristic of a_1 *B Pl* plants but in addition possessed from few to many narrow, longitudinal stripes of purple tissue on the husks and culms. The stripes were usually small, less than an inch in length, but occasionally one 6 to 8 inches long occurred. This small size indicated that the mutations to A_1 occur relatively late in the development of the tissues. The 150 plants from *dt* seed had the same brown pigment as their a_1 *B Pl Dt* sibs but no stripes of purple tissue were found on these plants.

The study of the effect of *Dt* on the mutability of a_1 in tissue of the culm and tassel was confined for the most part to brown plants of a_1 *B Pl* constitution since the brown pigment is distributed throughout the culm and sheaths and consequently offers a large area in which to detect mutations. However, the mutation hypothesis is subject to check in other plant color types. Plants of a_1 *b Pl dt* constitution are entirely green, whereas A_1 *b Pl* plants are also green except for purple colored anthers and some anthocyanin pigment at the base of the culm. A race of a_1 *b Pl Dt* individuals was synthesized. They had green culms and leaf sheaths but their green anthers had numerous small purple spots. Occasionally, though rarely, an entire anther was colored but the great majority of the colored areas were small, as was true in a_1 *B Pl Dt* plants. No attempt was made to find colored sectors at the base of the culm.

Plants with A_1 *B pl* constitute the sun red class. In this type anthocyanin is found in those portions of the culms, sheaths, and tassels which are exposed to light. Plants of a_1 *B pl dt* genotype possess no anthocyanin but a_1 *B pl Dt* individuals had the expected small colored regions in the culm, sheath, and anthers.

From the studies of the effect of *Dt* on aleurone and plant color it is seen that the production of the anthocyanin pigments can be interpreted on the assumption that a_1 becomes unstable in a cell in which *Dt* is present. A further test of this mutable gene hypothesis is afforded by a study of pericarp color. EMERSON and ANDERSON (1932) have shown that red pericarp color is produced when the dominant A_1 and the dominant *P* factors are present while a brown pericarp color is produced in a_1 *P* tissue. According to our hypothesis, ears on a_1 *P dt* plants should have brown pericarp with no red areas but those on a_1 *P Dt* plants should have red sectors on otherwise brown pericarp. This is precisely what was found. As in the

case of the experiments with aleurone and plant color, the size of the red sectors was small with the largest red area covering approximately one-third of a single kernel. Usually, the red sectors consisted of fine streaks running from the point of silk attachment towards the base of the kernel in a manner similar to the red stripes of variegated pericarp. In accordance with expectation no red sectors were found on a_1 *P dt* seeds.

The above data on the production of anthocyanin pigments in the three diverse tissues studied cannot be considered as proof of the mutation hypothesis even though the data are in agreement with such a hypothesis. A direct and conclusive test could be had only if a mutation occurred in sporogenous tissue which later gave rise to sex cells, enabling a genetic check to be made in subsequent experiments. Fortunately, such a test was possible.

It has been seen that a_1 *B Pl Dt* plants are brown with purple sectors. These purple areas are found in all parts of the plant which normally

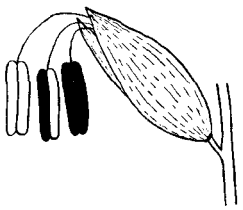

FIGURE 2.—A diagrammatic sketch of the three anthers of a single flower on a brown (a_1 *B Pl Dt*) plant. One of the anthers is entirely purple, one is half purple and half green, and the third is wholly green. Tests of the constitution of the pollen in purple anthers show that in some cases some of the pollen grains have the A_1 allele and some have the a_1 allele. The A_1 allele has been derived by a mutation of the a_1 allele.

develop purple color when the plants are A_1 *B Pl*. EMERSON (1921) has shown that the purple pigment on A_1 *B Pl* extends into the anthers if the r^r or R^r alleles are present. The a_1 *B Pl Dt* stock used in these experiments had the R^r allele. Not infrequently a_1 *B Pl Dt* individuals had small purple sectors in the tassel. Sometimes (fig. 2) all three anthers of a flower were purple but often one anther was purple with the other two either green or sectorial. In some of the partly colored anthers one-half of the bipartite anther was purple and the other green; in others only a portion of one-half was colored with the remainder of the tissue green. Purple sectors of various sizes were found, however, and by far the most frequent class was that in which a small area on individual anthers was pigmented. It may be reasonably assumed that a mutation which occurred early enough to affect all of the cells composing the anther wall might also, in some cases at least, affect the sporogenous tissue. If, for example, a mutation from a_1 to A_1 occurred in a cell whose descendants comprised the tissue of the

anther wall and also the sporogenous tissue within the anther it would be expected that one-half of the pollen grains would carry A_1 and the other half a_1. A ratio of 1 colored:1 colorless aleurone would be obtained when the pollen from such an anther was applied to a_1-tester silks. Varying ratios of colored:colorless seeds would be obtained if part of the sporogenous cells came from the cell in which the mutation occurred and part came from non-mutated cells. On the other hand, if there was no correlation between the genetic constitution of the cells of the anther wall and the sporogenous cells, or if the color of the anther resulted not from a mutation to A_1 but through some effect which simulates such a change, no colored seeds would be found when the pollen from purple anthers was used in a cross with a_1 A_2 C R plants.

TABLE 2

Summary of crosses with pollen from purple anthers occurring on a_1 B Pl Dt plants. Each purple anther listed came from a different plant. The Dt dt pair were segregating so that some of the ears had, in addition to colored seeds, only dotted or both dotted and colorless depending upon the Dt constitution.

COLOR OF ANTHER	COLORED SEED	COLORLESS OR DOTTED SEED	TOTAL
Purple	5	7*	12
Purple	19	18*	37
Purple	92	74*	166
Purple	4	4	8
Purple	2	49	51
Purple	0	173	173
Purple	0	110	110
Purple	0	18	18
¾ Purple	51	62	113
½ Purple	11	8*	19
½ Purple	13	34	47
½ Purple	8	114	122
½ Purple	1	67	68
½ Purple	0	142*	142
⅕ Purple	5	53*	58
Less than ⅕ Purple	2	36	38

* Only dotted seed.

In table 2 are listed the numbers of A_1 and a_1 seeds obtained when anthers either wholly or partly purple were tested in crosses with a_1-tester plants. Of the eight purple anthers used five produced seeds with colored aleurone. In four of the five cases the ratios suggest the presence of equal numbers of pollen grains carrying A_1 and a, respectively. This 1:1 ratio is expected if the cell which gave rise to all of the sporogenous tissue became heterozygous for A_1 following a mutation of one of the two a_1 genes

to A_1. The fifth purple anther produced only 2 A_1 to 49 a_1 seeds. The deviation from a 1:1 ratio is too great to be accounted for by errors in sampling and indicates that the genetic constitution of the cells of the anther wall is not necessarily identical with that of all of the microsporocytes. This reasoning is strengthened by the fact that three purple anthers produced no colored seeds when tested. It would appear that in ontogenetic development the separation of the sporogenous and anther wall tissue does not follow a precise pattern and that a mutation occurring in the cell giving rise to cells of the anther wall need not necessarily affect the sporogenous tissue. If the mutation occurs before the two cell lines have separated they will have the same constitution. The results from crosses using anthers sectorial for purple likewise show variation in A_1:a_1 ratios and point to a similar conclusion.[3] The fact that colored seeds were produced when pollen from purple anthers was used is proof that a_1 had mutated to A_1 in the cells of the tested anther and it follows that the anthocyanin color produced in the aleurone, plant, and pericarp tissues resulted from similar mutations.

As individual anthers were used in making the crosses and the amount of pollen therefore was limited, the possibility of contamination was increased. An occasional colored seed might result from this contamination. However, the presence of the recessive mutant genes lg_1, *y*, and *su* in the *Dt* stocks made it possible to check the origin of the colored seeds. In the tests made so far all colored seeds but one were the result of mutations to A_1.

Because the purple anthers were found on plants of a_1 *B Pl Dt* constitution the colored seeds produced by crossing with a_1-tester stocks gave rise to purple F_1 plants as they were A_1 a_1 *B b Pl pl* while the colorless and dotted seeds produced brown (a_1 a_1 *B b Pl pl dt dt*) and brown with purple stripes (a_1 a_1 *B b Pl pl Dt dt*), respectively.

Three of the wholly purple anthers came from plants homozygous for *Dt*. In crosses with a_1-testers they produced only colored and dotted seeds (table 2). The dotted kernels came from a_1 *Dt* pollen and the colored seeds from pollen carrying A_1 and, presumably, *Dt* unless the *Dt* gene is altered when it induces a mutation of a_1 to A_1. If no change had occurred in the *Dt* gene the colored seeds were of A_1 a_1 *Dt dt* constitution. However, if *Dt* changes to *dt* coincidentally with a mutation of a_1 the colored seeds will be A_1 a_1 *dt dt*. Some of these colored aleurone seeds were grown and self-pollinated. They yielded self-colored, dotted and colorless seeds in a 12:3:1

[3] It should be noted that rarely a self-colored seed is found on selfed ears of a_1 *Dt* constitution when bulked pollen is used. It seems probable that these self-colored seeds arise from the functioning of a gamete carrying an A_1 allele derived by mutation from the a_1 allele in either the sporogenous tissue or in the gametophyte.

ratio which is in accordance with the results expected from the selfing of A_1 a_1 *Dt dt* plants. If no dotted seeds had been produced the colored plants would have been A_1 a_1 *dt dt* and a ratio of 3 colored: 1 colorless would have been obtained. These results argue that no change occurs at the *Dt* locus when a mutation of a_1 to A_1 occurs even though the *Dt* gene is directly responsible for the change in the a_1 allele.

RELATIVE FREQUENCY OF MUTATION OF RECESSIVE a_1 TO THE a_1^p AND A_1 ALLELES

In the determination of the dosage effects of a_1 the various levels of a_1 were obtained by substituting the a_1^p allele for one or two a_1 genes. It was possible to do this because the a_1^p allele is not affected by *Dt* and the mutations to A_1 from a_1 are clearly evident on the pale color produced by the a_1^p gene. The relative dosage effects of a_1 would be subject to serious error if it were found that the a_1 allele mutated to a_1^p with a considerable frequency because these changes would not be visible on the

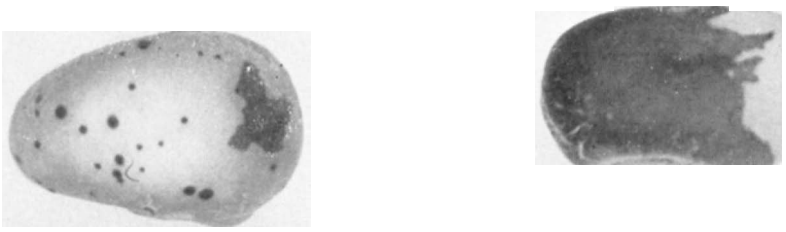

FIGURE 3.—The kernel on the left has a large a_1^p area in which is found a smaller, deeper colored A_1 dot. As a_1 mutates both to a_1^p and A_1 it is probable that one a_1 gene mutated to a_1^p early in development and somewhat later a second a_1 gene mutated to A_1. The rightmost kernel illustrates one of the mutations to A_1 which occur infrequently at an early stage.

pale background produced by the a_1^p gene initially present since the intensity of color produced by one, two or three a_1^p genes is not noticeably different. It was therefore considered pertinent to determine the frequency with which a_1 mutated to a_1^p. The dots on 2426 seeds of a_1 a_1 *Dt* constitution were examined with a low power binocular. There was a total of 80,955 dots of which 80 were a_1^p dots and the remainder A_1 dots. The distinction between the two classes of dots was clear. The size of the a_1^p dots varied in the same manner as the A_1 dots, that is, they were usually small but occasionally one was found covering a considerable portion of a seed. Such a mutation is shown in figure 3 where a pale colored sector is shown with an A_1 dot clearly discernible in this area. It seems probable that in this seed one a_1 allele mutated to a_1^p early in the development of

the aleurone and another a_1 allele mutated several cell generations later to A_1 to give the deeper colored spot.

It is apparent that the conclusions reached on the effects of different dosages of the a_1 allele are not seriously affected by the occurrence of a_1^p mutations.

Interestingly enough the a_1^p allele has been found only once in the great numbers of strains of maize collected in different parts of the world. Apparently mutations to this allele have occurred infrequently in the past. In these investigations the mutations from a_1 to A_1 occurred about 1000 times as frequently as the mutations to a_1^p.

There exists a fourth allele, A_1^b at the a_1 locus. The A_1^b allele is indistinguishable from A_1 in its effect on plant and aleurone color but produces a dominant brown pericarp with the *P* gene. No tests have been completed as yet to determine the relative frequencies of A_1 and A_1^b mutations. There is also a possibility that some of the mutations may be to new, previously unknown, alleles.

As reported in table 1 of the writer's 1936 paper $A_1\ a_1\ C\ R\ A_2\ Dt$ seeds have deep self color with no evidence of dots. If $A_1\ a_1\ a_1$ aleurone was lighter than $A_1\ A_1\ a_1$ we would expect in $A_1\ a_1\ a_1\ Dt$ aleurone to find areas or dots of a deeper color which would represent cells in which one of the a_1 alleles had mutated to A_1. However, it has not been possible, at least in the writer's experience, to detect a difference in intensity of aleurone color in seeds possessing one, two or three A_1 alleles.

While the a_1 allele is highly unstable with *Dt* there is no indication that the A_1 allele derived by mutation from a_1 is unstable either with *Dt* or *dt*. Purple plants of $A_1\ a_1\ B\ Pl\ Dt$ constitution, the A_1 allele being one derived by mutation, do not show the brown colored sectors expected if the A_1 gene mutated back to a_1. These observations are not extensive but there are, at present, no indications that the A_1 mutations from a_1 are unstable. While more data should be had on this point, it can be unequivocally stated that if *Dt* does affect the stability of reverted A_1 it does so with a low frequency.

GENES MODIFYING THE MUTATION FREQUENCY

The data in table 1 show considerable variation in the mean number of dots on the kernels from different ears possessing the same genetic constitution for a_1 and *Dt*. It is possible to ascribe these differences to certain genetic factors which modify the frequency with which the mutations to A_1 occur. While it appears likely that various stocks have different sets of lesser modifiers certain data were obtained which indicate that a single locus exerted a major effect on the frequency of mutation. Selfed ear 4347-7 was homozygous for a_1 and *Dt*. The a_1 alleles in this ear are de-

scendants of a common a_1 allele. The other aleurone genes were homozygous dominant so every seed on the ear was dotted. The number of dots on each of the 154 seeds was determined and the data represented graphically as a rectangular histogram. The mean number of dots per kernel was 31.1. The range was 93. As figure 4a shows, there was an indication of bimodality suggestive of a 3:1 ratio for a dominant modifier *M* which decreases the number of dots. To test this hypothesis, kernels were selected which had a high number of dots. Theoretically they should be a_1 a_1 *Dt Dt m m*. Seeds with an intermediate number close to the mean of 31.1 dots were also selected and planted. They should be a_1 a_1 *Dt Dt M m*. Finally, seeds possessing a low number of dots were picked. On the further assumption that the *M* factor only partially inhibits the mutation frequency and that there is a cumulative effect by increasing the dosage of *M* these seeds should be a_1 a_1 *Dt Dt M M*. Plants from these three classes of seed were grown and self-pollinated and the number of dots on each individual seed from ears in each of the three classes was determined. The seeds in the high class should, on the theory, give rise to offspring with a high mean number of dots and should give a unimodal frequency distribution since they would be homozygous for *m*. Figure 4b is representative of the results obtained with three selfed ears of this class. The mean number of dots is somewhat higher than on the parent seed but there is no tendency toward a bimodal distribution. Although it was desirable to have frequency distributions for additional ears of this class, the amount of labor involved in counting the dots on seeds with high numbers discouraged the accumulation of more data. However, an inspection of the other ears of this class indicated that they were of the same type as the three which were selected at random for classifying. Likewise, the ears from seeds with a low number of dots had uniformly a low average number of dots in the offspring and the data when plotted as a histogram showed no indication of a bimodal distribution. Figure 4c is representative of one ear of this class. They all had a unimodal distribution. Obviously the critical class for the hypothesis is the group with the intermediate number of dots. Individuals from this group should yield progeny with a frequency distribution similar to that obtained for the kernels of the parent ear 4347-7. Seeds from five ears of this class were classified for dot number and the data plotted. Figure 4d is typical of the results. They had frequency distributions closely resembling that of the parent ear. Furthermore, approximately one-fourth of the kernels fall into that portion of the histogram which presumably includes the *m m* class.

The difference in the mean number of dots between members of the *M M* class, for example, may be attributed to the action of numerous modifying genes whose effect, however, is relatively slight in comparison

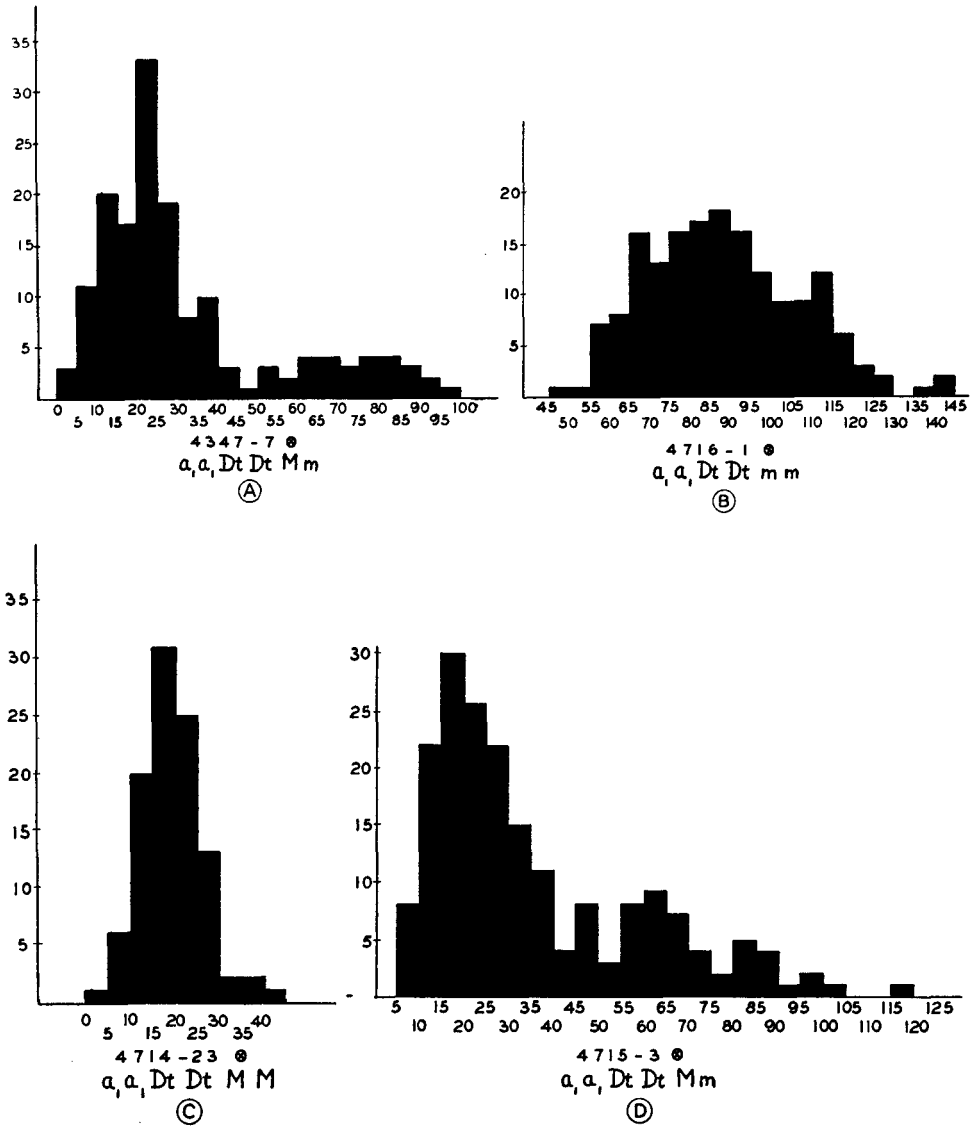

FIGURE 4.—Frequency histograms showing effect of *M* and *m* genes on the mutation rate of a_1. Number of dots per seed is plotted on the abscissa and the number of seeds on the ordinate. Figure 4a represents the data from selfed ear 4347-7 in which the presence of the *M* and *m* genes was suspected from the bimodality of the distribution. The data from selfed ear 4716-1 are shown in Figure 4b. The unimodality of the histogram and the high average number of dots suggest that the *m* gene is homozygous. The data from selfed ear 4714-23 is illustrated in Figure 4c. The homozygosity of the *M* factor is indicated by the unimodality of the histogram and the low average number of dots. Figure 4d from the data of 4715-3 has the bimodality expected upon selfing *M m* plants.

to the *M m* pair. Further work is necessary to prove the existence of these lesser modifiers but the material seems admirably suited for such a demonstration. In figure 5 the pedigree of the *Mm* line is shown. It is not certain which parent in the original cross brought in the *M* or *m* alleles.

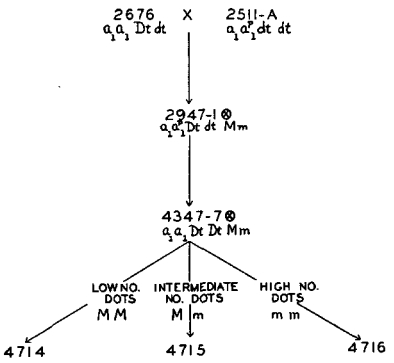

FIGURE 5.—Pedigree of ear 4347-7 in which the presence of the *M* and *m* alleles, which modify the effect of *Dt* on a_1, was first detected.

In table 3 the number of dots on each seed planted in families 4714–4716 is shown in column 2. There was a tendency for the mean number of dots on the seed of the progeny to exceed the number on the parental seed. Plant 4347-7 was grown under adverse conditions in the summer of 1936 at Ames, Iowa, while families 4714–4716 were grown under favorable conditions in 1937 at Arlington Experiment Farm, Arlington, Virginia. Whether or not the environment plays a role in the mutation frequency is unknown but these data suggest such a possibility.

LINKAGE RELATIONS OF THE *Dt* GENE

The four primary genes for aleurone color development, A_1, A_2, *C* and *R*, are located in different chromosomes. No indication has been found that *Dt* is linked with A_1, A_2, or *R*. There are some data which show linkage of *Dt* with *C* and suggest that *Dt* belongs in chromosome IX. When A_1 a_1 *C c Dt dt* plants are selfed a 36:9:19 ratio is expected with independence of the three segregating factors. Previous data of this nature closely approached this ratio but the average number of dots per kernel in these ears was low so that seeds genotypically *Dt* might be phenotypically colorless or *dt*. Therefore the test of linkage between *Dt* and *C* was repeated using a strain in which there was a much higher number of dots. With *Dt* and *C* in coupling phase a 9:3:4 ratio is expected with complete linkage of *Dt* and *C* as contrasted with the 36:9:19 ratio expected with independence. There resulted in F_2 from the selfing of A_1 a_1 *C-Dt c-dt* individuals

1761 colored: 501 dotted: 792 colorless seeds. The ratio of dotted and colorless seeds is far from a 9:19 ratio and approaches a 3:4 ratio. Any errors in classification should favor the colorless class at the expense of the dotted class. The *wx* gene was segregating in the above ears and gave a close fit to a 3:1 ratio. Since *wx* was brought in with *c*, with which it is linked, the excess of *Dt* seeds is probably not due to a failure of a portion of the *c–dt* gametes to function. However, if *Dt* is independent of *C* the

TABLE 3

Data on average number of aleurone dots per seed from selfed ears of M M, M m and m m constitution. The three families are descendants of selfed ear 4347-7.

PLANT	NO. DOTS ON PARENTAL SEED	INFERRED CONSTITUTION	NO. SEEDS COUNTED	MEAN NO. DOTS	TYPE OF FREQUENCY DISTRIBUTION
4714(2)	12	*M M*	75	16.4	unimodal
4714(9)	9	*M M*	100	8.8	unimodal
4714(12)	9	*M M*	200	16.8	unimodal
4714(13)	11	*M M*	75	11.4	unimodal
4714(14)	15	*M M*	75	19.6	unimodal
4714(23)	15	*M M*	101	18.9	unimodal
4714(24)	19	*M M*	100	16.8	unimodal
4715(7)	31	*M m*	158	31.7	bimodal
4715(3)	28	*M m*	193	34.4	bimodal
4715(22)	30	*M m*	168	39.9	bimodal
4715(10)	36	*M m*	124	38.2	bimodal
4715(5)	31	*M m*	100	33.8	bimodal
4716(1)	72	*m m*	169	87.6	unimodal
4716(24)	68	*m m*	106	83.9	unimodal
4716(3)	66	*m m*	125	76.9	unimodal

excess of dotted kernels could be explained by a gametophyte factor linked with *dt*. Linkage tests with other genes in chromosome IX, with *C* homozygous, will tell if the *Dt* factor belongs in this group. The *Dt* gene has failed to show linkage with any other tested genes which include from one to four members in each of the other nine groups.

Earlier data which gave a faint suggestion that the number of dots, that is, the mutation rate, was influenced by the dosage of *C* were not substantiated by more extensive tests. A similar conclusion is true for the *R* gene. These results are in accord with the mutation hypothesis. The dosage effect of A_2 was not determined but it should prove to be in the same category as *C* and *R*.

Self-colored aleurone depends upon the interaction of a set of complementary factors. This is also true for the dotted character since the colored dots are produced only when a_1 mutates to A_1 and the other mem-

bers of the complementary set are represented by a dominant allele. A strain was obtained which was a_1 a_1 *C C R R Dt Dt* and A_2 *Bt*/a_2 *bt*. Selfed plants of this genotype gave a ratio of 3 dotted to 1 colorless seed since the A_1 a_2 pair was segregating. The brittle endosperm (*bt*) gene showed 8 percent recombination with the dotted character. This would seem to indicate strong linkage between *Dt* and *bt*. Actually the linkage is between A_2 and *bt*. As the A_2 allele must be present before mutations of a_1 to A_1 can produce the dotted character most of the *Bt* seeds were dotted and most of the *bt* seeds were colorless. The 8 percent recombination value found for *Dt* and *bt* is the map distance between A_2 and *bt*. This example of pseudo-linkage suggests that certain linkages found by one investigator and not confirmed by another may be due to a similar genetic set-up in which one of the characters is determined by complementary genes and different members of the set are segregating in the two tests.

DISCUSSION

A consideration of the data presented in this paper as they relate to the behavior of unstable genes reported by other investigators shows several points of similarity as well as difference. Furthermore, these data have some bearing on the nature of gene mutation, especially since GOLDSCHMIDT (1938) has recently promulgated the revolutionary view that mutations are a consequence of position effects.

It has been held, most recently by GOLDSCHMIDT, that mutable or unstable genes constitute a class distinct from stable genes; that their relatively high mutation rate springs from some peculiarity in their structure and, therefore, that any conclusions reached with mutable genes concerning the nature of gene mutation might not be applicable to the mutation phenomenon in general. Inasmuch as mutable genes offer many advantages for the study of the mutation process it is desirable to ascertain if there is a fundamental difference between stable and unstable genes.

Let us see how the data in this paper bear on this question. The a_1 allele has been shown to be an extremely stable gene when *dt* is present but is highly mutable when the *Dt* allele replaces *dt*. The classification of the a_1 gene as mutable or stable depends, therefore, upon the presence or absence of the *Dt* gene. Whether or not the behavior of all so-called mutable genes is caused by a similar genetic situation cannot be stated at this time but the definite effect of *Dt* on the mutation frequency of a_1 certainly suggests that in some cases at least the difference between stable and unstable loci may be more apparent than real and results from the genetic environment rather than from some intrinsic dissimilarity.

Data have been presented in a previous section which indicate that the effect of *Dt* on the mutation rate of a_1 is influenced by the dominant gene

M and that there are also a number of lesser modifying genes. Somewhat comparable results have been reported by various workers with unstable genes. DEMEREC (1929) found three genes which affected the mutation frequency of unstable miniature-3 alpha and gamma of *Drosophila virilis*. DEMEREC also found one factor which increased the mutability of miniature-3 alpha in the germ cells. EMERSON (1929), HERTWIG (1926), and KIHARA (1932) have found the mutation rate of unstable loci to be markedly influenced by the presence of certain genes. These cases may not be strictly comparable with the effect of the *M* gene. Here we have a situation where the mutability of the a_1 allele is primarily controlled by *Dt* but the influence of *Dt* on the a_1 allele is to some extent modified by other genes. The a_1 allele is stable with *dt* irrespective of the presence or absence of these modifying genes. In the cases cited above the modifiers presumably affect the unstable loci directly.

The evidence that the mutability of certain loci is controlled, at least to some extent, by other genes, suggests that the relative mutation rate of different loci may differ according to the presence or absence of specific modifiers.

The size of a colored sector carrying A_1 is a fair indication of the relative time during ontogeny at which the mutation occurred, that is, larger colored areas descend from earlier occurring mutations than do smaller ones. The mutation rate at all stages of development of any tissue can be determined by a statistical study of the frequency with which areas of different size are found. Such a statistical study has not yet been made for the a_1 gene, but the rare occurrence of large colored regions on dotted kernels and the fact that the size range among the aleurone dots is relatively small (fig. 1) indicates that in the aleurone the mutations of a_1 occur predominantly in the later stages of development. The purple stripes on the culms and sheaths of brown (*a_1 B Pl Dt*) plants and the red sectors in the pericarp of *a_1 P Dt* plants had a size range which indicated that in these tissues also the great majority of the mutations occurred late in development. If mutations to A_1 took place consonantly throughout all tissues of the plant irrespective of the ontogenetic age of the different tissues we would expect to find many seeds with self-colored aleurone, since the culm has completely matured before fertilization, and mutations in the sporogenous tissue concomitant with mutations in the culm would give rise to groups of colored seeds on the ear. Inasmuch as the three tissues mature at different times and mutations occur late in the development of each tissue the possibility is indicated that the physiological age of the tissue is in some way concerned with the mutability of the a_1 gene.

Mutation rates for various unstable loci have been determined by several investigators. ANDERSON and EYSTER (1928) found that the rate of

change of the unstable variegated gene in maize pericarp increases toward the end of development of the pericarp. DEMEREC (1931) found that the unstable lavender gene of Delphinium had a high mutation rate in early and late stages but had a low rate in intermediate stages. IMAI (1934) reported a similar situation with yellow-inconstant-1 and flecked in Pharbitis. DEMEREC, however, states that the unstable rose gene of Delphinium mutates with constant frequency throughout all stages of development while, in direct contrast, the reddish-alpha gene of *Drosophila virilis* (DEMEREC 1928) mutates only at the maturation division in heterozygous females. If the mutability of unstable genes is conditioned by a peculiar physiological state, it seems that for the several mutable loci the threshold value of this state is reached at different stages in development.

The characteristic direction of mutation of unstable genes is from the recessive to the dominant, wild type allele. One exception is found in Pharbitis (IMAI 1934) where the unstable willow-leaf gene mutates to the maple-leaf allele which is dominant to willow-leaf but recessive to wild type. Recently DEMEREC and SLIZYNSKA (1937) reported an unstable cream allele, at the white locus of *Drosophila melanogaster*, which mutated both to the cherry and wild type alleles. The a_1 allele changes to a_1^p and A_1 but the frequency of mutation to the A_1 allele is a thousand times greater than that to a_1^p. In addition to mutating to the wild type allele, DEMEREC found that his unstable miniature genes in *Drosophila virilis* changed occasionally from one unstable allele to another. DEMEREC'S (1935) citation of the change of the near-self type to dark-crown variegation in maize pericarp as a case of a mutation of one unstable allele to another is erroneous. According to EMERSON, the difference between the heritable near-self and non-heritable dark-crown types is that in the near-self the mutation to red occurs in a sub-epidermal cell which develops into germinal as well as pericarp tissue, while in the dark-crown type the mutation occurs in epidermal tissue and the germ line is unaffected. We have obtained no evidence that the a_1 gene mutates to alleles varying in their response to *Dt* but they would be difficult to detect unless they occurred in a strain homozygous for all modifying genes.

As the aleurone is triploid tissue it was possible to have one, two or three a_1 genes present and the mutation rate was found to be proportional to the dosage of a_1. This increase in mutation rate with increase in number of genes able to mutate is in contrast with EMERSON'S results with variegated pericarp of maize in which he found the mutation rate of the variegated gene was greater in the heterozygous than in the homozygous condition. EMERSON considered the increased mutability in the heterozygous condition to be caused by modifying genes carried in the homologous chromosome. A similar result would be expected in the present study if

dominant modifiers for increased mutability of a_1 were located in the $a_1{}^p$ chromosome.

Cytological observations of microsporocytes of plants carrying *Dt* in the heterozygous condition have shown no chromosomal abnormalities. An examination of the pachytene stage of plants heterozygous for a dominant A_1 allele derived by mutation shows the chromosomes III to be normal in appearance. Likewise, chromosome IX of a *Dt* stock has no detectable structural change. The writer is not willing to state that a more intensive study might not reveal some minute difference between *Dt* and *dt* lines in the structure of the chromosomes, but there is no evidence of such at present and it can be stated that there are no gross structural differences in the *Dt* and *dt* lines. Further, there is no difference in the "stainability" of the chromosomes in *Dt* and *dt* stocks (SCHULTZ 1936).

As a result of his extensive studies with mutable genes, DEMEREC has advanced the view that changes or mutations in unstable genes are produced by a chemical process rather than through some mechanical shifting or rearranging of chromatin. He visualizes an unstable gene as one with a molecular group in a chemically labile state. DEMEREC also believes that there is no clear cut difference between stable and unstable genes. The data on a_1 and *Dt* interrelationships show clearly that this latter statement is true at least for this one example. Whether or not the a_1-*Dt* data can be held to support the theory that mutations of unstable genes are caused by chemical processes (for example, changes in the side chains of gene molecules) it is reasonably certain that they cannot be interpreted by the theories of the nature of variegation advanced by PATTERSON (1932), STERN (1935), and SCHULTZ (1936). The writer does not contend that these theories are inadequate to account for specific cases of variegation, but it is becoming evident that no one theory can satisfactorily explain the diverse types of variegation any more than one theory of gene mutation can account for all the different phenomena which are categorically called mutations. It is true that somatic crossing over (STERN), loss of a part of a chromosome carrying the dominant allele (CLAUSEN 1930, MCCLINTOCK 1932, PATTERSON), are responsible for certain cases of variegation but account only for the appearance of a recessive character that was present in a heterozygous condition. In the great majority of unstable genes the direction of mutation is from the recessive to the dominant. It is difficult, at least for the writer, to see how any mechanical theory as loss or rearrangement of chromosome pieces can account for the high mutability of a_1 in the presence of *Dt*. The following facts seem to negate such an explanation: (1) There is no visible chromosomal aberration as a translocation or ring chromosome in the *Dt* line. (2) The *Dt* gene remains unaltered when a mutation of a_1 takes place. On the theory of a rearrange-

ment of chromatin involving the a_1 and *Dt* loci we would expect a 1:1 correspondence between mutations of the a_1 allele and mutations of the *Dt* allele. (3) The dosage effect of *Dt* is not arithmetic as was true for the a_1 gene but is exponential. Numerous biochemical reactions such as the effect of a change of pH on enzyme activity offer an interesting parallel to the effect of changing the dosage of *Dt* if the possibility is considered that the *Dt* gene produces some chemical substance that accelerates the mutation rate of a_1 and that the amount of this substance produced by two *Dt* genes as compared with one causes the mutation rate to be more than twice as rapid. (4) Mutations of a_1 occur to at least two different alleles. (5) The loci of *Dt* and a_1 show independence in inheritance.

It also is difficult to reconcile the above statements with GOLDSCHMIDT'S view that mutations are position effects. Some dotted strains average over 200 dots or mutations per seed. On the position effect hypothesis every mutation of a_1 represents some kind of rearrangement at or near the a_1 locus. In the high dotted strains this would mean that breakages at this locus would be extremely frequent. It is difficult to visualize a mechanism by which a gene in one chromosome could produce so many breaks at or near a specific locus of another chromosome. Furthermore, unless all rearrangements are of a small intra-chromosomal type, evidence of gross structural changes should be found. The data offer no support for the position effect hypothesis as the cause of the mutability of a_1 in the presence of *Dt*.

The *Dt* gene increases, apparently, only the mutability of the a_1 allele. The mutation rates of other loci may be increased but studies made with the recessive aleurone genes, a_2, *c*, and *r*, and the recessive endosperm genes, *wx* and *su*, have shown no effect of *Dt* on the mutability of these loci. The specificity of *Dt* in affecting only the a_1 allele is in contrast to the results of DEMEREC (1937) and PLOUGH and HOLTHAUSEN (1937), who reported a general increase in mutation frequency in certain stocks of Drosophila where a mutability factor was present.

SUMMARY

1. The a_1 allele is a stable gene in the presence of *dt* but becomes highly mutable with dominant *Dt*. This pronounced influence of one gene upon the mutability of another suggests there may be no fundamental difference between stable and unstable genes.

2. The mutation frequency is proportional to the dosage of the a_1 gene.

3. Mutations of a_1 to the A_1 allele occur about a thousand times as frequently as to the a_1^p allele.

4. Mutations of a_1 occurred in the aleurone, culm and sheaths, and pericarp. The size of the mutated areas indicates that mutations of a_1

occur late in the development of each tissue. The evidence indicates that mutations do not occur consonantly in all parts of the plant.

5. Certain data indicate the existence of a dominant modifying gene which decreases the mutation frequency.

6. The a_1 and *Dt* genes are not linked. The a_1 gene is in chromosome III and *Dt* may be in chromosome IX.

7. Cytological observations of the *Dt* and *dt* stocks show no evidence of gross chromosomal aberrations.

8. The data do not support the view that mutations of a_1 induced by *Dt* result from a position effect. The *Dt* gene remains unaltered when a mutation of a_1 takes place.

LITERATURE CITED

ANDERSON, E. G., and EYSTER, W. H., 1928 Pericarp studies in maize. III. The frequency of mutation in variegated maize pericarp. Genetics **13**: 111–120.

CLAUSEN, ROY E., 1930 Inheritance in *Nicotiana tabacum*. X. Carmine-coral variegation. Cytologia **1**: 358–368.

DEMEREC, M., 1928 Mutable characters of *Drosophila virilis*. I. Reddish-alpha body character. Genetics **14**: 359–388.

1929 Genetics factors stimulating mutability of the miniature-gamma wing character of *Drosophila virilis*. Proc. Nat. Acad. Sci. Wash. **15**: 834–838.

1931 Behavior of two mutable genes of *Delphinium ajacis*. J. Genet. **24**: 179–193.

1935 Unstable genes. Bot. Rev. **1**: 233–248.

1937 Frequency of spontaneous mutations in certain stocks of *Drosophila melanogaster*. Genetics **22**: 469–478.

DEMEREC, M., and SLIZYNSKA, H., 1937 Mottled white 258-18 of *Drosophila melanogaster*. Genetics **22**: 641–649.

EMERSON, R. A., 1918 A fifth pair of factors *A a* for aleurone color in maize and its relation to the *C c* and *R r* pairs. Cornell Agric. Expt. Sta. Bull. **16**.

1921 The genetic relations of plant colors in maize. Mem. Cornell Agric. Expt. Sta. **39**: 1–156.

1929 The frequency of somatic mutation in variegated pericarp of maize. Genetics **14**: 488–511.

EMERSON, R. A., and ANDERSON, E. G., 1932 The *A* series of allelomorphs in relation to pigmentation in maize. Genetics **17**: 503–509.

GOLDSCHMIDT, RICHARD, 1938 Physiological Genetics. ix+361 pp., New York, McGraw-Hill.

HERTWIG, P., 1926 Ein neuer Fall von multiplem Allelomorphismus bei Antirrhinum. Z.i.A.V. **41**: 42–47.

IMAI, Y., 1934 On the mutable genes of Pharbitis, with special reference to their bearing on the mechanism of bud-variation. J. Coll. Agric. Tokyo Imp. Univ. **12**: 479–523.

KIHARA, H., 1932 Genetische studien an gestreiften Sippen von *Celosia cristata* L. Agric. Hort. **7**: 3–26.

MCCLINTOCK, BARBARA, 1932 A correlation of ring-shaped chromosomes with variegation in *Zea mays*. Proc. Nat. Acad. Sci. Wash. **18**: 677–681.

PATTERSON, J. T., 1932 A new type of mottled-eye Drosophila due to an unstable translocation. Genetics **17**: 38–59.

PLOUGH, H. H., and HOLTHAUSEN, C. F., 1937 A case of high mutation frequency without environmental change. Amer. Nat. **71**: 185–187.

RHOADES, M. M., 1936 The effect of varying gene dosage on aleurone colour in maize. J. Genet. **33**: 347–354.

SANDO, C. E., and BARTLETT, H. H., 1922 Pigments of the mendelian color types in maize: isoquercitrin from brown-husked maize. J. Biol. Chem. **54**: 629–645.

SANDO, C. W., MILNER, R. T., and SHERMAN, MILDRED, 1935 Pigments of the mendelian color types in maize: chrysanthemin from purple-husked maize. J. Biol. Chem. **109**: 203–211.

SCHULTZ, JACK, 1936 Variegation in Drosophila and the inert chromosome regions. Proc. Nat. Acad. Sci. Wash. **22**: 27–33.

STERN, CURT, 1935 The behavior of unstable loci—an hypothesis. Proc. Nat. Acad. Sci. Wash. **21**: 202–208.