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THE EFFECT OF VARYING GENE DOSAGE ON ALEURONE COLOUR IN MAIZE[1]

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(With One Text-figure)

INTRODUCTION

IT has not been possible, with the exception of a few outstanding cases (Sturtevant, 1925; Mangelsdorf & Fraps, 1931; Lindstrom & Gerhardt, 1926), to obtain reliable quantitative data on the change produced in a genetic character by varying the dosage of the major gene or genes determining the nature of that character. Data of this type are of value. A hypothesis which seeks to explain the role of a gene in ontogeny must take into account the change produced by varying the number of times it is present.

The aleurone tissue of the maize kernel offers exceptionally favourable material for the study of gene dosage and interaction. Several hundred seeds are produced on a single ear under the same environment. The aleurone is triploid in nature, since it arises from the union of a haploid sperm with two haploid polar nuclei. The seed on one ear may be of several genotypes. It is possible to vary the dosage of any allel from one to three in disomic strains, and the use of the proper primary trisome allows the dosage to range from one to five. In spite of these advantages there has been little work on the effect of gene dosage on aleurone colour because none of the previously known aleurone characters have been adapted to the obtaining of quantitative data. This paper presents a study of the effect of gene dosage on a new aleurone character in maize.

Four major genes are known to be involved in the development of aleurone colour in the maize kernel (East & Hayes, 1911; Emerson, 1918; Jenkins, 1932). These are $\mathbf{A}_1$, **C**, **R**, and $\mathbf{A}_2$. The $\mathbf{A}_1$ locus is in chromosome 3, the **C** locus in chromosome 9, the **R** locus in chromosome 10

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and the $\mathbf{A}_2$ locus in chromosome 5. Aleurone colour is not formed if the recessive allel of any one of the four primary loci is homozygous, i.e. at least one dominant allel of all four loci must be present. The alternative of purple or red aleurone colour depends upon the **Pr pr** genes, purple being dominant to red. A ratio of 3 coloured to 1 colourless seed is obtained in F_2 when one of the four primary loci is heterozygous; a 9 : 7 ratio when two loci are heterozygous, etc.

In a selfed ear of Black Mexican sweet corn obtained from Dr L. F. Randolph, aleurone colour segregated into 12 self-coloured: 3 dotted: 1 colourless seeds. The Black Mexican line had been maintained for several generations by sibbing. The occurrence of colourless aleurone in a strain of corn homozygous for all the dominant allels for aleurone

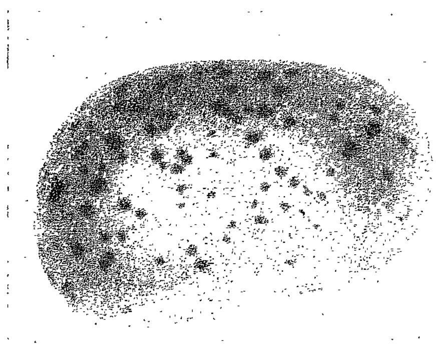

Fig. 1. Photograph of crown of maize seed showing the dotted aleurone character. The genetic constitution of this seed is $a_1a_1a_1\mathbf{DtDtDt}$ and it is homozygous for the other dominant genes necessary for aleurone colour.

colour was unexpected, but more surprising was the appearance of a new dotted aleurone character. The dots or spots of colour were distributed apparently at random over the aleurone layer. They were small and fairly uniform in size (Fig. 1).

The colour of the dots is either purple or red depending upon the **Pr pr** constitution. The intensity of the colour is greatest in the central portions of the dots with a gradual fading towards the surrounding colourless tissue.

The 12 : 3 : 1 ratio of the original ear was correctly interpreted as the segregation of one of the four primary genes, to give 12 self-coloured: 4 non-self-coloured (a 3 : 1 ratio), and a dominant gene which interacted with the recessive primary gene to give dots of aleurone colour in three-fourths of the non-self-coloured class. The segregating primary aleurone factor proved to be $\mathbf{A_1a_1}$. The dominant gene interacting with $\mathbf{a_1}$ to give the dotted character has not previously been reported. It has been designated **Dt dt**, the **Dt** allel giving dots of aleurone colour with $\mathbf{a_1}$, whereas **dt** does not.

The conclusion that the segregation of $\mathbf{A_1a_1}$ occurred following a mutation of $\mathbf{A_1}$ to $\mathbf{a_1}$ seems unescapable, because the genetic residuum of the Black Mexican line was unchanged and out-crossing would have been detected. The **Dt** gene was thought, at first, to have arisen by mutation in the same gamete in which the mutation from $\mathbf{A_1}$ to $\mathbf{a_1}$ occurred, but tests of sister plants showed the **Dt** gene to be segregating in the Black Mexican strain, its presence not having been previously detected because the strain was homozygous for the dominant allels of genes producing aleurone colour.

TABLE I

Interaction of **Dt** *with the different aleurone genes*

Genotype	Aleurone colour
$\mathbf{a_1CRA_2DtPr}$	Purple dots on colourless background
$\mathbf{a_1CRA_2Dtpr}$	Red dots on colourless background
$\mathbf{A_1cRA_2Dt}$	Colourless—no dots
$\mathbf{A_1CrA_2Dt}$	Colourless—no dots
$\mathbf{A_1CRa_2Dt}$	Colourless—no dots
$\mathbf{a_1CRA_2dt}$	Colourless—no dots
$\mathbf{A_1CRA_2Dt}$	Strong self-colour—no dots
$\mathbf{a_1^pCRA_2Dt}$	Pale self-colour—no dots
$\mathbf{A_1a_1CRA_2Dt}$	Strong self-colour—no dots
$\mathbf{a_1^pa_1CRA_2Dt}$	Pale self-colour with dots
$\mathbf{a_1cRA_2Dt}$	Colourless—no dots
$\mathbf{a_1CrA_2Dt}$	Colourless—no dots
$\mathbf{a_1CRa_2Dt}$	Colourless—no dots

Dt interacts with $\mathbf{a_1}$ to give dots of aleurone colour only if all the remaining three primary genes have at least one dominant allel present. **Dt** interacts in this manner only with $\mathbf{a_1}$. Seeds homozygous for $\mathbf{a_2}$ or for **c** or for **r**, the remaining three genes in each case being present in dominant form, have colourless aleurone in the presence of **Dt**. The interaction of **Dt** with the different aleurone genes is given in Table I.

The linkage group to which the **Dt** gene belongs has not been established. **Dt** is, however, independent in its inheritance of the $\mathbf{A_1}$, **C**, **R**, $\mathbf{A_2}$ and **Pr** loci, genes all affecting aleurone colour, as well as of the $\mathbf{Lg_1}$, **Su**, **Y** and $\mathbf{F_1}$ loci.

RELATION BETWEEN DOSAGE OF $\mathbf{a_1}$ AND NUMBER OF ALEURONE DOTS

There are four allels at the $\mathbf{A_1}$ locus. Two of these, $\mathbf{A_1}$ and $\mathbf{A_1^b}$, produce deep-coloured aleurone; $\mathbf{a_1^p}$ produces pale-coloured aleurone and $\mathbf{a_1}$ produces colourless aleurone. Nothing is known concerning the interaction of $\mathbf{A_1}$ and **Dt**, as dots of colour, if formed, are invisible in the deep aleurone colour produced by $\mathbf{A_1}$. Seeds homozygous for $\mathbf{a_1^p}$ have no dots of colour in the presence of **Dt**, although the intensity of colour in the dots on seeds of $\mathbf{a_1Dt}$ constitution is greater than in the pale aleurone colour and they would be visible if present. However, seeds carrying $\mathbf{a_1^p}$ and one or two $\mathbf{a_1}$ allels have dots of aleurone colour which are clearly evident on the pale-coloured background. Therefore it is possible to determine the effect of different dosages of $\mathbf{a_1}$ on the aleurone dots by varying the ratio of the $\mathbf{a_1}$ and $\mathbf{a_1^p}$ allels. As the aleurone is triploid the dosage of genes in it may be varied from one to three. The dosage of $\mathbf{a_1}$ may be varied while that of **Dt** is held constant, or the dosage of **Dt** may be varied while that of $\mathbf{a_1}$ is held constant.

Crosses of $\mathbf{a_1a_1^pDtDt} \times \mathbf{a_1a_1dtdt}$ were made to determine the effect of three $\mathbf{a_1}$ allels as compared with one $\mathbf{a_1}$ allel on the aleurone dots. Two classes of seed were produced on the same ear having the following constitution:

(1) $\mathbf{a_1a_1^pa_1^pDtDtdt}$ = pale aleurone with dots,
(2) $\mathbf{a_1a_1a_1DtDtdt}$ = colourless aleurone with dots.

These two genotypes occur at random on the ear. Their environment is identical. They have the same genetic residuum except for the possibility of segregating genes linked with $\mathbf{a_1}$. The other aleurone factors were present in a homozygous dominant condition. The two classes, therefore, may be considered as differing only in that one of them has one $\mathbf{a_1}$ and the other three $\mathbf{a_1}$ allels.

The number of dots of aleurone colour was determined on each seed under a low-power binocular after the seed coat had been removed to increase the accuracy of counting. Counts on the seeds in both classes were made independently by two investigators. The agreement was always close. Counts were made on the kernels from four ears, approximately fifty seeds of each class being counted from each ear. No appreciable increase in the reliability of the mean number of dots per seed was obtained by counting more than fifty seeds.

The average number of dots per seed of $\mathbf{a_1a_1a_1}$ constitution was about three times as great as on seeds of $\mathbf{a_1a_1^pa_1^p}$ constitution which is the same ratio as the number of $\mathbf{a_1}$ allels in the two classes. The data from one ear are given in Table II with a statistical analysis. The data from the three other ears also were treated statistically but the deviations from a 3 : 1 ratio were not significant. The ratio of the mean numbers of dots per seed in the two classes for all four ears was 3·1 : 1·0.

The effect of three a_1 alleles as compared with two a_1 alleles was studied by analysing the data obtained from the cross $\mathbf{a_1a_1DtDt} \times \mathbf{a_1a_1{}^{p}dtdt}$. The two classes of seed produced have the following genotypic constitution:

(1) $\mathbf{a_1a_1a_1{}^{p}DtDtdt}$ = pale aleurone with dots,

(2) $\mathbf{a_1a_1a_1DtDtdt}$ = colourless aleurone with dots.

Data on the two classes were obtained from three ears. The ratio of the average number of dots on seeds of $a_1a_1a_1$ constitution to the average number on seeds of $a_1a_1a_1{}^{p}$ constitution was 3 : 2. This is the same ratio

TABLE II

Numbers of aleurone dots on 55 *seeds with three* a_1 *genes and* 55 *seeds with one* a_1 *gene from an ear* (*Pedigree* 2946–6 × 2944–1) *of the cross* $\mathbf{a_1a_1{}^{p}DtDt} \times \mathbf{a_1a_1dtdt}$

$a_1a_1a_1DtDtdt$ class				$a_1a_1{}^{p}a_1{}^{p}DtDtdt$ class			
39	54	47	79	24	15	19	10
111	88	65	59	20	25	16	37
55	73	45	64	26	27	16	18
68	43	48	39	37	8	23	12
35	35	75	64	32	42	16	21
75	53	63	106	24	43	21	1
66	47	47	47	32	20	27	23
57	55	75	46	20	23	17	2
52	54	56	79	14	18	17	16
15	12	75	22	31	21	23	29
71	81	54	43	19	40	10	17
51	52	113	55	18	13	18	22
53	39	53	29	12	23	24	14
57	75	51	—	38	47	18	—
Mean = 58 dots per seed				Mean = 21·8 dots per seed			

t = 1·6442. (108 D/F). Not significant.

as the dosage of the a_1 allel in the two classes of seed. Data from one ear are given in Table III. The ratio of the averages for the two classes of seed for the three ears was 3·06 : 2·0. The deviations from a 3 : 2 ratio for the individual ears were not significant.

The above data indicate that the effect on the dotted aleurone character of increasing the number of recessive a_1 alleles, as determined by the number of dots, is additive or linear.

In obtaining the data on the a_1 gene the number of **Dt** genes was held constant. Crosses therefore were made in which the number of a_1 genes remained constant while the **Dt** gene varied in dosage. The following cross was made reciprocally: $\mathbf{a_1a_1DtDt} \times \mathbf{a_1a_1dtdt}$. When the dotted parent was the female the seeds were all $\mathbf{a_1a_1a_1DtDtdt}$. In the reciprocal cross, however, the seeds were $\mathbf{a_1a_1a_1Dtdtdt}$. These crosses are exact reciprocals and were made between lines originating from the ear on which the dotted character first appeared so that they have much the same genetic make-up. It is important to emphasize that these reciprocal crosses should be made between lines related as closely as possible because the seed produced on two different plants is to be compared. The dosages of any genes modifying the number of aleurone dots would be different in reciprocal crosses between unlike lines, as we are dealing with triploid tissue in which two-thirds of the heredity is contributed by the female parent. The mean number of dots per seed in the $\mathbf{a_1a_1a_1DtDtdt}$ class was about four times that for seeds of

TABLE III

Numbers of aleurone dots on 76 *seeds with three* $\mathbf{a_1}$ *genes and* 60 *seeds with two* $\mathbf{a_1}$ *genes from an ear* (*Pedigree* 2676 × 2511–*a*) *of the cross* $\mathbf{a_1a_1DtDt \times a_1a_1{}^pdtdt}$

$\mathbf{a_1a_1a_1DtDtdt}$ class						$\mathbf{a_1a_1a_1{}^pDtDtdt}$ class				
6	25	27	19	8	20	15	3	18	18	10
11	29	29	21	30	20	8	11	19	10	9
8	17	3	26	23	29	16	10	25	10	7
25	11	19	37	21	14	6	1	5	8	10
13	25	7	32	17	4	7	5	18	3	15
21	8	20	23	7	20	9	19	3	11	7
12	9	19	38	14	17	9	6	19	13	—
5	29	9	8	6	16	31	13	12	7	—
27	15	36	16	14	12	14	10	9	5	—
5	11	17	16	7	15	4	11	10	9	—
10	9	37	12	20	16	15	14	6	13	—
22	26	20	10	14	—	3	13	5	7	—
33	8	8	23	20	—	9	14	14	20	—
Mean = 17·6 dots per seed						Mean = 11·0 dots per seed				

$t = 0{\cdot}8532$. (134 D/F). Not significant.

$\mathbf{a_1a_1a_1Dtdtdt}$ constitution. Data on one reciprocal cross are presented in Table IV. The data are not as extensive as might be desired but the deviation from a 4 : 1 ratio of the means for the two kinds of seeds is not significant. The ratio of the means of the two seed classes for the three reciprocal crosses approximated 4 : 1, the actual ratio being 4·2 : 1·0. There is no *a priori* reason for assuming theoretically that the ratio of these two means be exactly 4 : 1. The data from the three ears ranged about this value so it was arbitrarily chosen. The ratio of dosages of **Dt** in the reciprocal crosses was 2 : 1. However, the effect on the number of aleurone dots was not additive as was the case for the $\mathbf{a_2}$ gene. There were four times as many dots when two **Dt** genes were present as there were where only one **Dt** gene was present.

Data on the effect of three **Dt** genes can be obtained from a selfed plant homozygous for the **Dt** gene. Obviously these data cannot be compared with those for two and one doses of **Dt** unless the different lines used in obtaining the three different dosages of **Dt** were very closely related. The only data available for three **Dt** genes came from seed obtained by selfing a plant in a line derived from the original dotted ear. It is, therefore, related to the other lines, but some differences in genetic constitution could and undoubtedly did exist. The data should be considered with discretion. The average number of dots per seed on this selfed ear was 185·0. The average numbers of dots on seeds with two and one **Dt** genes were 45·4 and 10·4, respectively. The data on the three **Dt** genes are, perhaps, not strictly comparable with the others, but it is possible to state, considering the data on the three classes of seeds,

TABLE IV

Numbers of aleurone dots on individual seeds from reciprocal crosses, one cross yielding seeds with two **Dt** *genes and the other yielding seeds with one* **Dt** *gene*

Pedigree 2951–9 × 2949–8 $\mathbf{a_1a_1DtDt \times a_1a_1dtdt}$ $\mathbf{a_1a_1a_1DtDtdt}$ seeds				Pedigree 2949–8 × 2951–9 $\mathbf{a_1a_1dtdt \times a_1a_1DtDt}$ $\mathbf{a_1a_1a_1Dtdtdt}$ seeds			
55	77	55	37	8	16	30	16
29	69	19	28	4	12	15	6
45	69	32	49	7	8	7	10
20	42	35	36	8	3	8	3
17	57	56	—	14	11	10	—
20	52	52	—	12	17	9	—
78	16	46	—	9	1	7	—
87	27	37	—	14	10	18	—
100	20	45	—	6	12	10	
Mean = 45·4 dots per seed				Mean = 10·4 dots per seed			

$t = 0{\cdot}7075$. (60 D/F). Not significant.

that the effect of increasing the dosage of the **Dt** gene is more nearly exponential than linear. More data will be obtained on this problem when the proper lines have been synthesized.

If the conclusions reached on the dosage effects of $\mathbf{a_1}$ and **Dt** are valid, it should be possible to predict the outcome of previously untried combinations. A plant of $\mathbf{a_1a_1^{P}Dtdt}$ constitution was self-pollinated. The resulting seeds were classified into 102 pale coloured: 92 pale coloured with dots: 50 colourless with dots: 16 colourless. This is a close approximation to the expected ratio of 6 : 6 : 3 : 1. It was predicted before the dots were counted that the mean number per seed in class $\mathbf{a_1Dt}$, colourless background with dots, would be twice that of the class $\mathbf{a_1^{P}a_1Dt}$, pale background with dots. The mean number of dots on the fifty seeds comprising the three $\mathbf{a_1Dt}$ classes was 45·0, while the mean number of dots on the ninety-two seeds of the six $\mathbf{a_1^{P}a_1Dt}$ classes was 23·0.

The dotted aleurone character is expressed only in seeds of $\mathbf{a_1CRA_2Dt}$ constitution. Dominant **C**, **R** and $\mathbf{A_2}$ are, therefore, as necessary for the expression of the dotted character as recessive $\mathbf{a_1}$. The number of dots has been shown to be dependent upon the number of doses of $\mathbf{a_1}$. What the effect would be if the number of doses of dominant **C** or **R** or $\mathbf{A_2}$ varied is a problem for the future as little information has been obtained. Certain data, however, suggest that seeds with one dominant **C** gene have fewer dots than seeds with two dominant **C** genes. This problem will, of course, be prosecuted, as it promises to open up an extensive field of investigation on the dosage effects of the several genes affecting aleurone colour.

Conclusion

1. A new dominant gene **Dt** interacts with recessive $\mathbf{a_1}$ in the presence of the dominant allels of the other factors concerned with aleurone colour to give coloured dots scattered over the aleurone layer. **Dt** is specific in its interaction with recessive $\mathbf{a_1}$.

2. The effect on the dotted character of varying the dosage of recessive $\mathbf{a_1}$ was additive. Data on three dosages of $\mathbf{a_1}$ were obtained.

3. The effect on the number of dots of varying the dosage of the **Dt** gene was non-additive.

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