THE ASSOCIATION OF SIZE DIFFERENCES WITH SEED-COAT PATTERN AND PIGMENTATION IN *PHASEOLUS VULGARIS*[1]

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INTRODUCTION

The work of EAST, EMERSON, HAYES and others with plants, and CASTLE's work with animals, show that inherent size differences are apparently dependent on Mendelizing factors. In some cases, such as habit of growth in peas and beans and certain abnormal characters in maize, the quantitative characters involved are dependent on single factor differences, although in most cases many factors are involved and simple ratios are not obtained. In fact there is some evidence to show that quantitative differences may sometimes depend on genetic factors in most or all of the chromosomes.[2]

The multiple-factor hypothesis of size inheritance does not necessarily mean that the various factors are equal in effect or that they are interdependent for their expression. The relative effect of various factors and the cumulative effect of factor combinations have been difficult to analyze because the size factors could not readily be isolated and studied independently. If, however, certain size factors can be found linked with factors for qualitative characters it should be possible to study independently the size factor or factors within each linkage group. This is now possible in a limited way with the size differences in beans.

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[2] In a recent paper, GATES (1923) maintains that increased variability of petal size in the F_2 of Oenothera hybrids is not due entirely, at least, to Mendelian size factors, and suggests that "perhaps sizes in general, where repeated in the same organism, are not Mendelian in their inheritance."

The work of TSCHERMAK, EMERSON, SHULL and SPILLMAN on the inheritance of vine and seed-coat characters in the bean has been reviewed by SHAW and NORTON (1918) who find the earlier hypotheses in accord with the results of their own extensive studies. Pigmentation is dependent on a factor *P*. Total pigmentation is dependent on an extension factor *T*, the recessive of which results in a partially pigmented or "eyed" bean. Mottling is, according to the theory of SPILLMAN and EMERSON, dependent on two factors *Y* and *Z* in the same chromosomes. *Y* and *Z* may also exist separately in non-mottled beans and when brought together in crosses give mottled beans which seldom if ever become homozygous for mottling. Other factors control type of mottling and color of pigmentation. The inheritance of pattern and pigmentation in beans is often complex because of the numerous factors usually involved.

The parental varieties used in the present study include two varieties classed under the local names of Improved Yellow Eye and Dot Eye. In the Improved Yellow Eye the pigment is uniform or "solid" (self-colored) and is restricted to the area around the hilum and covers from a quarter to a third of the surface of the bean. The Dot Eye has only a very small pigmented area at either end of the hilum. In both of the eyed varieties the color of the pigment is yellow. For the non-pigmented parents several pure lines of small white beans were used. In all cases the pigmented varieties were about twice as large as the white varieties.

The parents and F_1 plants and all F_2 plants which were continued to F_3 were grown in an insect-proof cage to prevent uncontrolled cross-pollination, although cross-pollination does not occur frequently in the field. All F_2 beans for which weight records were made were grown in 1920. A glass scale designed by the writer (SAX 1921) was used for obtaining individual bean weights. The average seed weight in centigrams was obtained for F_2 and F_3 segregates. Most of the field work and description of F_2 segregates has been done by H. C. MCPHEE. The parental varieties were obtained from selections made by Doctor FRANK SURFACE.

GENETIC BEHAVIOR OF CROSSES

The genetic behavior of some of the crosses is shown in table 1. In the cross Improved Yellow Eye×White 1333 the F_1 was dark mottled and the F_2 gave a ratio of 27 mottled : 9 self-colored : 12 eyed : 16 white. The χ^2 test for goodness of fit gives a value of P = .83. The proportion of mottled and self segregates indicates that both of the mottling factors, *Y* and *Z*, were carried in the same chromosome by the white parent. The extension factor and factors for black, brown and purple color were also carried by the white parent but could not be brought into expression until combined with the pigmentation factor P.

In the cross Dot Eye × White 1228 the F_1 was mottled. The approximately equal number of mottled and self-colored beans in F_2 indicates that the mottling factor Y came from one parent and factor Z from the other parent. The proportion of eyed beans is somewhat too large to result from two recessive factors and too small to result from a single factor, but otherwise the theoretical ratio fits very well (P = 0.57). It is assumed that two extension factors are present in 1228 because such a hypothesis is in accord with the results in the following cross.

TABLE 1

Segregation of pattern types in F_2 of eyed×white crosses.

CROSS	MOTTLED	SELF-COLORED	EYED	WHITE	TOTAL	GOODNESS OF FIT
Improved Yellow Eye 1310×White 1333	150	51	68	80	349	
Theoretical for 27 : 9 : 12 : 16 ratio	147	49	65	87		P = .83
Dot Eye 1902×White 1228	93	101	28	67	289	
Theoretical for 90 : 90 : 12 : 64 ratio	102	102	14	72		P = .001
Improved Yellow Eye 1317×White 1228	82	44	12	41	179	
Theoretical for 45 : 3 : 16 ratio	126		8	45		P = .32

The cross Improved Yellow Eye × White 1228 gave a purple mottled F_1 and a very complex segregation in F_2. The entire F_2 was carried to the F_3 and it was found that no mottled beans bred true. If Y came from one parent and Z from the other then no homozygous mottled beans should be obtained except by crossing over, but the ratio of mottled to self-colored should be 1 : 1. By grouping all completely pigmented beans a fairly good 45 : 3 : 16 ratio is obtained. Total pigmentation is apparently effected by two extension factors the double recessives of which result in eyed beans.

SEED WEIGHT OF VARIOUS CLASSES OF SEGREGATES

In all crosses the pigmented parent was at least twice as large as the non-pigmented parent. The large parents contributed large size of bean, the pigmentation factor P, the recessive or recessives of the extension factor which results in eyed beans, and one of the mottling factors. If size factors are present in most or all of the chromosomes, then there should be some correlation between pigmentation and pattern of F_2 beans and size. In all crosses of large pigmented varieties with small white varieties, involving 3865 progeny, the white segregates were significantly smaller than the pigmented segregates. The relation between pigmentation and pattern and average bean weight of F_2 plants is shwno for several of the larger populations in table 2.

TABLE 2

Showing average seed weight in centigrams, of parents, and mean seed weight of various classes of segregates in F_2 and F_3 of certain bean crosses.

PARENTS				F_2 SEGREGATES				
♀	*Weight*	♂	*Weight*	*Mottled*	*Self*	*Eyed*	*White*	*Total*
I. Y. E. 1310	56. ± .5	White 1333	28 ± .9	39.1 ± .4	36.5 ± .5	39.0 ± .4	33.8 ± .4	349
Dot Eye 1902	58. ± 1.2	White 1228	21 ± .2	27.8 ± .4	27.7 ± .4	26.6 ± .5	23.4 ± .5	150
I. Y. E. 1317	48. ± .5	White 1228	21 ± .2	28.8 ± .4	28.6 ± .6	31.3 ± 1.1	26.4 ± .5	179
I. Y. E. 1317	48. ± .5	White 1228F_3	21 ± .2	29.2 ± .1	29.0 ± .1	30.2 ± .2	25.8 ± .1	2438

In the cross Improved Yellow Eye ($P\ t\ \widehat{yZ}$ or $P\ t\ \widehat{Yz}$) × White 1333 ($p\ T\ \widehat{YZ}$) all pigmented classes are significantly larger than the white F_2 segregates. The mottled and eyed F_2 segregates are also significantly larger than the uniformly pigmented or self-colored beans. It might be expected that large size would be associated with the eyed factor as large size and eyed came in from one parent, but the increased size of the mottled segregates is unexpected as the mottling factors were apparently contributed by the smaller parent. However, if the mottling hypothesis is correct then two-thirds of the mottled beans will carry the Improved Yellow Eye chromosome which carries one of the mottling factors and probably a factor for large size. It is also possible that the chromosome from the small white bean which carries the mottling factor carries a factor for large size. The small bean could carry several factors for large size and yet be relatively small due to the predominance of factors for small size. No significant correlation was found between bean weight and color of pigment in this cross.

There is no significant difference in size of the pigmented classes of F_2 segregates in the cross Dot Eye ($P\ t\ t\ \widehat{yZ}$) × White 1228 ($p\ T\ T\ \widehat{Yz}$). The mottled and solid segregates are somewhat larger than the eyed segregates but this difference is only twice the probable error and is of doubtful significance. All classes of pigmented segregates are significantly larger than the white segregates. The difference between the weight of eyed and white segregates is 4.6 times the probable error. The fact that there is no difference between size of mottled and self-colored segregates indicates that the chromosomes which carry the mottling factor in this cross carry different size factors than those in the preceding cross.

The weights of the different classes of segregates in both F_2 and F_3 are shown for the cross Improved Yellow Eye $(P\ t\ t\ \widehat{yZ})$ $\times$ White 1228 $(p\ T\ T\ \widehat{Yz})$. The pigmented classes are all heavier than the F_2 white segregates although the differences are only about 3 times the probable error in case of the self-colored and mottled segregates. The eyed segregates are larger than the other pigmented classes although the difference is of doubtful significance in F_2 because of the few individuals. In F_3, however, it is clear that the eyed segregates are significantly larger than the mottled or self classes. There is also some correlation between bean weight and eye pattern in F_3. The eye types were divided into 8 classes ranging from dot eye to almost completely pigmented. The correlation between eye type and bean weight for 307 eyed segregates gave a correlation ratio of $.30 \pm .04$. The dot-eyed and types similar to Improved Yellow Eye were smallest, while intermediate types were largest. It was also found that among the self-colored and mottled F_3 classes the yellow and brown segregates were heavier than the purples and reds. The difference is more than twelve times the probable error. The genetic behavior of the various colors is very complex and has not been completely worked out.

TSCHERMAK (1922) finds some evidence for segregation in size of beans borne on the F_1 plant. In the above cross the coefficient of variability for weight of beans on the F_1 plants was $18.0 \pm .7$ as compared with $10.8 \pm .9$ and $11.7 \pm .7$, respectively, for the parents. There is, however, little indication that the increased variability of F_1 is due to genetic factors for bean size. The correlation between bean weight of F_1 and bean weight in F_2 was found to be $.08 \pm .06$ (η). For F_1 bean weight and F_2 plant height $\eta = .36 \pm .05$, but this relation may be due to greater reserve food in the larger beans. There was no significant correlation between F_1 bean weight and F_2 pattern $(\eta = .10 \pm .06)$.

Bean size is apparently not greatly influenced by plant height. In the above cross (Improved Yellow Eye $\times$ 1228) the correlation between F_2 bean weight and F_2 plant height gave a value of $r = .05 \pm .06$. For F_2 pattern and F_2 plant height $\eta = .12 \pm .06$. These correlations were determined because it was thought that since pigmented beans often have darker foliage than white beans that there might be an increase in plant size with darker foliage and perhaps some relation between plant size and bean weight. However, the large eyed beans used in this cross have white blossoms and pale green foliage,—a characteristic of all eyed and white beans used as parents.

Crosses of pigmented beans which differ in size also show association of size differences with pattern. In the cross of Improved Yellow Eye 1318 (weight = 52.1 ± .8) × Cranberry 1906 (weight = 70.0 ± 1.6) the F_2 mottled class had a mean weight of 56.9 ± .9; self-colored beans weighed 55.3 ± .7; while the eyed segregates weighed only 50.5 ± .8 centigrams.

THE NATURE OF FACTORS INVOLVED IN SIZE DIFFERENCES IN BEANS

So far as data are available, the behavior of the inheritance of size differences is in accord with the eight requirements proposed by EAST (1916) to test the multiple-factor hypothesis. No data on weight of F_1 beans were taken and even if F_1 weights were available they would be of little value due to paucity of numbers. The parents were pure lines and had been grown in a screened cage for six years so that the F_1 should be no more variable than the parents. The variability of the F_2 is greater than that of the parents. The grandparental types are occasionally recovered. There is a significant correlation between size of F_2 and resulting F_3 segregates. For Improved Yellow Eye × White 1228 this correlation gave a value of $r = .33 \pm .01$.

The number of size factors involved was estimated by CASTLE'S (1921) formula and by several other methods. These various methods give the number of size factors as about 4 to 6 in two of the crosses and more than 6 in the third cross (Dot Eye × 1228). However, the various assumptions necessary in estimating the number of size factors, based on F_2 distribution, make the results obtained of little or no value as SHULL (1922) has pointed out.

It is possible in these bean hybrids to determine the effect of a single factor or a group of closely linked factors. In this discussion an independent size factor may be taken to mean a single factor or several closely linked factors. In the cross Improved Yellow Eye × 1228 the pigmented beans had a mean weight of 29.0 while the white segregates weighed 26.4 centigrams. The pigmented F_2 segregates consist of homozygous and heterozygous individuals in the ratio of 1: 2. The genetic constitution of F_2 pigmented segregates was determined from F_3 behavior. The failure of a white segregate to appear in 8 or more F_3 segregates from a single F_2 pigmented plant was taken to indicate that the F_2 plant was homozygous for *P*. The chances are ten to one that an F_2 plant is homozygous for pigmentation if no whites appear in 8 F_3 progeny (see MULLER 1923). The F_2 segregates homozygous for pigmentation, and therefore homozygous for at least one size factor, except for crossing over, were significantly larger than heterozygous individuals. The average weight of the 45 homozygous segregates was 30.7 ± .6, while the mean weight of 80 F_2 segregates heterozygous for size was 28.3 ± .3. The difference in weight of these classes is more than three times the probable error. The homozygous segregates (*PP*) were 4.3 ± .8 centigrams heavier than the white segregates (*pp*), while heterozygous F_2 individuals (*Pp*) were 1.9 ± .6 centigrams heavier than the non-pigmented class. Thus, both allelomorphs for large size which are associated with *P* control about 16 percent of the parental difference, while the factor in a heterozygous condition controls about half of this amount. In this case two factors have approximately double the effect of one factor within a single homologous pair of chromosomes, indicating that there is no dominance of size factors. The size factor or factors associated with the eye factors also cause an increased weight above that of the totally pigmented beans. The size factor or factors associated with the extension or eye factors have only about half the effect on seed weight as the size factor linked with the pigmentation factor, indicating that different size factors may not have an equivalent effect. This difference in effect of size factors may, however, be attributed to greater frequency of crossing over between the eye factor and size factors than between pigmentation factors and size factors.

In the cross Improved Yellow Eye × 1333 the pigmented F_2 segregates have a mean weight of 4.8 ± .5 centigrams heavier than the mean weight of the white segregates. It was not possible to compare the size factors in the heterozygous and homozygous condition since the F_3 was not grown to test the genetic constitution of F_2 segregates. Size factors are also associated with mottling and extension factors in this cross.

In the cross Dot Eye × White 1228 the pigmented segregates are 4.2 ± .6 centigrams heavier than the white segregates, so that about 11 percent of the parental difference is controlled by the factors associated with pigmentation, two-thirds of which are heterozygous.

In these crosses the factor or factors associated with pigmentation control about 4 or 5 centigrams of seed weight or about one-fifth or one-sixth of the difference in weight of the parental varieties. If the size factors in other linkage groups have a similar effect then the number of factors involved in the various crosses is about 5 or 6. However, the size factors in different linkage groups may not have the same effect on seed weight as shown by the comparison of the mean weight of totally pigmented, eyed, and white segregates in the cross Improved Yellow Eye × 1228.

The size factors in beans are independent in causing increased seed weight and several factors have a cumulative effect. Even within a single homologous pair of chromosomes the allelomorphic factors in the homozygous condition have double the effect of a single factor in the heterozygous condition. These results are not in accord with behavior of size factors in rabbits. CASTLE (1922) finds that size factors are present in most or all of the chromosomes and yet there is no association of size difference with any one of four independent color and pattern factors. Accordingly, CASTLE concludes that the size factors in rabbits are interdependent for their expression.

The fact that a size factor in the $1n$ or heterozygous condition has only half the effect that it does in the $2n$ or homozygous condition is of interest in connection with JONES'S (1917) heterosis hypothesis. If all of the size factors in Phaseolus behave in a similar way then no hybrid vigor would be expected. As a matter of fact TSCHERMAK'S (1922) data indicate that in bean crosses the F_1 seed weight is less than that of the mid-parent. As shown in table 2 the mean weights of F_2 classes are less than the weight of the mid-parent. The decreased weight may be due to the dominance of some of the weight factors contributed by the smaller parent, or it may be attributed to heterozygosis. Since there is an inverse ratio between number of beans per plant and bean weight it is possible that heterozygosis increases fecundity and thereby decreases seed weight.

SUMMARY

Crosses between certain eyed (partially pigmented) and white beans resulted in completely pigmented mottled beans in F_1 and mottled, self-colored, eyed, and white beans in F_2. Mottling is dependent on two factors in the same linkage group, both of which are necessary to produce mottling. Completely pigmented beans are dependent on either one or two extension factors, the recessives of which result in eyed beans. Pigmentation is dependent on a single factor.

In all crosses of large pigmented beans with small white beans the pigmented F_2 segregates had a mean seed weight greater than that of the white segregates. In the cross Improved Yellow Eye × White 1228 the difference in average seed weight between homozygous pigmented F_2 segregates and white segregates was found to be about one-sixth of the F_2 range. The difference between the weight of heterozygous pigmented F_2 segregates and white segregates is only about half as great as the difference between homozygous pigmented individuals and the white segregates. Thus, a size factor (or group of closely linked factors) in the $1n$ or heterozygous condition has only about one-half of the effect that it has in the $2n$ or homozygous condition. This lack of dominance is of

interest in connection with the hybrid-vigor hypothesis. Factor differences for seed weight are also associated or linked with one or both of the eye factors, with eye pattern factors, and with factors which determine the color of the pigment. Size differences even in case of blending inheritance where several factors are involved, may be effected by the independent action of the size factors in different linkage groups. These factors, when combined, have a cumulative effect. The size factors in different chromosomes may not be equal in their effect.

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