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The Effects of Complementary Epistasis on the Inheritance of a Quantitative Character, Seed Size in Lima Beans[1]

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SYNOPSIS

The inheritance of seed size in a lima bean cross was influenced by a form of complementary epistasis. This was evidenced by depression of means with inbreeding, significance of scaling statistics, skewness of frequency distributions and mean arrays, and distortion of other genetic effects. Distortion was most apparent in the results of a variance component analysis and a factorial analysis.

THE effects of genetic interaction in quantitative inheritance are complex and difficult to analyze. Until more powerful and refined analytical methods are devised and tested, we will be greatly restricted in our detection, measurement, and interpretation of these effects. Present methods are largely inadequate beyond mere detection of certain interactive mechanisms.

Some of the difficulties involved became apparent in the course of a study on the inheritance of seed size in a lima bean cross. The experiment was originally designed to compare two methods of analysis of quantitative data. However, a form of genetic interaction, complementary epistasis, contributed to the expression of the seed size character and this resulted in unforeseen complications. This paper presents the concept of complementary epistasis, its detection in the lima bean experiment, and its effects on the manifestation of other genetic mechanisms in the experiment. This discussion is to serve as a particular illustration of an apparently general phenomenon in quantitative inheritance.

REVIEW OF LITERATURE

Genetic interaction in quantitative inheritance has been discussed by Hutchinson, Panse, and Govande (7) who suggested that epistasis as well as dominance might contribute to heterosis. Mather (11) differentiated two types of interaction: one giving rise to metrical bias, which could be eliminated by proper scaling of the data, and epistasis, which often could not be scaled out. Khambanonda (10) studied fruit size in red pepper and tentatively attributed his results either to dominance or to complementary gene effects.

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Anderson and Kempthorne (2) presented a model designed to detect epistatic effects and applied it to two sets of data. In both cases they found interaction present. Allard (1), in a diallel study of nine lima bean strains, indicated that heterotic effects were possibly due to a form of complementary epistasis. Cockerham (3) described the use of analyses of variances and covariances among relatives in detecting epistatic biases and set up a generalized scheme applicable to all epistatic situations.

In addition, Hayman (5), Dickinson and Jinks (4), Jinks (8), Kempthorne (9), and others have discussed the problems of detection and measurement of non-allelic interaction.

Two methods of analysis of quantitative data to which reference will be made are a variance component method as developed by Mather (12) and a factorial method described by Powers, Locke, and Garrett (13).

MATERIALS AND METHODS

A cross between two lima bean strains, L96 and L44, from the University of California accession series provided the material for this study. L96 is a short determinate type with non-variegated leaves and white seed coats. L44 is a taller determinate type with variegated leaves and red seed coats. It matures somewhat later than L96.

The original cross was made in 1949. Seven generations were included in the main planting of 1952: P_1(L96), B_1 (first backcross to L96), F_1, F_2, F_3 families, B_2 (first backcross to L44), and P_2 (L44). These were grown in three replicates of 133 plots each, randomized within replicates. Each replicate consisted of the following number of plots per generation: P_1 — 4, P_2 — 4, F_1 — 3, F_2 — 15, B_1 — 2, B_2 — 2, F_3 — 103 (families), for a total of 133 plots.

Each F_3 family was represented once in each replicate. Plots were 16 feet long, and were originally thinned to one foot between plants or 17 per plot. Many plants did not survive, however, and the number of harvested plants actually contributing to the calculations varied from plot to plot. The final mean number of plants per plot was 12. Of the F_3 families, selected at random from a previous F_2 population, two were later eliminated because of insufficient numbers.

Whole full seeds from each plant were counted and weighed to the nearest 5 grams. A seed index was calculated as grams per seed and log grams per 100 seed.

Means and standard errors were calculated for all generations both on arithmetic and log scales (table 1). Plot and replication differences were inconsistent as to significance and for convenience were largely ignored. Standard errors for the gram scale were based on generation error variance and for the log scale on generation grand total variance.

The procedures of calculation for the variance component and factorial methods were substantially as described by Mather (12) and Powers, Locke, and Garrett (13), respectively. Changes from the original procedures stemmed from certain deficiencies and changes in the design of the experiment.

RESULTS AND DISCUSSION

A complementary relationship may be defined as one in which all members of a complex must be present for the complex to function. The function may be a narrow one or quite variable once the threshold is passed. The genetic phenomenon of complementary epistasis has been reported definitely in the literature only in the narrow sense, in which its most prominent feature is the all-or-nothing effect typified by the clear-cut 9:7 ratio in a dihybrid F_2.

In other words, the genes operating in such a complementary complex contribute to two phenotypes only. All genotypes having at least one dominant at each locus are phenotypically identical. All genotypes with at least one locus having a pair of recessives are also phenotypically alike.

It will be shown in this paper that complementary epistasis need not be so restricted in its function, but may act in a more generalized manner as a mechanism contributing to a measurement character, i.e. a character whose phenotypic variation is continuous and reflects the action of several genes of small, similar effects. Furthermore, these genes may show additive and multiplicative effects as well as dominance once the complementary requirement is satisfied.

Models of Genetic Action. To illustrate and compare the effects of various genetic mechanisms, six models of genetic behavior in a dihybrid cross *(AABB × aabb)* were constructed. Genes *Aa* and *Bb* are equal in effect. The genotype *aabb* has a value of 1 unit and *AABB* a value of 5 units. The midparental value is three units. The magnitudes of the other genotypes vary according to the mechanism illustrated by the particular model. The six models are: *(1)* additive, *(2)* dominant, *(3)* geometric, *(4)* additive complementary, *(5)* dominant complementary, and *(6)* geometric complementary. Table 2 summarizes the ratios of the various genotype groups in the F_2 and F_3 generations and the values for each genotype in each of the six models. Table 3 gives the means for the F_1 and the 4 segregating generations for each model.

Model 1 illustrates a simple additive mechanism in which each capital allele adds one unit. The F_1 mean is identical to the midparent and there is no change with inbreeding. The backcross means are midway between the F_1 mean and the respective parents.

When each large allele is dominant to its small partner and with inter-allelic independence, model 2 results. In this model the F_1 mean is identical to the large parent and there is a fall with inbreeding of one-half the difference between the midparent and the previous generation mean. The B_1 mean is closer to the midparent than in model 1 and the B_2 mean is equal to the large parent.

In model 3 the addition of capital alleles multiplies the value of the previous genotype by a constant factor of 1.5. The F_1 mean is now smaller than the midparent. There is regression with inbreeding towards the midparent, or, more precisely, approaching the value 2.63. Both the backcrosses have comparatively low values.

Table 1.—Means and standard errors for all generations on gram and log gram scales. M = midparent.

Generation	N	Gram scale	Log gram scale
P_1	171	0.4032 ± 0.0019	1.6045 ± 0.0022
B_1	34	0.4521 ± 0.0112	1.6515 ± 0.0097
F_1	69	0.5942 ● 0.0049	1.7729 ± 0.0036
F_2	644	0.5690 ± 0.0035	1.7498 ± 0.0027
F_3	3193	0.5389 ± 0.0013	1.7241 ± 0.0014
B_2	46	0.7046 ± 0.0226	1.8382 ± 0.0136
P_2	83	1.1824 ± 0.0097	2.0712 ± 0.0041
M		0.7928	1.8879

Table 2.—Genotype values for each of six dihybrid models. F_2 and F_3 ratios calculated after grouping genotypes of identical values (*AaBB = AABb*, etc.).

Genotype	Ratio		Model values					
	F_2	F_3	1	2	3	4	5	6
AABB	1	9	5	5	5	5	5	5
AaBB	4	12	4	5	3.38	4	5	3.38
AaBb	4	4	3	5	2.25	3	5	2.25
AAbb	2	18	3	3	2.25	1	1	1
Aabb	4	12	2	3	1.5	1	1	1
aabb	1	9	1	1	1	1	1	1

Table 3.—Mean values for five generations for each of six dihybrid models.

Model	Generation means				
	F_1	F_2	F_3	B_1	B_2
1	3	3	3	2	4
2	5	4	3.5	2.5	5
3	2.25	2.43	2.53	1.56	3.5
4	3	2.5	2.25	1.5	4
5	5	3.25	2.56	2	5
6	2.25	2.16	2.09	1.31	3.5

The next three models illustrate the effects of complementary epistasis superimposed upon each of the first three mechanisms, respectively. In those genotypes in which the complementary requirement is satisfied, model 4 is an additive case as in model 1. Although the F_1 mean is equal to the midparent, there is regression with inbreeding away from this value, towards the small parent, specifically towards the value 2. The B_2 mean is the same as in the "normal" additive model, while the B_1 mean is quite small.

If dominance is also present, its effects are shown as in model 5. This is similar to the familiar case often cited. The F_1 mean is like the large parent, but with inbreeding there is a strong regression past the midparent, again towards the value 2. The B_2 mean has the same value as the large parent. However, the B_1 mean is halfway between the small parent and the midparent, as in the additive model 1.

Finally, model 6 incorporates epistasis and geometric gene action. The F_1 mean is smaller than the midparent. Inbreeding does not cause regression towards the value 2.63 as in model 3, but in the opposite direction, again approaching the number 2. Both backcross means are quite low.

Models 4 and 6 are, of course, the departures from expected complementary action and represent the possible broader function discussed earlier.

It is clear from these two gene models that the most striking characteristic of complementary epistasis is the depression of the generation means in the process of inbreeding. This marked effect can also be demonstrated in various combinations in models of three or more gene pairs. No other type of epistasis is known which has the same range of effects. The mechanism is non-operative in the first backcross to the large parent and in the F_1 and cannot be recognized in these generations. The depression of the means is also evident in the backcross to the small parent in comparing models 4, 5, and 6 with 1, 2, and 3, respectively.

The key to recognition in this type of comparison is in the inbreeding process. Other effects of the epistasis are obvious in comparisons among several models, but in any single experiment the set of means comprise only one model and the most reliable comparisons are among them. At least the first two filial generations are necessary to detect a downward trend; in certain cases, viz. dominance plus complementary epistasis as in model 5, advanced filial generations are needed to show the trend through the midparent.

Lima Bean Experiment.—In the 1952 lima bean experiment, seed indices for the various generations were in terms of grams per seed for the factorial analysis as shown in table 1. It became immediately apparent that the F_1, F_2, and F_3 means bore an unusual relationship to each other and to the parental values, i.e. there was apparent regression away from the midparent towards the small parent. A "t" test (table 4) disclosed that in fact the means differed highly significantly from each other: the F_1 mean was greater than the F_2 mean and the F_2 mean was greater than the F_3 mean. In addition, both backcross means were markedly depressed; the B_1 mean exceeded the P_1 mean only slightly, and the B_2 mean was actually smaller than the midparent. The influence of a form of complementary epistasis was thus indicated.

Table 4.—Comparison of F_1, F_2, and F_3 means, using 1952 data.

Comparison	F_1 versus F_2	F_2 versus F_3
t value	4.00**	7.12**

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{V_1}{N_1} + \frac{V_2}{N_2}}}$$

, where $\bar{x}_i$ = generation mean, V_i = generation error variance, N_i = degrees of freedom of error variance.

** Significance at 1% level.

Table 5.—Means and standard errors of P_1, P_2, F_1, and F_2 generations planted in 1951. Gram scale. M = midparent.

Generation	N	Mean
P_1	15	0.4386 ± 0.0037
F_1	8	0.6341 ± 0.0150
F_2	50	0.5740 ± 0.0119
P_2	11	1.1274 ± 0.0270
M		0.7830
t value (F_1 versus F_2)		3.47**

** Significant at 1% level.

A preliminary planting had been in the field during the previous summer (1951). Included were plants of the P_1, P_2, and F_1 generations, set out in several rows with single plants alternating in the following sequence: P_1 F_1 P_2 P_1 F_1 P_2 etc., and a separate group of F_2 plants. This planting served mainly for observation and seed increase, but some data were taken on seed size. These are shown in table 5. Although relatively meager, they show the same trend: regression of the F_1 and F_2 means away from the midparent towards the small parent. The difference between the F_1 and F_2 means was highly significant. The premise that this type of regression is characteristic only of complementary epistasis, together with the close agreement of the data of both years, appears to warrant the conclusion that this epistasis is a contributing mechanism to the genetic basis for the seed size character in this cross.

Two other aspects of the data support this conclusion, although they are not of critical value. First, in the tests suggested by Mather (12) for determining the proper scale prior to analysis, the statistics A, B, C, and D were all significantly different from zero. This indicated the presence of non-allelic interaction and, since a simple log transformation (see table 6) did not change the results, may be interpreted as reflecting the complementary interaction.

Table 6.—Results of scaling tests. Scale satisfactory of A, B, C, and D are not significantly different from zero. Mather (12).

Scaling statistic	Gram scale	Log gram scale
A	−0.0932 ± 0.0222	−0.0745 ± 0.0199
B	−0.4675 ± 0.0470	−0.1677 ± 0.0277
C	−0.4980 ± 0.0206	−0.2223 ± 0.0137
D	−1.6280 ± 0.0381	−0.7801 ± 0.0193

All significant at 1% level.

The second aspect concerns the generation frequency distributions and the array of generation means. Both the F_2 and the F_3 on the gram scale were non-normal, i.e. they were skewed with the tail extending to the right. Probably the backcrosses were similarly skewed, though this could not be shown statistically. The array of means was shifted strongly to the left, so that all generations except the large parent were smaller than the midparent. A log transformation did not markedly alter the relationship. Again, complementary epistasis seemed responsible (figure 1).

The relationships of figure 1 show greater similarity to models 4 or 6 than to 5. The evidence is not sufficient to enable one to definitely ascribe the action of the lima bean size genes to either of those two mechanisms. However, the possibility certainly exists and suggests the operation of the complementary mechanism in more than one restricted form as discussed earlier.

The insufficiency of the evidence was probably due to the masking and distortion of other genetic effects by the epistatic mechanism, particularly in the results of the component and factorial analyses.

For example, in the variance component analysis, of the four components (D, H, E_1, E_2) only H (dominance deviations) was significantly different from zero. The other three, although positive, had standard errors sufficiently large to void them. Discussion of statistically non-existent components is of course difficult, but the model makes the actual existence of these three necessary.

Perhaps, then, the effect of the epistasis was to distort the biological model so that it did not fit the postulated mathematical model. This could have shown up in at least two ways. First, it might have caused an abnormal inflation of the variances of some or all of the components resulting in their being statistically zero. Second, any residual interaction after scaling would have appeared in one or more of the components of the variance equation, since there was no component labeled "Interaction," and the sum of the components must equal the total variance. Therefore, the H component, which by implication actually measures the effect of unbalanced deviations, might have included part or most of the complementary effects.

Similar difficulties appeared in the analysis using the factorial method. It was attempted to fit the data to several 3-, 4-, and 5-gene models, incorporating complementary epistasis in various genetic schemes. However, no satisfactory fit was obtained, which also might be ascribed to the complexity of the genetic basis for the character and the inability of the method to handle such complexity.

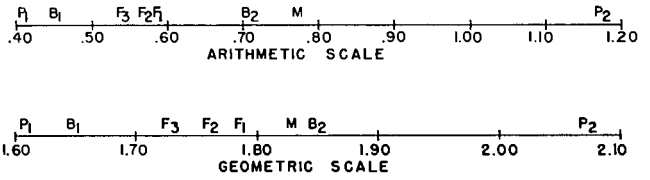

Fig. 1.—Distribution of generation means. Arithmetic scale in grams per seed. Geometric scale in log grams per 100 seed. M = midparent.

SUMMARY

Data on seed size from a cross between two lima bean strains were subjected to analysis. Depression of means with inbreeding indicated that a form of complementary epistasis contributed to the inheritance of the character. The magnitude of the scaling statistics and the skewness of the generation frequency distributions and means also suggested the influence of non-allelic interaction.

Distortion of the effects of other genetic mechanisms was noted in the results of the variance component and factorial analyses. In the component analysis this was evidenced by the significance of only the H component, possibly caused by inflation of the variances and appearance of residual interaction in the H component.

In the factorial analysis no satisfactory fit was obtained, probably due to the complexity of the genetic basis of the character.

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