

SELECTION FOR PUPA WEIGHT IN *TRIBOLIUM CASTANEUM*.
 II. LINKAGE AND LEVEL OF DOMINANCE¹

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ROBERTSON (1967) in discussing areas of quantitative genetics where our knowledge is still very limited pointed out that we need to know more about the kinds of gene action at the level of the individual locus and to know more about the linkage relationships among the loci affecting quantitative traits. COMSTOCK and ROBINSON (1952) described an experimental approach (Experiment III and referred to in later publications as Design III) which could be used to estimate average levels of dominance for genes affecting a quantitative character. This design has been incorporated into a long term selection experiment (see ENFIELD, COMSTOCK and BRASKERUD 1966) to obtain evidence on the importance of linkage in the inheritance of a quantitative trait and to determine the degree of dominance for genes affecting the trait. While this experiment does not pinpoint gene action at the level of a single locus it can be used to give information on whether there is an overdominant class of genes which as a group play a significant role in the genetic variation observed for a quantitative trait.

DESCRIPTION OF EXPERIMENT

The general approach of the Design III analysis was described in the earlier paper of this series (ENFIELD *et al.* 1966) and will not be repeated here. The use of the design in conjunction with a selection experiment gives it a utility not previously exploited by GARDNER *et al.* (1953), GARDNER and LONNQUIST (1959) or MOLL, LINDSEY and ROBINSON (1963). Critical evidence is provided by estimation of two variance components ($\hat{\sigma}_m^2$ and $\hat{\sigma}_{ml}^2$) and their ratio. Given two alleles per locus and no epistasis the compositions of the two components, given by COMSTOCK and ROBINSON (1952), are as follows:

$$\sigma_m^2 = \frac{1}{2} \sum_i \bar{q}_i (1 - \bar{q}_i) u_i^2 + \sum_{i,k} (pt - rs)_{ik} u_i u_k \quad (1)$$

$$\sigma_{ml}^2 = \sum_i \bar{q}_i (1 - \bar{q}_i) a_i^2 u_i^2 + 2 \sum_{i,k}^c (pt - rs)_{ik} a_i u_i a_k u_k + 2 \sum_{i,k}^r (rs - pt) a_i u_i a_k u_k \quad (2)$$

where \bar{q}_i is the frequency of the favorable allele at the i^{th} locus, u_i is half the difference between the effects of the two homozygous genotypes, a_i is the level of dominance at the i^{th} locus, p is the frequency of gametes with the favorable allele at both the i^{th} and k^{th} locus, t is the frequency of gametes with the unfavorable allele at both loci, r and s are the frequencies of the two repulsion

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type gametes and $\sum_{i,k}^c$ and $\sum_{i,k}^r$ indicate summation over pairs of loci at which the original linkage was in the coupling phase and repulsion phase, respectively. Note that the quantity, $pt - rs$, is a measure of linkage disequilibrium and that the magnitude of either σ_m^2 or σ_{ml}^2 may vary with amount of linkage disequilibrium. Note also that the direct contributions of segregating loci (those not dependent on linkage disequilibrium) to σ_m^2 and σ_{ml}^2 vary with gene frequency, becoming less as the frequency of either allele approaches 1.0. The quantity $k (= \sqrt{\sigma_{ml}^2 / 2\sigma_m^2})$ provides information about average level of dominance.

This investigation enables comparisons of σ_{ml}^2 , σ_m^2 and their ratio between populations and between generations within populations. Procedure and rationale are as follows. Two highly inbred lines of *Tribolium castaneum* (38 generations of brother-sister mating) were crossed to produce a segregating population.* The F_3 generation was subdivided into four populations of which two were selected for heavier pupa weight (S-populations) and two served as control lines (C-populations). Effective population size has been maintained at about 75 in all four populations. Selection by generation 20 had increased average pupa weight from 2459 to 3416 μg in one S-population and from 2412 to 3442 μg in the other. In the F_2 generation gene frequencies at all loci that were homozygous for different alleles in the two inbred lines would have been very close to 0.5 and $pt - rs$ for pairs of linked loci would have been close to maximum. (The expectation of $pt - rs$ in the F_2 from the cross of homozygous lines is $\pm \frac{1}{4}(1-2X)$ where X is the probability of recombination between the loci.)

In the control populations, where directional selection is not practiced, the expectation is (1) that gene frequencies will remain at 0.5 except for changes due to drift but (2) that all values of $pt - rs$ will diminish. Therefore differences, larger than could be expected as a result of drift, between Design III estimates obtained using F_2 males and those obtained using males from advanced generations of the C-populations could result only from linkage disequilibrium effects. Change in σ_m^2 associated with reduction in linkage disequilibrium is not expected unless linkage among genes pertinent to pupa weight is combined with imbalance between coupling and repulsion in the initial linkage disequilibrium. The situation is different in the case of σ_{ml}^2 , where all contributions to the last two terms of equation (2) are positive when a_i and a_k have the same algebraic sign. The presence of heterosis indicates more positive than negative a 's. Assuming them to be predominantly positive, lower values of σ_{ml}^2 in advanced generations of the C-populations should result if there is much linkage among the pertinent genes. The observation of such reduction would support the inference (a) that there is a significant amount of linkage and (b) that dominance is largely in one direction.

In the S-populations changes in gene frequencies as well as reductions in linkage disequilibrium are anticipated. Moreover, gene frequencies are expected to change most rapidly at the loci where dominance of the favorable allele is absent or slight. In particular at any overdominant loci ($a > 1$) selection will not lead to fixation; these loci will remain segregating unless fixation occurs as a consequence of random drift (ROBERTSON 1962), an event that has low probabilities in populations as large as those in question. Considering equations (1) and (2) and the quantity $k (= \sqrt{\sigma_{ml}^2 / 2\sigma_m^2})$ in the light of effects of selection on gene frequencies the following facts are apparent.

- (a) At the F_2 stage k will pertain to all the loci affecting pupa weight that were homozygous for different alleles in the foundation inbred lines and will probably contain a positive component due to linkage among those genes.
- (b) In advanced generations of the S-populations k will tend to become smaller because of reduction in linkage disequilibrium and to become larger because changes in gene frequencies will eventually mean proportionately greater contributions to σ_m^2 and σ_{ml}^2 by loci with larger a -values (greater dominance). The early balance between these op-

* Assuming homozygosity of the inbred lines, more than two alleles per segregating locus would occur only by mutation and, in general, the frequency of mutant alleles would be too low for their presence to be of consequence.

posing tendencies appears unpredictable but eventual increases in k should occur unless the variation of a among loci is small.

Thus comparison of advanced generation estimates obtained using C-population males on the one hand and S-population males on the other should indicate whether there is much locus to locus variation in degree of dominance and, in particular, eventual estimates of k in excess of 1.0 from S-population data would establish the presence of some overdominant loci.

The results presented in this paper are based on Design III data obtained using males from the following five populations:

F₂ —the base from which all other experimental populations were derived.

C₇ —seven generations of recombination but no selection.

C₁₇ —seventeen generations of recombination but no selection.

S₁₀ —ten generations of mass selection and recombination.

S₂₀ —twenty generations of mass selection and recombination.

The preceding discussion of the experiment assumes that the inbred lines employed were homozygous or at least very nearly so. Two-way selection experiments established that this homozygosity assumption was valid for one of the lines but revealed a moderate amount of genetic variation within the other. We believe that the appropriate interpretation of our data is modified only slightly by the variation indicated. The basis for this judgment will be explained after the results have first been reported and discussed as if both lines had been completely homozygous.

RESULTS AND DISCUSSION

Mean squares from the Design III analysis, conducted separately on male and female, are given in Table 1.

TABLE 1
Mean squares from Design III analysis

Population	df†	Male progeny pupa weight‡		Error
		Mean squares Males	Males × line	
F ₂	152	27,546	20,343	10,902
C ₇	106	17,505*	10,987**	10,174
C ₁₇	96	25,656	13,142*	10,457
S ₁₀	107	23,765	15,239	12,636
S ₂₀	81	22,345	14,024*	12,377
Pooled C	202	21,379*	12,011**	10,311
Pooled S	188	23,153	14,715*	12,523
		Female progeny pupa weight‡		
F ₂	152	30,774	18,474	12,470
C ₇	98	19,484**	12,158*	8,730*
C ₁₇	95	27,285	14,364	12,096
S ₁₀	108	28,386	18,386	13,413
S ₂₀	81	21,767*	15,021	17,169*
Pooled C	193	23,324**	13,244*	10,406
Pooled S	189	25,549	16,944	15,041

* Differs significantly from F₂ at .05 level of probability.

** Differs significantly from F₂ at .01 level of probability.

† The degrees of freedom given are for the "males" and "males × line" mean squares. Degrees of freedom for error were twice that given in the Table in each case.

‡ Unit of weight was in micrograms.

TABLE 2

Estimates of variance components and average level of dominance for pupa weight

Population		σ_m^2	σ_{ml}^2	k^*
Male	F	4161 ± 815	4720 ± 1235	.75
	C ₇	1832 ± 645	406 ± 888	.33
Progeny	C ₁₇	3799 ± 959	1342 ± 1071	.42
	S ₁₀	2782 ± 863	1302 ± 1193	.48
	S ₂₀	2492 ± 936	824 ± 1279	.40
	Pooled C	2767 ± 558	850 ± 688	.39
	Pooled S	2657 ± 635	1096 ± 874	.45
	F ₂	4576 ± 912	3002 ± 1156	.57
Female Progeny	C ₇	2688 ± 726	1714 ± 963	.57
	C ₁₇	3797 ± 1031	1134 ± 1192	.39
	S ₁₀	3743 ± 1013	2486 ± 1394	.57
	S ₂₀	1150 ± 966	-1074 ± 1484	negative
	Pooled C	3229 ± 619	1419 ± 760	.47
	Pooled S	2627 ± 706	951 ± 1014	.42

$$* k = \sqrt{\sigma_{ml}^2 / 2\sigma_m^2}$$

The quantities estimated by these mean squares, i.e. their expectations, are as follows:

Males	$\sigma^2 + 4\sigma_m^2$
Males × line	$\sigma^2 + 2\sigma_{ml}^2$
Error	σ^2

where σ^2 is the experimental error variance in the mean weight of five pupae. Estimates of σ_m^2 , σ_{ml}^2 and k are presented in Table 2.

If the error mean square could be considered constant from one population and generation to another, differences between mean squares would provide direct information concerning differences in σ_m^2 and σ_{ml}^2 . However, the error variance is obviously not constant in the female data. Accordingly, interpretations will be based on estimates recorded in Table 2.

When the estimates of σ_m^2 are considered, the issues are (1) do they show the time trend in the C-population that is expected as the result of genetic drift or is there a deviation from the expected that suggests an effect of linkage? (2) what does the comparison between estimates for the C- and S-populations indicate concerning effects of selection? The estimates for the C-population are all smaller than those for the F₂. In the absence of a linkage effect, change in σ_m^2 would be restricted to the first term of equation (1), i.e. to the quantity $\frac{1}{2}\Sigma \bar{q}(1-\bar{q})u^2$. The expectation of the variable portion in generation n is

$$E[\bar{q}(1-\bar{q})] = \bar{q}_0(1-\bar{q}_0) - \sigma_q^2 \quad (3)$$

where \bar{q}_0 represents the initial gene frequency in the F₁ and σ_q^2 is the variance of \bar{q} among individual loci in generation n . FALCONER (1960) gives the variance in gene frequency due to drift as

$$\sigma_q^2 = \bar{q}_0(1-\bar{q}_0) \left[1 - \left(1 - \frac{1}{2N}\right)^t \right] \quad (4)$$

where N is effective population size and t is the number of generations of random mating. Substituting (4) for σ_q^2 in (3) we then have

$$E[\bar{q}(1-\bar{q})] = \bar{q}_0(1-\bar{q}_0) \left(1 - \frac{1}{2N}\right)^t$$

With an effective population size of 75, $\left(1 - \frac{1}{2N}\right)^t$ equals .993 for the F_2 and .880 for C_{17} (which was 19 generations beyond the F_1) and the expected reduction resulting from drift is between 11 and 12%. Averaging for males and females the estimate of σ_m^2 was 13% lower for the C_{17} than for the F_2 generation, a reduction very close to that expected. On the other hand, the C_7 estimates of σ_m^2 are too low to be explained by drift.

In comparison of estimates for the C and S-populations, the most advanced generations have greatest interest. The S_{20} estimate is lower than the C_{17} estimate in both sexes, suggesting a reduction in σ_m^2 as a consequence of selection. Equation (1) makes it apparent that this should ultimately occur. However, it is probable that the data for females exaggerate the effect. The error mean square for S_{20} (see Table 1) was considerably larger than in any other case. If it was too large, as this suggests, the estimates of both σ_m^2 and σ_{ml}^2 were biased downward. The lower estimates for C_7 than for C_{17} were not anticipated and cannot be explained with certainty. It is possible that the variance was lower in C_7 as a consequence of a unique time pattern of recombination events. It seems at least equally probable that the unexpected difference in estimates reflects sampling error. The estimates of sampling errors cast doubt on this latter explanation but do not preclude it.

The significant feature of the estimates of σ_{ml}^2 is that all estimates for advanced generations are much smaller than those for the F_2 . Again a small reduction of approximately 12% would be expected as a result of drift. The pooled estimates in advanced generations range from 55 to 80% less, a much greater reduction than can be attributed to drift. The most plausible explanation for this reduction is linkage between segregating genes with effects on pupa weight and that linkage disequilibrium was the source of a substantial contribution to σ_{ml}^2 in the F_2 generation. This is consistent with results presented in the first paper of this series where the pattern of continuing response to selection together with no apparent reduction in additive genetic variance indicated that pupa weight is affected by a large number of genes.

All estimates of k were smaller than 1.0 and there was no evidence of an upturn in k in the selected population. These results indicate that the average level of dominance is in the partial dominance range, i.e. that $\bar{a} < 1.0$. The remaining question is whether there are any loci with a -values greater than one, whether after further generations of selection an estimate of k in excess of 1.0 is likely to be obtained from S-population data. A complete answer cannot be provided but implications worthy of note can be derived assuming (1) no epistasis and (2) that

a and u are completely independent in their variations from locus to locus. Then the sums over loci of au and a^2u^2 would be

$$\Sigma au = \bar{a} \Sigma u$$

and

$$\Sigma a^2u^2 = (\bar{a}^2 + \sigma_a^2) \Sigma u^2 \quad (5)$$

From the second of these

$$\bar{a}^2 + \sigma_a^2 = \Sigma a^2u^2 / \Sigma u^2 \quad (6)$$

These expressions provide a basis for estimating \bar{a} and σ_a^2 if information on Σu , Σau , Σu^2 and Σa^2u^2 is available.

The difference between the average of mean pupa weight in the two inbred lines and the average pupa weight in the F_1 cross of the lines provides an estimate of Σau . ENFIELD *et al.* (1966) reported an estimate of about 250 μg . The lines employed were those pertinent to the data of this report.

Thirty-two generations of selection beginning with the F_2 generation have increased the mean pupa weight of the S-populations by about 1800 μg . The expected F_2 mean and the limit of performance assuming fixation of the better allele at every locus may be symbolized as

$$\alpha + \frac{1}{2} \Sigma au \quad \text{and} \quad \alpha + \Sigma u,$$

respectively. Thus

$$\Sigma u - \frac{1}{2} \Sigma au \geq 1800$$

and substituting $\Sigma au = 250$,

$$\Sigma u \geq 1925$$

Finally, information on the relative sizes of Σa^2u^2 and Σu^2 is available from estimates in Table 2. Consider the estimates of $\sigma_{m_i}^2$ and σ_m^2 for C_{17} . The average of the $\sigma_{m_i}^2$ estimates is no less in C_{17} than in C_7 . Thus it seems safe to assume that linkage disequilibrium has decreased to an unimportant amount by C_{17} . Assuming no linkage disequilibrium $2\sigma_m^2$ and $\sigma_{m_i}^2$ become $\Sigma \bar{q}(1-\bar{q})u^2$ and $\Sigma \bar{q}(1-\bar{q})a^2u^2$, respectively (see equations 1 and 2). Since selection was random in the C-population the expectation of $\bar{q}(1-\bar{q})$ in C_{17} is the same for all loci. Hence the expectations of $2\sigma_m^2$ and $\sigma_{m_i}^2$ can be written as

$$E[\bar{q}(1-\bar{q})] \Sigma u^2 \quad \text{and} \quad E[\bar{q}(1-\bar{q})] \Sigma a^2u^2.$$

The ratio of these expectations is $\Sigma a^2u^2 / \Sigma u^2$. Substituting estimates from Table 2, averaged for the two sexes, we then obtain $\frac{1}{2}(1342 + 1134) / 2(3798) = .163$ as an estimate of $\Sigma a^2u^2 / \Sigma u^2$. This, by equation (6), is also an estimate of $\bar{a}^2 + \sigma_a^2$. If we use the pooled estimates from all four advanced generations the result is similar; in that case, .192.

From equation (5), $\bar{a} = \Sigma au / \Sigma u$. Using estimates described earlier, it appears that $a \leq 250 / 1925 = .13$ and that $\bar{a}^2 \leq .017$. When this is subtracted from .163 or .192 we have σ_a^2 estimated in the range .146 to .175 and about 0.4 as the standard deviation of a . If \bar{a} is 0.13 and σ_a is 0.40, the fraction of loci with $a > 1.0$ must be very small (0.05 or less if the distribution of a is somewhere near normal).

Let us now continue the no epistasis assumption while considering that a and u may not be independent. Note in particular that if these quantities are independent in their variation the standard deviation of au is directly proportional to u , i.e. it approaches zero as u approaches zero. However, in a real situation the

distribution of au may remain relatively broad at low values of u . In that event the proportion of overdominance (if there is any) would be larger at loci with smaller u -values and the over-all proportions of overdominant loci could be considerably larger than seems possible assuming independence of a and u . Even so, their total impact measured by the sum of associated dominance deviations could not be very large. It is true that some fraction of the au -values may be negative and hence that the sum of the positive values may be larger than the 250 μg estimate of Σau . However, in the light of the bounds placed on the distribution of au by our estimate of Σa^2u^2 and the fact that a substantial portion of the positive au -values must be associated with non-overdominant loci, the sum of au 's associated with loci where $a > 1.0$ must be small.

Finally, we must consider how inferences must be changed when epistasis is contemplated. HORNER, COMSTOCK and ROBINSON (1955) examined the effects of various types of epistasis on Design III estimates for the case of linkage equilibrium with all gene frequencies equal to one-half in the segregating population. Thus their results have meaning for the C_{17} data reported here unless the gene frequency deviations from one-half resulting from random drift have an important bearing on effects of epistasis. They found no bias due to epistasis in the estimate of \bar{a} in three of the four genetic models examined. Moreover, the result for the fourth model (optimum number, see HORNER *et al.* 1955 and WRIGHT, 1935) indicated that underestimation is very unlikely, that it will occur only when the number of plus genes contributed to the segregating population by the two inbred lines is exactly the same. Thus from information available there is nothing to suggest that the C-population data are misleading as the result of epistasis. The joint effects of epistasis and gene frequency changes in response to selection need theoretical investigation but, by the intrinsic nature of epistasis, it seems likely that they would contribute more to σ_{ml}^2 , an interaction variance, than to σ_m^2 . If so, what has been deduced concerning the possible amount of overdominance would not be biased in the direction of indicating too little overdominance.

The obvious first question concerning the genetic variation discovered in one of the inbred lines has to do with its magnitude. Judging from responses to recurrent selection, the additive genetic variance was about three-eighths as large as in the S-populations. Assuming no categorical difference between the inbred line and the S-populations in amount of genetic variation per segregating locus, this suggests that about three-eighths as many loci were segregating in the inbred line. The contributions of these loci to σ_m^2 and σ_{ml}^2 are much more complex algebraically than in the ideal case (inbred lines homozygous for different alleles which in turn are the only ones present in the S- or C-population). This is because there may be three alleles involved and because the contributions are functions of gene frequencies in the variable line as well as those in the S- or C-population. However, it is still true that the contribution to σ_{ml}^2 depends on the presence of dominance and, other things being equal, increases as the level of dominance increases. On the other hand, it is easily shown that while the contribution to σ_{ml}^2

as a multiple of the contribution to σ_m^2 may be as large as in the ideal case, it will often be less. The effect is toward underestimation of the average level of dominance. Let the ratio of single locus contributions to $\sigma_{m_i}^2$ and σ_m^2 when there is segregation in one of the inbred lines be $c \hat{a}^2$ (as compared to a^2 in the ideal case) where \hat{a}^2 is an appropriate average of three a 's when there are three alleles involved or simply a^2 when only two alleles are involved. Then if the loci segregating in the inbred line are not unique with respect to level of dominance and p is the proportion of loci segregating in the inbred line out of all those segregating in the S- or C-population, the downward bias (from lack of homozygosity in the inbred line) in the estimate of average dominance should be $p(1-c)$. Remembering that c is positive, though less than one, and accepting three-eighths as a satisfactory approximation to p , it seems likely in the present case that the downward bias in the estimate of \bar{a} is no more than 15–20%. In arriving at this conclusion it has been assumed that so far as effects on pupa weight are concerned the loci segregating in the inbred line are similar in (1) genetic variation produced per locus and (2) level of dominance, to those segregating in the S- or C-populations. There seems no basis to argue differently without invoking an effect of selection. However, natural selection (the only form to which the inbred line had been exposed) relates to "fitness" and there is good reason to believe that pupa weight in the inbred line was near optimum for "fitness." In the light of these facts, it is unreasonable to propose that selection could have been responsible for a substantial categorical difference between the loci segregating in the inbred line and those segregating in the S- or C-populations.

SUMMARY

An experimental approach described by COMSTOCK and ROBINSON (1952) as Experiment III was employed to investigate levels of dominance in the action of genes affecting pupa weight in *Tribolium castaneum*. The experiment employs two inbred lines and a segregating population derived from their cross and focuses on genetic variances among progenies obtained by crossing individuals from the variable population with each of the inbred lines. In the work reported five segregating populations (an F_2 ; populations advanced 7 and 17 generations, respectively, without selection; and populations advanced 10 and 20 generations, respectively, with distinctly effective selection for pupa weight), all derived from the same pair of inbred lines, were employed. Contrasts provided enabled inferences concerning both linkage and dominance of genes affecting pupa weight. It was concluded (1) that linkage had an important bearing on F_2 results, probably because the number of genes is too great to allow their location in the chromosomes with insignificant linkage, (2) that the average dominance level was below 0.5, on a scale where 1.0 is complete dominance, and (3) that the distribution of dominance levels may include some overdominance but not enough to have any practical consequence.

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