

Quantitative Genetic Variation from New Mutations in *Tribolium*

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SUMMARY

Directional selection for heavier pupa weight in *Tribolium castaneum* was practiced for ten generations in two replicates of an inbred line that had been separately maintained in population cages for more than 90 generations. The response to selection was used in a prediction equation developed by Hill (1982) to estimate mutational variance in the two populations. Estimates of the ratio of mutational to environmental variance ranged from .0002 to .0015 depending upon the assumptions made concerning the effective population sizes that had been maintained in the population cages. The results support the argument that mutation may have played a significant role in supplying new genetic variation for the long continuing response to selection in experiments reported earlier. A second experiment designed specifically to test the predictive theory on response to directional selection from new mutations is described.

INTRODUCTION

Established dogma concerning the nature of quantitative genetic variation has been challenged in recent years with two areas of research activity. First, several long term selection experiments have demonstrated that response to directional selection may continue for 50 to 100 generations before there is any indication that a plateau is being approached. (Dudley, 1977; Enfield, 1980; Yoo, 1980; and Bell, 1981). In contrast with many of the earlier selection experiments, these results suggested that a large number of genes must be segregating for the traits under selection to produce the long continuing response. Hill (1982a, 1982b) made an important extension to existing theory in the first of a series of papers evaluating the importance new mutations might play in providing useful variation for continuing response to selection for quantitative traits. His work suggests that the long term response of these recent experiments may be a function of recurrent new mutations rather than variation that was present in the foundation populations. While mutation has always been viewed as a major element for natural selection on the evolutionary time scale it has been a common view that the time scale was too short and population sizes too small in artificial selection experiments or practical breeding programs for new mutations to make a very significant contribution to selection response.

One of the important questions in quantitative genetics that has persisted since the early development of the science concerns the number of genes affecting quantitative traits. While it seems apparent at this point that there is likely a great deal of variation in gene number from trait to trait the question does remain whether some important measures of phenotype are affected in a significant way by genes numbering in the dozens to hundreds. The question has two direct practical implications both of which are related to the ability to make directional changes in mean phenotype. As you all know, conventional selection procedures will potentially lead to a much greater change in mean phenotype when variation is due to many genes with small effects rather than a few genes of large effects given the same amount of initial additive genetic variation. On the other hand the potential use for recombinant DNA technology as a tool for improving quantitative traits will be a much more difficult task in the case of many genes with small effects. A critical assumption in the procedures for estimating gene number from total selection response has been that the total change results from changes in allelic frequencies for only those genes segregating in the foundation population. Thus if

recurrent mutation is an ongoing source of useful variation for selection the methodology leads to an over estimate of gene number unless the amount of response associated with new mutations can be estimated. There is undoubtedly a certain amount of circularity and confounding of gene number estimates and mutation effects as the greater the number of genes the greater the opportunity for contribution from new mutations. The ability to sort out response to directional selection that is a result of preexisting variation versus new mutation is further complicated by the interaction of effective population size with total response to selection for either source of variation.

Given that mutation may be important in providing new variation for response to directional selection I would like to briefly evaluate existing experimental data on this point and then describe a new set of experiments which have been initiated this past year using *Tribolium* as the experimental organism to test the mutation theory developed by Hill.

HISTORICAL BACKGROUND

Two experimental approaches have commonly been used to evaluate the role of newly arisen mutations affecting quantitative traits. One method has been to evaluate the effectiveness of selection in lines where preexisting variation has been eliminated either by inbreeding or a selection process. The inherent problem with this approach has been the counterbalancing effects of natural selection in preventing the elimination of existing variation either by inbreeding or selection. Alternatively many experiments have utilized radiation treatments to determine whether increasing mutation rate increases the availability of favorable alleles that can be utilized in directional selection. Unfortunately this method is most likely to produce a class of mutations skewed in the unfavorable direction so it may provide limited information about the contributions of naturally occurring mutations.

Early work by Durant and Mather (1954) and Paxman (1957) provided information on the significant accumulation of mutant effects on bristle number in inbred lines of *Drosophila* derived from a common origin. This was followed by an elaborate series of mutation accumulation experiments by Mukai (1964) and Onisha (1977) where viability in *Drosophila* was the quantitative trait of interest. Again, considerable mutational variation was observed.

Selection experiments initiated from an inbred line base followed by an opportunity for mutations to accumulate have provided mixed results on the importance of new selectable variation from mutation. Mather and Wigan (1942) and Tanaka *et al.* (1958) both reported essentially unlimited progress in selection initiated from an inbred base. A certain amount of caution is needed in interpretation of results where inbreeding is the tool for producing homozygosity. It may or may not be effective in eliminating preexisting genetic variation (Goodwill and Enfield, 1971). Kitagawa (1967), Hollingdale and Barker (1971), and Clayton and Robertson (1955) showed little response to selection from an inbred base unless irradiation was used to increase mutation rate. In all cases an increase in response resulted from radiation treatment. In many of these experiments population sizes were too small to provide much opportunity for accumulation of favorable mutations. Hill (1982b) emphasizes that at least 20 generations at intermediate population sizes (100 to 250) will be needed for mutations to accumulate for selection to be very effective. A more recent experiment of Frankham (1980) provides some of the best direct evidence for the role of mutation in supplying genetic variation that is utilized in directional selection. He was, however, dealing with a trait in *Drosophila* (abdominal bristles) that may be somewhat unique because of its relationship to the rRNA loci and their ability to generate new mutation at a higher than normal rate by unequal crossing over in a tandemly repeated region.

SELECTION EXPERIMENTS WITH TRIBOLIUM

I want to very briefly summarize the results of the long term selection experiments with *Tribolium* that were initiated in my lab more than 20 years ago since these experiments serve as the foundation for the work which is now underway. When this work was first initiated our primary interest was centered on gene action issues but as response continued over

a long period of time interest gradually shifted to the many factors involved in reaching selection limits. It is worthwhile to keep in mind that at the time these experiments were begun most directional selection experiments had reached selection response plateaus in 25 to 30 generations.

In the directional selection experiments for increased 21 day pupa weight two replicated populations were selected for 130 generations before the project was terminated. Initial population means were near 2450 micrograms with an additive genetic standard deviation of approximately 110. The highest individual means attained were 5980 for replicate 1 in generation 116 and 5790 for replicate 2 in generation 124. These two replicates, which had originated from the same base population were crossed in generation 74 to form two new replicates which were also selected to generation 130 for heavier pupa weight. The highest means attained in these two populations were 6300 micrograms (generation 125) and 6140 (generation 119). Even though response to selection was very slow in the latter generations, pooled statistical estimates of heritability for the last third of the experiment were still $.18 \pm .02$ as compared to $.28 \pm .02$ in the early generations indicating a great deal of genetic variation still remained. Obviously at this stage of the experiment reproductive fitness was the limiting factor in selection response. The data emphasizes several important points. First, as compared with many selection experiments, tremendous changes were possible even when a relatively narrow base resulting from the cross of two inbred lines served as the foundation. Second, peaks of performance were not attained until after more than 115 generations of selection, and even then, considerable genetic variation remained. Third, crossing of the two replicates produced a modest increase in response (about 5%) over what was obtained in the original replicates suggesting a divergence between the lines that could be a function of either drift or mutation. The question posed by these results is what role mutation was playing in the total selection response.

Two experiments are now underway to ascertain the importance of new mutation as a source of variation for pupa weight. The first experiment is aimed at providing estimates of mutation variance as defined by Hill(1982). The second experiment is designed to test Hill's prediction equation concerning the asymptotic rate of response to selection from new mutations in finite populations.

The foundation populations used in the original selection experiments were initiated from the cross of two highly inbred lines that had been brother-sister mated for 38 generations before the cross was made. Recognizing that level of inbreeding was often not truly indicative of level of homozygosity because of natural selection, a two-way selection experiment was initiated early in the program to test for residual heterozygosity in the inbred lines (Goodwill and Enfield, 1971). The results indicated that one of the lines (CSI-10) possessed a moderate amount of variation while the other line (CSI-5) appeared to be homozygous or nearly so for genes affecting pupa weight. Heritability estimates in CSI-5 ranged from 0 to .03 depending on the method of analysis. The major point of emphasis here is that we do have a population where it is possible to begin an experiment with little or no genetic variation for the trait.

The CSI-5 line was maintained for many additional generations by brother-sister mating until it was finally expanded into mass mating population cages more than ten years ago. The population cages were replicated in two separate growth chambers as separate populations and in each case discrete generations were maintained. Population size has been somewhat variable from generation to generation with the extremes in the range being between 100 to 250 breeding individuals per generation. These two populations are now being used to provide estimates of mutation variance as defined by Hill (1982).

For additive genes with symmetrical effects around zero the asymptotic rate of response reduces to $2Ni\sigma_m^2/\sigma_e^2$ in a range of population sizes and mutation rates where it is assumed that simultaneous segregation of more than two alleles can be ignored. In the absence of linkage and negative correlations with fitness the equilibrium rate of response ultimately becomes then a function of N (effective population size), i (selection intensity in standard deviation units), σ_m^2 (the mutational variance contributed by new mutations immediately after

their occurrence), and σ_e (the phenotypic standard deviation or environmental standard deviation in homozygous lines). Assuming certain values for effective population sizes, mutation variance can then be estimated from the response to selection, assuming that a sufficient number of generations has elapsed for an equilibrium state to have been reached between population size and mutation. Directional selection has now been practiced for ten generations in the two CSI-5 lines that had been mass mated without artificial selection for more than 90 generations.

The equilibrium rate of response has been estimated in the two replicates in two ways. The first is an adjusted direct response estimate obtained from the regression of response as measured in terms of deviations from an unselected control on generations of selection. These estimates were 36 ± 4 in Replicate 1 and 17 ± 2 in Replicate 2. Since selection was practiced on a within half-sib family basis the estimates were adjusted to an expected value based on mass selection to 46 and 23 respectively (Enfield *et al.*, 1966). This represents a realized heritability of .18 and .11 in the two replicates. Estimates of heritability from a sire-offspring regression analysis were $.26 \pm .04$ and $.10 \pm .04$ respectively in the two replicates. Using these values the predicted response to selection in the two replicates would be 65 and 21. Note that in Replicate 1 the heritability is very near the estimated value in the long term selection experiment when selection was no longer effective. The estimates in the second replicate are about half the magnitude of plateau estimates. Figures 1 and 2 show the accumulated response to selection as measured by the regression on generations of selection. In both replicates the response is remarkably linear. Table 1 provides the estimates of the mutational variance and the ratio of mutational to environmental variance from the various methods of analysis. Final estimates of mutational variance depended on assumptions being made concerning the effective population sizes maintained while the replicates were maintained as population cages over the 90 generations. Extreme values of 100 and 200 were used to put upper and lower limits on the estimates of mutational variance.

Hill (1982b) summarized the extensive data on the ratio of new mutational variance to environmental variance from several studies on bristle number in *Drosophila melanogaster*. These estimates were all quite near 10^{-3} with a range of values between .2 to 2.2×10^{-3} . All eight of the estimates for pupa weight in *Tribolium* in the two replicates of this experiment fall within this range. The magnitude of these estimates are such that they provide credence to the argument that mutation may have been important in providing new genetic variance for the long continuing response to selection in the earlier experiments (Enfield, 1980).

There is always a concern when populations are maintained over a long period of time in the laboratory that contamination of stock from migrants might also be a source of new variability. We cannot rule this out with certainty but feel the results, especially from replicate 2, are quite consistent with a mutation hypothesis. We do have evidence that there has been no wild population contamination since the inbred lines are marked with the sooty gene and still show no segregation for this gene. Second, contamination of an inbred line from an outside migrant would be expected to increase genetic variability very rapidly in a population cage because of the much higher reproductive rates of outcross progeny. Thus I would expect a heritability much higher than the estimate of .10 in replicate 2 if there had been contamination from other material. The estimates of .18 to .21 in replicate 1 make this argument somewhat more shaky for this population but even here the heritability is much less than estimates in the range of .28 to .35 in the foundation material of earlier experiments which was produced by crossing two inbred lines (Enfield *et al.*, 1966).

I want to briefly describe the second experiment which is just getting underway and which is designed primarily as an experimental check on the predictive theory developed by Hill but will also provide a further check on the estimates of mutational variance obtained in the experiment just described. The second experiment consists of a long term selection experiment initiated from highly inbred lines that have been continuously brother-sister mated so that there

FIGURE 1. REGRESSION OF ACCUMULATED RESPONSE ON GENERATIONS OF SELECTION (REPLICATE 1)

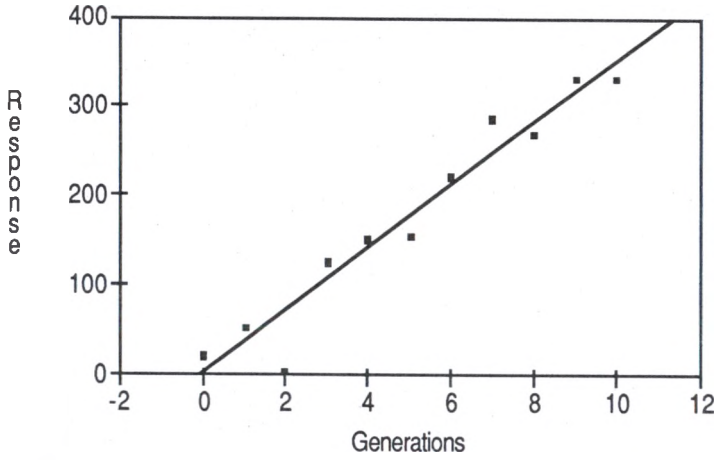


FIGURE 2. REGRESSION OF ACCUMULATED RESPONSE ON GENERATIONS OF SELECTION (REPLICATE 2)

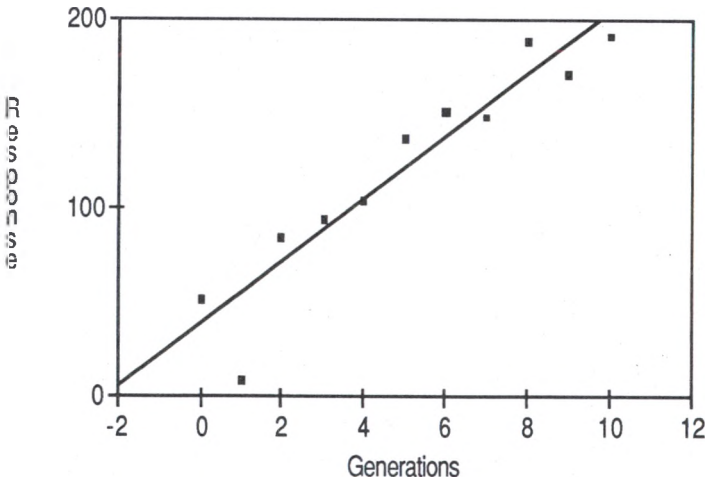


Table 1. Estimates of Mutational Variance

	Replicate 1		Replicate 2	
	σ_m^2	$\sigma_m^2/\sigma_e^2^*$	σ_m^2	$\sigma_m^2/\sigma_e^2^*$
Assuming N= 100				
(a) Response estimated from realized heritability	46	1.10	27	.60
(b) Response estimated from sire-offspring regression	62	1.50	24	.50
Assuming N = 200				
(a) Response estimated from realized heritability	23	.55	13	.30
(b) Response estimated from sire-offspring regression	31	.75	12	.20

* The ratio of mutational to environmental variance $\times 10^{-5}$

has been no opportunity for mutational variance to accumulate within the lines. These lines are being initially tested by short term selection experiments to insure that we are starting with lines with a heritability for pupa weight as close to zero as possible. The second experiment will consist of three different inbred lines each maintained at three different population sizes (40, 70, and 100) with a constant selection intensity maintained for all nine populations. In addition random selected control populations will be maintained for each line and population size.

Based on Hill's theory and simulation results it is expected that a minimum of 20 to 30 generations will be required before the rate of response stabilizes. The theory predicts that rate should be increasing until an asymptote is reached. The rate should be relatively faster initially with larger effective population size. The asymptotic rate is directly proportional to population size for both additive and dominant genes. The randomly selected controls will not only provide a measure of generation to generation fluctuations but also will provide a second set of base populations for comparison in advanced generations when the asymptotic rate is approached. A direct comparison of the response to selection at that time in previously selected and unselected populations with the same effective population size will provide a second check of the validity of the prediction equation since the asymptotic rate is independent of previous selection intensities but not effective population size. This second experiment should also provide more precise estimates of mutational variance since all the variables required for estimation can be controlled or estimated with a high degree of accuracy.

This work is supported by National Science Foundation grant BSR 8415873. The author also wishes to thank Ove Braskerud for technical assistance in this and earlier experiments.

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