Theor Appl Genet (1989) 77:416-420

Mutational variance for pupa weight in *Tribolium castaneum*

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Received July 4, 1988; Accepted October 28, 1988
Communicated by K. Sittmann

Summary. Directional selection for heavier pupa weight in *Tribolium castaneum* was practiced for 18 generations in two replicates of an inbred line, each separately maintained in small population cages for more than 90 generations. Mutational variance was estimated in two ways, based on Hill's (1982a) prediction equation for response to directional selection where an equilibrium state between effective population size and variation created by new mutation is assumed. Estimates of mutational variance based on response to selection in a selected population and from a sire-offspring regression analysis in an unselected control population were in strong agreement within each replicate population. Significant differences between the two replicates were observed. Estimates of the ratio of mutational variance to environmental variance ranged from 0.0002 to 0.0012, depending upon the assumptions made about effective population sizes maintained in the two replicate lines. Estimates of realized heritability from the 18 generations of selection were 0.23 ± 0.02 and 0.12 ± 0.02 in the two replicates. The results support the hypothesis that mutation may have played a significant role in supplying useful genetic variation for long-continuing response to selection for this trait in experiments reported earlier.

Key words: Mutational variance – *Tribolium* – Pupa weight – selection

Introduction

An evaluation of the importance of new mutations in maintaining quantitative genetic variation has been an area of major research activity in both theoretical and experimental quantitative genetics in recent years. Examples of significant theoretical contributions include Bulmer 1972; Lande 1975, 1983; Turelli 1984; Barton and Turelli 1987; Hill 1982a, b; Lynch and Hill 1986. One of the questions addressed by Hill (1982a, b) was whether mutation could explain the continuing response to directional selection in selection experiments, where effective population size was of the magnitude of that usually applicable to plant and animal breeding programs or laboratory selection experiments. This question became of particular consequence when the results of several more recent selection experiments demonstrated the presence of considerable genetic variation and continuing response after a long history of directional selection (Dudley 1977; Enfield 1980, Yoo 1980; Bell 1981). These results were in contrast with many of the earlier selection experiments summarized by Falconer (1960), where selection response plateaus were usually reached in 25-30 generations. One of the primary differences between the more recent experiments and those reported prior to 1960 is that recent experiments had much larger effective population sizes. If effective population size is the major variable responsible for these differences, an important issue arises as to whether its impact is through the loss of preexisting variation due to drift, or to the accumulation of potential new variation from mutation, or to both. This paper will address the importance that mutation may have played in maintaining genetic variability for pupa weight in *Tribolium* in two experiments reported earlier (Enfield 1980; Kaufman et al. 1977). Preliminary results and rationale for the present study were first reported at the 3rd World Congress on Genetics Applied to Livestock Production (Enfield 1986).

Materials and methods

The data for this study comes from one of the two highly inbred lines of *Tribolium castaneum* that were crossed to produce the segregating populations used in the directional selection experiment (Enfield 1980), as well as in the stabilizing selection experiment (Kaufman et al. 1977). These two lines had been brother-sister mated for 38 generations before the cross was made. A two-way selection experiment for pupa weight was initiated within both of these inbred lines at the start of the experiment to test for residual heterozygosity in the inbreds (Goodwill and Enfield 1971). The results indicated that one of these 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 0.00 to 0.03 depending on the method of analysis. This line provides the source of data for the experiment to be reported here.

After the initiation of the selection experiments from the cross of the two inbreds, both lines were maintained for several additional generations of brother-sister mating before being expanded into small population cages. The CSI-10 line was lost but two replicates of the CSI-5 inbred line were maintained in two separate growth chambers for more than 90 discrete generations. Population size was somewhat variable from generation to generation with the extreme range being from 100 to 200 individuals per generation.

An 18-generation selection experiment for heavier pupa weight in both replicates of CSI-5 provides the data for evaluating the role of new mutations as a source of genetic variation. Interpretation and analysis of the data are based on the prediction equation for the asymptotic rate of response to selection in finite populations developed by Hill (1982a). For additive genes with symmetrical effects around zero, the equilibrium rate of response to selection is $2Ni\sigma_m^2/\sigma_e$ for 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 correlation with fitness, the equilibrium rate of response is then a function of N (effective population size), i (selection intensity in standardized units), σ_m^2 (mutational variance contributed by new mutations immediately after their occurrence), and σ_e (the phenotypic standard deviation or environmental standard deviation in homozygous lines). Under the assumption that the CSI-5 line was homozygous for genes affecting pupa weight at the time the population cages were established and that 90 generations were sufficient to establish an equilibrium state between mutation and population size, estimates of mutation variance were obtained in the following two ways.

First, response to selection for heavier pupa weight was measured for 18 generations in the two replicates to provide an accurate estimate of response. Given these estimates and maximum and minimum values for effective population size, σ_m^2 was estimated directly from the prediction equation. Since individual selection was practiced within half-sib families, the response estimates were adjusted to an expected value based on mass selection in the total population by a method described earlier (Enfield et al. 1966).

Second, it can be demonstrated from the theoretical formulations of Hill (1982a) that, given the assumption that the CSI-5 population is in mutation-population size equilibrium additive genetic variance for this population will be equal to $2N\sigma_m^2$. Once again, this assumes that the population was initially homozygous for genes affecting pupa weight, that all new variation is the result of mutation, and that gene effects are additive. Estimates of additive genetic variance were obtained for the two control population replicates from the heritability estimates obtained from a sire-offspring regression analysis. These control populations were carried each generation with random selection. This same method of estimation was not used with the selected populations, since it has been demonstrated that selection within families will potentially bias estimates of heritability and additive genetic variance obtained from a parent-offspring regression analysis (Kaufman et al. 1977). Using the data from the unselected control populations to estimate heritability also provides for a completely independent estimate of mutation variance for the same foundation populations in the two separately maintained replicates.

In the selection experiment, 36 males were each mated to 2 females per generation in each of the 4 populations. Response to selection was measured in several ways with nearly identical results. The method presented in this paper provided the smallest standard error on the estimate of response. A multiple regression equation was used, where the select population generation mean was the dependent variable. Generations of selection and the control population mean served as the independent variables. Response was measured as the partial regression of the selected population mean on the number of generations of selection.

Sire-offspring regression analyses for pupa weight were done separately for the two sexes of offspring on a family mean basis. Each single generation estimate served as a separate estimate. Empirical standard errors were then calculated from the 18 separate estimates for each control population for both the estimate of heritability and the estimate of mutation variance obtained from this method of analysis. Data from the two sexes were pooled for a final estimate when the two did not differ significantly in either replicate.

Results and discussion

The response to selection for each replicate is shown graphically in Fig. 1. A summary of the realized heritability estimates in the select (S) populations and the sire-offspring regression estimates from the control (C) populations is given in Table 1. There is strong agreement between the two methods of estimation within the replicates and obvious differences between the two replicates. It should be reemphasized that the two replicates had been separately maintained in different growth chambers for more than 90 generations so there had been considerable opportunity for divergence.

The two most obvious explanations for the differences between the replicates are a difference in mutation variance between the two populations or different effective population sizes, or both. Also, there is always a concern, when populations are maintained over a long period of time in the laboratory, that contamination from migrants might also be a a source of new variability. We cannot rule this out with certainty, but feel the results are not consistent with this possibility and are much more in line with the mutation hypothesis. Contamination of an inbred line with an outside migrant would be expected to increase genetic variability very rapidly in a population cage because of the much higher reproductive rate of outcross progeny. Thus we would expect a much higher heritability for pupa weight than was observed in either replicate. This would typically be no less than 0.3 and may be as high as 0.6–0.8 when starting with broad-

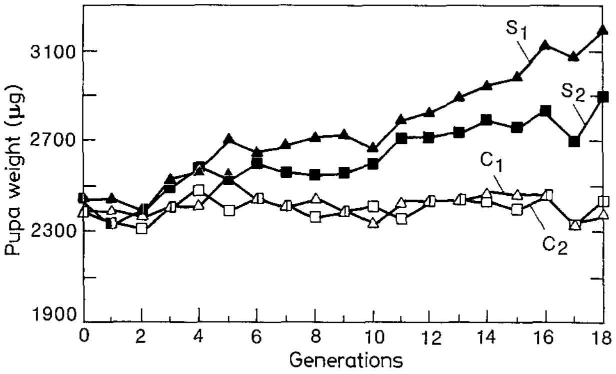

Fig. 1. Response to selection for pupa weight in the directional selected populations (S_1 and S_2) and the in randomly selected controls (C_1 and C_2)

Table 1. Summary of heritability estimates

Method of estimation	Heritability	
	Replicate 1	Replicate 2
Realized heritability from the select population	0.23±0.02	0.12±0.02
Sire-offspring regression from the control population	0.19±0.03	0.07±0.04

Table 2. Estimates of mutational variance and the ratio of mutational to environmental variance

	Replicate 1		Replicate 2	
	σ^2_m	σ^2_m/σ^2_e	σ^2_m	σ^2_m/σ^2_e
Assuming $N=100$				
Estimate based on selection response	49±3	1.2×10^{-3}	25±3	0.50×10^{-3}
Estimate from sire-offspring regression	45±7	1.2×10^{-3}	16±5	0.40×10^{-3}
Assuming $N=200$				
Estimate based on selection response	24±2	0.6×10^{-3}	12±7	0.25×10^{-3}
Estimate from sire-offspring regression	22±4	0.6×10^{-3}	8±3	0.20×10^{-3}

er base genetic material (Enfield et al. 1966; Wilson et al. 1963; Bell and Moore 1958).

Estimates of mutational variance and the ratio of mutational variance to environmental variance are presented in Table 2. The absolute value of mutational variance per se provides little insight, unless expressed as a component of the heritability parameter or as a ratio to the environmental variance. Lynch (1988) has called this ratio "mutation heritability". The ratio enables a comparison of the relative importance of new mutations as a source of variation in different traits and species. Lynch (1988) did an extensive survey of the literature and estimated this ratio for a number of traits in a diversity of both plant and animal species. There can be some problems in this type of retrospective analysis since the data has usually been collected for different purposes and may fall short of meeting the needed assumptions to provide an unbiased estimate. However, taking the estimates from his study at face value, the range of estimates was from approximately 10^{-4} to 5×10^{-2}. Hill (1982b) summarized the several estimates for the ratio of mutational variance to environmental variance for bristle number in *Drosophila* and concluded the estimates tend to be near a value of 1×10^{-3}. Our estimates for pupa weight in *Tribolium* are very near those summarized by Hill for *Drosophila* bristle number, especially under the assumption of an effective population size of 100, and they fall in the middle of the range of the traits analyzed by Lynch.

As indicated in the introduction of this paper, one of the primary purposes of this experiment was to reevaluate the conclusions reached in two of our earlier papers involving long-term selection experiments. In both of those papers, and in most other selection experiments, it has generally been assumed, either stated or implied, that mutation rates are too low to have an appreciable effect on the results. Thus, the interpretation has been based on the assumption that the observed results can be attributed to the effects of selection and drift on genetic variation that existed at the start of the experiment.

In the long-term directional selection experiment (Enfield 1980), selection for pupa weight was practiced for 130 generations in 2 replicates before the experiment was terminated. Initial population means were near 2,450 μg with an additive genetic standard deviation of approximately 110. The highest individual generation means attained were 5,980 μg for replicate 1 in generation 116 and 5,790 μg for replicate 2 in generation 124. Of particular significance was the result that even though response to selection had nearly ceased becaue of reproductive fitness problems, estimates of heritability for the last third of the experiment were still 0.18±0.02 as compared with estimates of 0.28±0.02 in the early generations. This indicates that a great deal of genetic variability was still present after the long history of selection. Effecitve population size based on coefficient of inbreeding data was about 100 in these 2 populations.

In a study designed to estimate gene number from this data (Comstock and Enfield 1981) we concluded, using a model that assumed equal effect of genes, no dominance, multiplicative effects, and no mutation, that the observed response could only be explained if at least 150 genes that affected pupa weight were segregating initially. It is of interest to note at this point that the estimate of heritability after 100 generations of selection was nearly identical to the estimate from replicate 1 of the CSI-5 inbred line after it had been maintained in a population cage for about the same period of time. One of the intuitively difficult ideas to grasp in the prediction equation developed by Hill is that the asymptotic rate of response is independent of previous selection intensities. Thus under his model, and based on mutational variance estimates from the CSI-5 inbred line population cage and effective population sizes used in the long-term experiment, the results are quite consistent with the idea that persistent genetic variation in the long-term directional selection experiment could be explained by mutation.

A reevaluation of the results presented in the paper of Kaufman et al. (1977) is equally interesting. In this paper, one of the populations is a randomly selected population that also originated from the cross of the two inbred lines described earlier. Two replicates of this population had been maintained at an effective population size of 80 for 67 generations, at the time the work was published. One of the questions posed in this paper was how much reduction in additive genetic variance should occur as a function of drift for a population starting out with initial allelic frequencies of 0.5, when gene effects are largely additive. Most studies on pupa weight have indicated that there is very little dominance for this trait. Additive genetic variance contributed by any locus will initially be a function of $q_0(1-q_0)$ and will be maximum at $q=0.5$. After t generations of random mating, the expectation of $q_t(1-q_t)$ will be $q_0(1-q_0)\ (1-1/2N)^t$ where N is effective population size (Enfield et al. 1969). On this basis, the expected reduction in additive genetic variance due to drift would be 34% after 67 generations. There was no evidence of any decline in additive genetic variance over this period. Our original interpretation of the data was that natural selection may well have been maintaining the allele frequencies at an intermediate optimum, since stabilizing selection on pupa weight leads to an improvement in fitness while directional selection leads to a decline. Results of our present experiment would indicate that mutation could have played an equally important role in supplying new genetic variation in a population of size 80 over a period of 67 generations.

Our results emphasize that effective population size and mutation may have played a more important role in total response to selection in many of the experiments reported in the literature than has generally been recognized. In many of the early selection experiments where population sizes were small, estimates of gene number affecting traits also tended to be small. Was this because of fixation of genes initially segregating because of drift rather than fixation of favorable alleles by selection? On the other hand in some of the more recent experiments where selection response has gone on for a long period of time, we are confronted with the problem of sorting out response from variation existing in the foundation populations with that originating from recurrent mutations that accumulate in suffcently large effective populations during the course of an experiment. Long-term directional selection experiments which vary in effective population size, but start with homozygous material, seem needed at this point to determine the relative importance of the mutation-population size equilibrium state in breeding and directional selection programs.

Acknowledgements. This work is supported by National Science Foundation grant BSR 8415873.

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