

Response to selection for increased pupa weight in *Tribolium castaneum* as related to population structure*

By ALAN J. KATZ† AND FRANKLIN D. ENFIELD

*University of Minnesota,
Department of Genetics and Cell Biology,
St Paul, Minnesota, U.S.A. 55108*

(Received 23 February 1977)

SUMMARY

The effectiveness of selection for increased pupa weight in *Tribolium* was compared for three different selection systems. In all three systems the same number of breeding individuals was used each generation. Population L was a large random mating population with 24 males and 48 females selected each generation. The C₄ and C₈ populations were each divided into 6 subpopulations (lines) consisting of 4 males and 8 females. Each of the three populations was replicated. In C₄, selection for pupa weight was within lines for three generations, followed by a generation of among-line selection when the best two out of six lines were selected. These lines were then crossed to produce 6 new subpopulations, and the cycle was repeated. The C₈ population was handled in exactly the same manner except that seven generations of selection within lines were practised before each generation of among-line selection. Selection response for the 42-generation period was significantly greater in the L population than in either subdivided population. No consistent differences among the selection systems were apparent when evaluating short-term response for the first 12 generations of the experiment. The results were interpreted as indicating that the influence of multiple-peak epistasis was not of major importance for this trait in determining ultimate response to selection when starting from a base population of previously unselected lines and utilizing a within- and among-line selection regime.

1. INTRODUCTION

This paper is concerned with a question relative to the importance of population structure in natural and artificial populations: is there a selective advantage in dividing large breeding populations into subdivisions (lines) with repeated cycles of within- and among-line selection? Theoretically the question of a selective advantage in subdivided populations focuses on two underlying genetic issues. Wright (1931, 1970) has suggested that for those traits in which specific gene

* Supported by USPHS grant GM-16074. The senior author was supported by USPHS training grant GM-01156.

† Present address: Department of Biological Sciences, Illinois State University, Normal, Illinois, U.S.A. 61761.

complexes are the units of selection, subdivision of a large population into partially isolated lines accompanied by selection within the lines may be a more efficient selection programme than mass selection within a large population. The greater efficiency of selection expected upon subdivision rests primarily upon the assumption that the trait under consideration is being influenced by multiple-peak epistasis. Hence the existence of multiple-peak epistatic effects is of prime importance. Secondly, there is the issue of whether inbreeding and genetic drift in the absence of epistasis can be used to alter the genetic variance in a subdivided population in a way that will increase the effectiveness of selection.

It is important to examine the possible advantages and/or disadvantages associated with subdividing a population prior to selection, since this procedure is now being employed in domestic breeding programmes. A standard selection regime involves n generations of within-line selection followed by a generation of among-line selection during which the better lines are interbred. Sub-lines are then re-established and the selection procedure is repeated. This cyclic regime of within- and among-line selection has been suggested by Wright (1939) as a practical means by which breeders might utilize the effects of multiple-peak epistasis to enhance response to selection.

Madalena & Hill (1972) have examined the effects of subdivided population structure on response to selection using computer simulations. They compared the selection responses of large populations to those of subdivided populations that were subjected to a cyclic selection regime of within- and among-line selection. They concluded that the only possible advantage associated with the subdivided populations is the more rapid fixation of recessive alleles when the recessive alleles were at low frequencies in the base populations. It should be emphasized that this simulation work did not include epistasis in the genetic model, and as a result was not testing an important component of Wright's theory. However, from the simulation studies and other theoretical work that has considered the probability of fixation of favourable alleles in finite populations (Robertson, 1960; Maruyama, 1970), it does seem clear that without multiple-peak epistasis the *limit* reached at a plateau will usually be greater in large populations that have not been subdivided.

Fraser & Burnell (1970) have reported that although the selection limit may be lower in populations which are subdivided and periodically crossed, the rate of fixation of favourable alleles is greater in the subdivided populations. Baker & Curnow (1968) have suggested that if short-term response is of critical importance, as it may be to the commercial crop breeder, there can be an advantage in selecting within very small inbred lines followed by crossing of the better sub-lines.

In our study the effectiveness of three different selection systems was compared. Two of the systems involved repeated cycles of within- and among-line selection. These populations were compared with genetic changes in a large mass-selected population. The same number of breeding individuals were used in all populations, and each population was twice replicated. The experiment was conducted for 42 generations, and both short-term and long-term responses to artificial selection

were evaluated. The results are compared with expectations when multiple-peak epistatic and non-epistatic models are considered.

2. MATERIALS AND METHODS

(i) *Experimental procedures*

Two highly inbred lines of *Tribolium castaneum* (CSI-5 and CSI-10) were twice crossed to each other to produce two segregating populations. The two populations were randomly bred beyond the F_2 for 22 and 26 generations, respectively. At this time replicates 1 and 2 were initiated from the two segregating populations. Any differences in genetic variability in the two base populations from which the replicates were established might be due to either (1) the effects of random drift during the period of random mating without selection prior to the establishment of the base populations or (2) variability that may have been present in the original cross if the inbred lines were not completely homozygous.

Seventy-two males and 144 females were sampled from each of the segregating populations to serve as foundation material for the experimental populations in each replicate. Twenty-four males and 48 females were randomly assigned to each of three populations which are designated L, C_4 and C_8 . Population L was a large random-mating population with 24 males and 48 females selected as parents each generation. The C_4 and C_8 populations were both subdivided into 6 lines, with each line consisting of 4 males and 8 females. In C_4 selection was within lines for three generations, followed by a generation of among-line selection when the best two out of the six lines were selected. These selected lines were then crossed to each other to produce 6 new lines and the cycle was repeated. The C_8 population was handled in exactly the same manner except that seven generations of selection within lines was practised before each generation of among-line selection.

In each population a male was mated to two females. A maximum of 5 male and 5 female pupa progeny were sampled from each full-sib family and weighed to the nearest microgram. Selection was for individual 21-day pupa weight. In all three populations selection was practised within half-sib families. In the ideal situation this would mean that the heaviest male out of ten and the heaviest two females out of ten would be selected within each family. Selection was practised within half-sib families to prevent confounding the effects of population structure with the expected selection differential. However, it was occasionally necessary to select less intensely in the L population to maintain approximately equal selection differentials in the three populations. This was a function of greater reproductive problems in the subdivided populations, where the rate of inbreeding was much greater.

An attempt was made to keep constant the number of breeding individuals contributing progeny each generation in each of the three populations. Thus a surplus of matings was made with the goal of weighing 18 sire families from an original 24 matings. In the large population if more than 18 sires were fertile the

number was reduced to 18 before weighing. In the C_4 and C_8 populations the goal was to weigh three of the four sire groups within a sub-line. In several generations this goal was not attained due to increased sterility. In a limited number of instances in the advanced generations of the experiment reproductive problems also impaired the effectiveness of selecting the best lines during generations of among-line selection.

(ii) *Statistical techniques*

Complete pedigree data were available on all individuals in all populations. This enabled us to calculate mean inbreeding coefficients each generation and effective population sizes based on calculated coefficients of inbreeding.

Estimates of heritability were obtained each generation within each population from both parent-offspring regressions and components of variance analysis. In both the C_4 and C_8 populations the data were analysed within lines and then pooled to obtain an estimate for the population.

Realized heritabilities were obtained each generation. Single-generation estimates were pooled for the entire experiment by treating each estimate as a separate statistic. Empirical standard errors were calculated for the pooled estimate. Single-generation estimates were based on response to selection from the mean of the previous generation. Adjusted selection differentials were calculated by weighting the pupa weights of the parents by the number of progeny they contributed to the next generation.

Since selection was practised on a within-half-sib family basis, corrections were applied to all methods of heritability estimation to take into account the selection process. The method of Rahnefeld *et al.* (1963) was used to adjust the sire component of variance in the analysis of variance to correct for the selection of sires. Parent-offspring regression estimates of heritability may be biased upward when selection is practised within families. The theory on this point and the appropriate corrections are discussed by Kaufman, Enfield & Comstock (1977). Realized heritabilities were adjusted to a total population basis, taking into consideration that only three-quarters of the additive genetic variance was utilized in the selection process, and that the phenotypic variance represents the variance within half-sib families.

3. RESULTS

The generation means averaged over sexes for the three populations in each replication are shown graphically in Fig. 1. It is apparent from Fig. 1 that a clear pattern had emerged for long-term response by the time the experiment was terminated. The large undivided population (L) was superior in both replicates followed by the subdivided population (C_4) which was recycled every fourth generation. For the first 12 generations of the experiment no particular pattern was evident and no consistency was observed between the two replicates. By the end of the experiment each of the populations of replicate 1 was superior to that of replicate 2.

Table 1 gives the linear regression coefficients of mean pupa weight on genera-

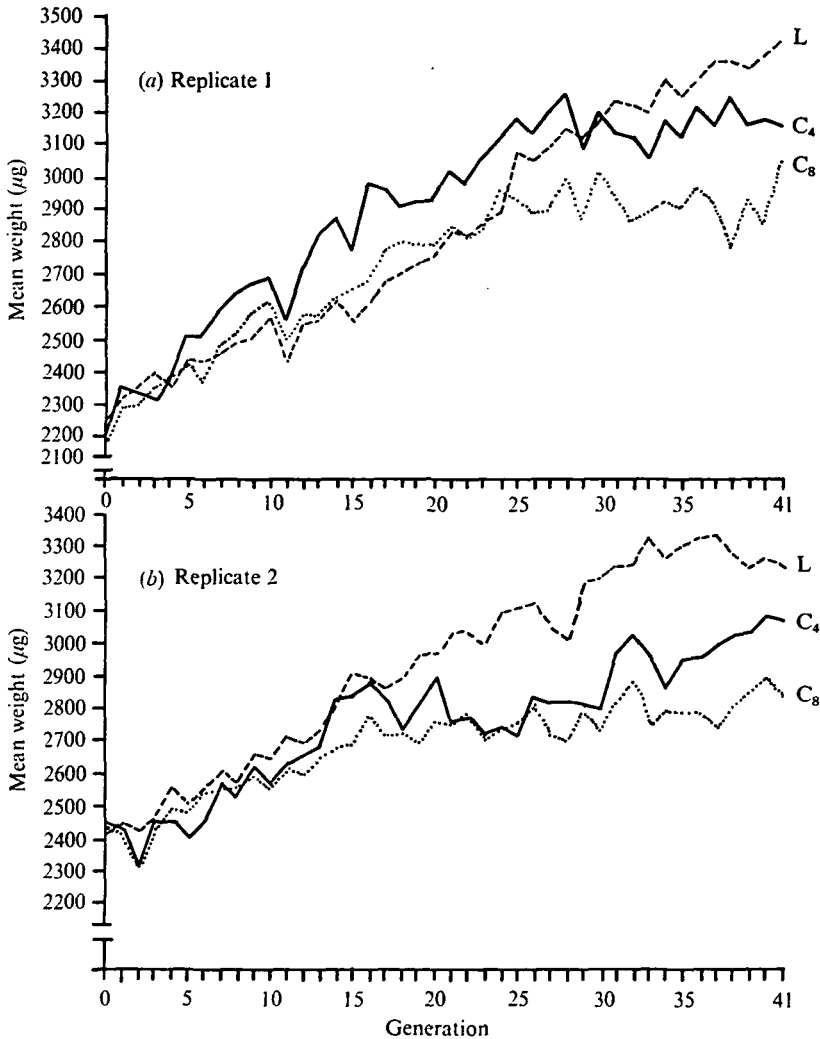


Fig. 1. Mean pupa weights of selected populations (L, C_4 , C_8) plotted against generation. (a) Replicate 1; (b) replicate 2.

tion of selection for each population within each replicate. The difference between the two replicates observed in Fig. 1 may be partially explained by the fact that the linear regression of response to selection on generation was greater for all selection systems in replicate 1 than replicate 2. This suggests that there was more genetic variability in the base populations for replicate 1 than 2. A difference in heritability of 0.04 is all that would be required to account for the replicate differences. The observed differences in the statistical estimates of heritability were of this magnitude, but the standard errors associated with the separate estimates preclude making a strong case for differences of this size. The standard errors presented in Table 1 were determined in the usual manner for a regression coefficient. As such they contain only a fraction of the drift variance which should be

included when making comparisons between the populations. The expectation of the standard error for the L populations based on the method of Hill (1972) which includes drift would be 1.32. No theory has been developed that permits a comparable estimate in the C_4 and C_8 populations.

Table 1. *Linear regression coefficients of mean pupa weight on generations of selection*

Population	Replication	$b \pm$ Standard error
L	1	29.35 ± 0.76
C_4		22.69 ± 1.46
C_8		17.01 ± 1.22
L	2	23.37 ± 0.87
C_4		15.15 ± 1.01
C_8		9.84 ± 0.76

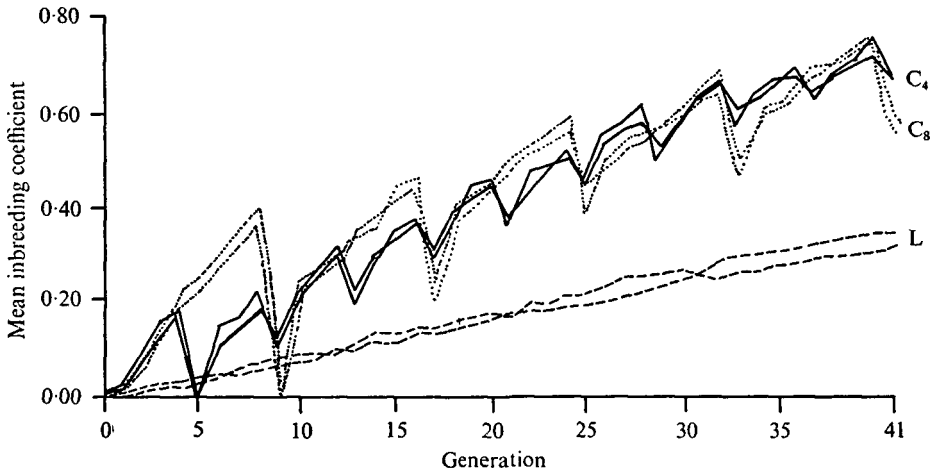


Fig. 2. Mean inbreeding coefficients of both replicates of each selected population (L, C_4 , C_8) plotted against generation.

An analysis of variance of the regression coefficients in Table 1 provides an additional way of comparing the populations where the results are not complicated by the correlated error structure. This analysis (not presented) further indicates a highly significant difference among both populations and replications.

Fig. 2 shows graphically the average coefficient of inbreeding (F) for all populations each generation. Estimates of mean effective population sizes (N_e) based on coefficients of inbreeding are given in Table 2.

Estimates of heritability within the three populations pooled over replicates are compared with realized heritabilities in Table 3. The realized heritability for the L population is considerably below the statistical estimates and is also much lower than we have observed in other experiments with large populations and similar foundation material (see Enfield, Comstock & Braskerud, 1966). The selection intensity was considerably less in this experiment than in our other

work because of an attempt to maintain a selection differential in population L similar to that of the subdivided populations. Meyer & Enfield (1975) have shown that decreasing the selection intensity may result in a lower realized heritability. The realized and estimated heritabilities are in closer agreement in the subdivided populations. The estimated values are lower than in the large population which would be expected based on the difference in average level of inbreeding between the populations. The very close agreement in realized heritabilities among the three populations is an important consideration in interpretation of the final results of this experiment. In the case of C₄ and C₈, the realized heritabilities are for those generations when selection was practised within lines.

Table 2. *Estimates of mean effective population sizes (N_e)*

	Population		
	L	C ₄ †	C ₈ †
Replicate 1	62.1	9.9	10.8
Replicate 2	58.7	10.0	10.2
Pooled	60.4 ± 1.7	10.0 ± 0.5	10.5 ± 0.2

† N_e refers to population size within subpopulations.

Table 3. *Estimates of heritability of pupa weight in selected populations*

	Population		
	L	C ₄	C ₈
Statistical estimates			
Parent-offspring regression	0.21 ± 0.02	0.09 ± 0.06	0.13 ± 0.06
Sib analysis	0.23 ± 0.04	0.11 ± 0.05	0.20 ± 0.05
Pooled	0.21 ± 0.02	0.10 ± 0.04	0.17 ± 0.04
Realized heritability	0.14 ± 0.03	0.14 ± 0.05	0.13 ± 0.04

Since the linear regression of response on time was significantly greater in L for the total period, the realized heritabilities would indicate either a greater selection differential in L or possibly a lack of effectiveness of selection among lines in the recycling generations in C₄ and C₈. The average adjusted selection differentials (in units of micrograms) for L, C₄ and C₈ were 200.2 ± 4.8, 201.1 ± 7.1, and 190.0 ± 5.3, respectively, ruling out selection intensity as a major source for differential response. In the 10 generations of among-line selection in C₄, the average response was -4 ± 17 with an average among-line selection differential of 50 ± 10. In C₈ the average response in the 5 recycled generations was 10 ± 24, with an average selection differential of 40 ± 30. It should be emphasized that in replicate 2 there were severe reproductive problems in generations 32, 36 and 40. The best lines for pupa weight were not selected in these generations, and as a result the average selection differential for the recycled generations was reduced. The lack of effectiveness of selection during recycling is, at least in part, a possible explanation for a greater response in L than in either C₄ or C₈. The results are not

totally consistent with this explanation, however. A greater response occurred in C_4 than in C_8 , although the average response in recycling generations was less in C_4 and it was recycled more often. The differences between C_4 and C_8 cannot be explained by differences in inbreeding depression since the average levels of inbreeding in C_4 and C_8 are almost identical (Fig. 2).

4. DISCUSSION

There are several important issues that need to be addressed in interpreting the results of this experiment in the light of existing theory and previous experimental results. Attention will be focused first on observed responses, both short and long term, as they relate to previous work. Secondly, the results will be interpreted in the light of theoretical expectations given the characteristics of the foundation populations.

Our results indicate no distinct differences in response among the different population structures when considering short-term response. The results are directly comparable with the work of Goodwill (1974) since the selection regimes were the same in the two experiments. His results also showed no differences between the large population and the subdivided populations after adjustments were made for differences in selection differentials. Similar experiments by Madalena & Robertson (1975) and Rathie (1976) also failed to demonstrate any distinct advantage for selecting in a subdivided population when compared with a large random breeding population.

Using a somewhat different experimental approach to examine the question of selection advance in subdivided populations, Katz & Young (1975) compared the selection response in a large population with two subdivided populations where a limited amount of migration was permitted among the subdivisions each generation. Their results showed significantly greater responses in both subdivided populations than in the large population. This suggests that perhaps a greater utilization of multiple-peak epistatic effects may be obtained through limited migration among the lines rather than through among-line selection.

One of the more striking experimental results that have suggested a real utility in inbreeding followed by selection and crossing is the work of Falconer (1971). A strain of mice which had already reached a selection limit for litter size was the foundation material for this experiment. The conclusions were that the inbreeding, crossing and selection process eliminated a considerable number of recessive genes which had been segregating at low frequencies. These results are consistent with predictions from the computer simulations of Madalena & Hill (1972).

In considering our selection results in light of theoretical expectations and in comparison with other experimental results, the characteristics of the foundation populations becomes an important factor. The simulation results of Madalena & Hill (1972) indicate that the cyclic sub-line structure is likely to be effective in the absence of epistasis only when recessive alleles are segregating at low frequencies. Since our base populations were established from the cross of only

two inbred lines, we do not expect large numbers of alleles to be segregating at low frequencies.

Certainly the results of this experiment do not support the hypothesis that multiple-peak epistasis is an important type of gene action for this trait, since our long-term response favours the large population in both replicates. However, these results are a function of the initial base populations and the specific selection regime employed. Different results might have been obtained if the base populations had been plateaued populations possessing residual genetic variation (Falconer, 1971) or if limited migration had been practised among the lines of C_4 and C_8 rather than among-line selection (Katz & Young, 1975). Given the breeding structures that were compared in this experiment, a more definitive interpretation of the results would be that if multiple-peak epistatic effects are important, either they were not efficiently utilized through among-line selection, or they could not compensate for the loss of favourable non-epistatic genes that may have occurred in the subdivided populations due to increased levels of inbreeding. We have some preliminary evidence (Enfield, 1976) that the residual variability for pupa weight in plateaued populations may include considerable epistasis. A new series of experiments is now underway to examine this possibility.

The relatively low estimates of heritability and response to selection for pupa weight found in this experiment for all populations are inconsistent with the commonly accepted idea that the trait is highly heritable (see King & Dawson, 1972, for a review on this point). There is increasing evidence, however, that the heritability estimates, both realized and estimates from parent-offspring regressions, may be a function of selection intensity. Low estimates of heritability for this trait (Meyer & Enfield, 1975; Goodwill, 1974; and this paper) have come from data where relatively weak selection was practised. This may indicate that the heritability for the trait is not a constant over the total range of phenotypic expression for the trait. This would be consistent with the earlier observations of Clayton, Morris & Robertson (1957), where lower realized heritabilities were associated with lower selection intensities.

In general, the results of this experiment and other laboratory experiments of this kind raise serious questions concerning the commonly accepted practice of employing recurrent cycles of within- and among-line selection for the improvement of breeds or strains of the domesticated species of livestock and poultry. The exceptional cases might be those where mass selection within a population is no longer effective and significant residual genetic variability remains.

We gratefully acknowledge Ove Braskerud and Pamela Kaufman for technical assistance throughout the experiment. We also thank Nancy Hartung and Robert Goodwill for their most useful comments on the manuscript.

REFERENCES

- BAKER, L. H. & CURNOW, R. N. (1968). Choice of population size and use of variation between replicate populations in plant breeding selection programs. *Crop Science* **9**, 555-560.
- CLAYTON, G. A., MORRIS, J. A. & ROBERTSON, A. (1957). An experimental check on quantitative genetic theory. I. Short term responses to selection. *Journal of Genetics* **55**, 131-151.
- ENFIELD, F. D. (1976). Selection experiments in *Tribolium* designated to look at gene action issues. *Proceedings of the International Conference on Quantitative Genetics*.
- ENFIELD, F. D., COMSTOCK, R. E. & BRASKERUD, O. (1966). Selection for pupa weight in *Tribolium castaneum*. I. Parameters in base populations. *Genetics* **54**, 523-533.
- FALCONER, D. S. (1971). Improvement of litter size in a strain of mice at a selection limit. *Genetical Research* **12**, 215-235.
- FRASER, A. & BURNELL, D. (1970). *Computer Models in Genetics*. New York: McGraw-Hill.
- GOODWILL, R. (1974). Comparison of three selection programs using *Tribolium castaneum*. *Journal of Heredity* **65**, 8-14.
- HILL, W. G. (1972). Estimation of genetic change. I. General theory and design of control populations. *Animal Breeding Abstracts* **40**, 1-15.
- KATZ, A. J. & YOUNG, S. Y. Y. (1975). Selection for high adult body weight in *Drosophila* populations with different structures. *Genetics* **81**, 163-175.
- KAUFMAN, P. K., ENFIELD, F. D. & COMSTOCK, R. E. (1977). Stabilizing selection for pupa weight in *Tribolium castaneum*. *Genetics* (in press).
- KING, C. E. & DAWSON, P. S. (1972). Population biology and the *Tribolium* model. *Evolutionary Biology* **5**, 133-223.
- MADALENA, F. E. & HILL, W. G. (1972). Population structure in artificial selection programmes: stimulation studies. *Genetical Research* **20**, 75-99.
- MADALENA, F. E. & ROBERTSON, A. (1975). Population structure in artificial selection: studies with *Drosophila melanogaster*. *Genetical Research* **24**, 113-126.
- MARUYAMA, T. (1970). On the fixation probability of mutant genes in a sub-divided population. *Genetical Research* **15**, 221-225.
- MEYER, H. H. & ENFIELD, F. D. (1975). Experimental evidence on limitations of the heritability parameter. *Theoretical and Applied Genetics* **45**, 268-273.
- RAHNEFELD, G. S., BOYLAN, W. J., COMSTOCK, R. E. & SINGH, M. (1963). Mass selection for post-weaning growth in mice. *Genetics* **48**, 1567-1583.
- RATHIE, K. A. (1976). Artificial selection with differing population structures. Unpublished Ph.D. thesis, University of Sydney.
- ROBERTSON, A. (1960). A theory of limits in artificial selection. *Proceedings of the Royal Society of London B* **153**, 234-249.
- WRIGHT, S. (1931). Evolution in Mendelian populations. *Genetics* **16**, 97-159.
- WRIGHT, S. (1939). Genetic principle governing the rate of progress in livestock breeding. *Proceedings of the American Society of Animal Production* **32**, 18-26.
- WRIGHT, S. (1970). Random drift and the shifting balance theory of evolution. In *Mathematical Topics in Population Genetics* (ed. K. Kojima), pp. 1-31. Berlin: Springer.