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Artificial Selection for 18-day Pupa Weight and Opposing Simulated Naturel Selection in *Tribolium castaneum*

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Summary. The effect of simulated opposing natural selection on the response to mass selection for 18-day pupa weight of *Tribolium castaneum* was studied for 10 generations of selection. Natural selection was simulated in replicated treatment lines by imposing a negative relationship between mid-parent genetic value for pupa weight and fertility. Responses to selection and realized heritabilities were smaller ($P < 0.05$ and $P < 0.10$, respectively) for the treatment lines than for control lines under selection for pupa weight only. One treatment, line E3G1, reached an intermediate selection plateau by generation 10, and responded linearly to 4 generations of artificial selection after natural selection had been discontinued. Possible explanations for the different behaviors of the replicate lines E3G1 and E3G2 were also discussed.

Key words: Natural selection – Artificial selection – *Tribolium*

Introduction

Artificial selection for a quantitative trait may be opposed by natural selection to such an extent that response to artificial selection ceases: the population remains at an intermediate selection plateau (Lerner 1954).

Indirect evidence for such an action of natural selection has been provided in selection experiments on *Drosophila* (Mather and Harrison 1949; Reeve and Robertson 1953; Clayton and Robertson 1957), *Tribolium* (Bell and Moore 1972; Orozco 1972; Enfield 1977), and on the mouse (Falconer 1955, 1973; Bradford 1971; La Salle et al. 1974). The action of natural selection is not limited to laboratory species however. Egg production in the chicken, a major economic trait, also seems to be plateauing (Harris 1977) partly due to the influence of natural selection.

Simple genetic models of the interaction between mass selection and natural selection have been developed by several workers (Carmelli and Karlin 1975; Karlin and Carmelli 1975; Sved 1977; Minvielle 1979). Their findings were in general agreement with the conclusions based on experimental evidence reviewed by Al-Murrani (1974), Kress (1975) and Barria (1976): natural selection can arrest and even overpower artificial selection; most intermediate plateaus are reached in 10 to 80 generations.

The objectives of the present work were to simulate and compare different hypothetical modes of natural selection opposing artificial selection for high 18-day pupa weight in *Tribolium castaneum*.

Material and Methods

For each one of 3 experiments (E1 to E3), three groups (G1 to G3) were set up by sampling and single-pair mating pupa from a wild-type base population of *Tribolium castaneum* described previously (Gall 1970). Their progeny constituted generation 0 of these nine lines.

The life cycle was as follows:

step 1		step 2		step 3		step 1	
artificial → selection	selected 18-day pupa	single pair → mating	progeny 10 F.S. families	natural → selection	surviving pupa to be selected for weight	artificial → selection	selected 18-day pupa →

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Within each line, the 10 heaviest 18-day pupa of both sexes were kept (step 1) and paired at random (step 2). Thus, the progeny constituted 10 full-sib (F.S.) families. The contribution of each family to the pool of pupa to be selected for high weight was restricted by simulated natural selection (step 3).

Within each experiment Ei, the two lines EiG1 and EiG2 were treatment replicates, submitted to the same mode of opposing natural selection. At each generation simulated natural selection was imposed on these lines as follows: (1) Ten pupa were randomly sampled from each family of full-sib progeny at day 18 and their average weight was calculated as an estimation of their mid-parent genetic value. (2) Within each line the 10 families were ranked accordingly and the pupa that had been weighed were reunited with their full sibs (3) Pupa were randomly sampled from each family in numbers determined by their rank, as shown in Figure 1, to simulate the opposing natural selection. (4) The pupa surviving natural selection were pooled to make up a single population for each line.

The planned and the average realized population sizes prior to artificial selection were respectively 110 and 95, 140 and 100, 80 and 70 for each of the two lines in experiments E1, E2 and E3, respectively. These differences were due to the adjustments made to the modes of natural selection because of matings not producing progeny (2 per generation per line, on the average). Consequently, the actual population sizes were similar in E1 and E2, but lower in E3.

The pupa were mass selected in each line. The 10 heaviest pupa of each sex – or 21%, 20% and 28% of the population, respectively in E1, E2 and E3 – were saved and randomly paired at day 18. Eleven days later (day 0) 36-hour egg collections were started to produce the 10 full-sib families of the next generation.

In control lines E1G3, E2G3 and E3G3 natural selection was neutral; at step 3 the same number of pupa was sampled from each full-sib family of the line EiG3. The actual number of pupa retained was equal to the average number of pupa per family surviving after natural selection had been imposed in the 2 corresponding treatment lines EiG1 and EiG2. Then, mass selection as described above was practiced in the control lines. Equivalent pressures of artificial selection were achieved for all 3 lines within each experiment.

The selective process was terminated after 10 generations in all lines except E3G1 and E3G3. These were continued without natural selection for four more generations to further study an apparent plateau reached in line E3G1 by generation 10.

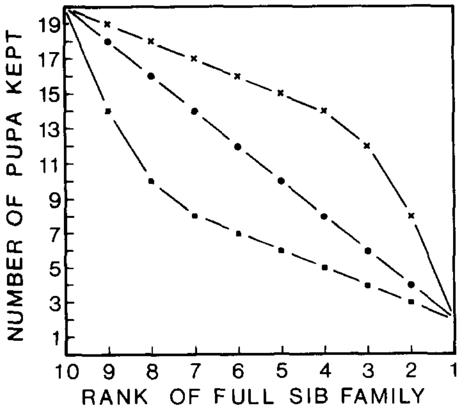

Fig. 1. Modes of simulated natural selection: number of pupa surviving in each full-sib family after natural selection in the treatment lines of experiments E1 (•————•), E2 (X————X) and E3 (■————■)

Selected parents and full-sib progeny were kept in 6 dram vials containing about 5 grams of a mixture of 90% unbleached flour and 10% dried brewer's yeast. The lines were kept in an incubator at 33°C and 70 percent relative humidity.

Selection results were analyzed by linear regression of pupa weight on generation number and accumulated selection differential. Slopes of the regression lines were compared (Snedecor and Cochran 1967) to test the effectiveness of natural selection. The 8 degrees of freedom (df) available to compare the slopes of the regression lines were partitioned as follows: 2 df were used to compare the 3 control lines, and, for each experiment Ei, ldf was used to compare each of EiG1 to EiG2, and EiG1 + EiG2 pooled to EiG3. This analysis assumes that errors are uncorrelated. But Richardson et al. (1968) and Hill (1972) showed that this assumption is violated because of random genetic drift. Little change was found in the standard errors of the regression coefficients when Hill's approximations were used, therefore, the effects of the correlated errors on our statistical analysis were certainly small.

Results and Discussion

Overall Responses

Overall responses to selection for pupa weight in E1, E2 and E3 are shown in Figures 2-4, respectively. All the lines responded to selection in a fairly linear way except for line E3G1 which appeared to have reached a plateau in less than 10 generations.

Average response per generation, estimated by linear regression, and its standard error is given for each line and for each treatment in Table 1. The control lines (G3) had

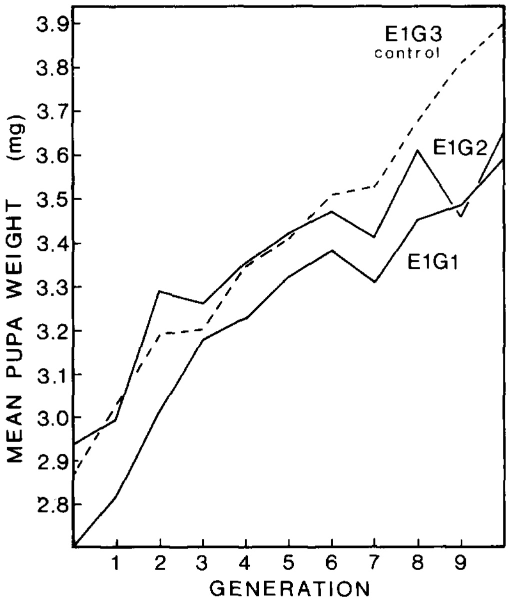

Fig. 2. Response to selection for 18-day pupa weight in experiment E1

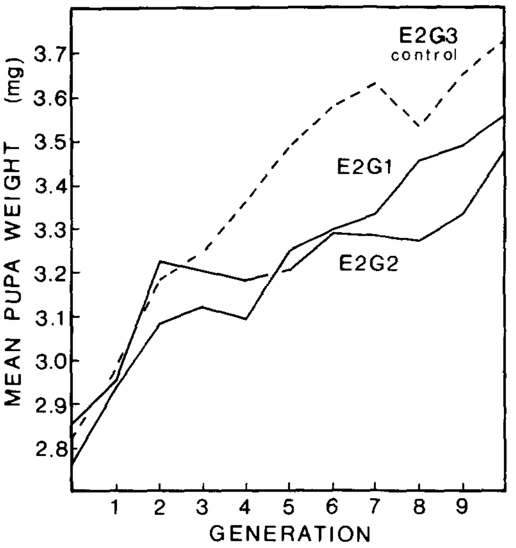

Fig. 3. Response to selection for 18-day pupa weight in experiment E2

Table 1. Response to selection, estimated by the regression of the mean pupa weight (mg), before artificial selection, on the generation number for each line and for pooled treatment lines

Line	Regression coefficient	Standard error
E1G1	0.080†	0.008
E1G2	0.062	0.009
E1G3	0.096	0.004
E2G1	0.072	0.005
E2G2	0.045	0.008
E2G3	0.084	0.009
E3G1	0.017††	0.008
E3G2	0.080	0.006
E3G3	0.093	0.010
(E1G1, E1G2)	0.071	0.008
(E2G1, E2G2)	0.059	0.005
(E3G1, E3G2)	0.049	0.012

† = all coefficients greater than 0 at $P < 0.001$ except as indicated
†† = $0.05 < P < 0.10$

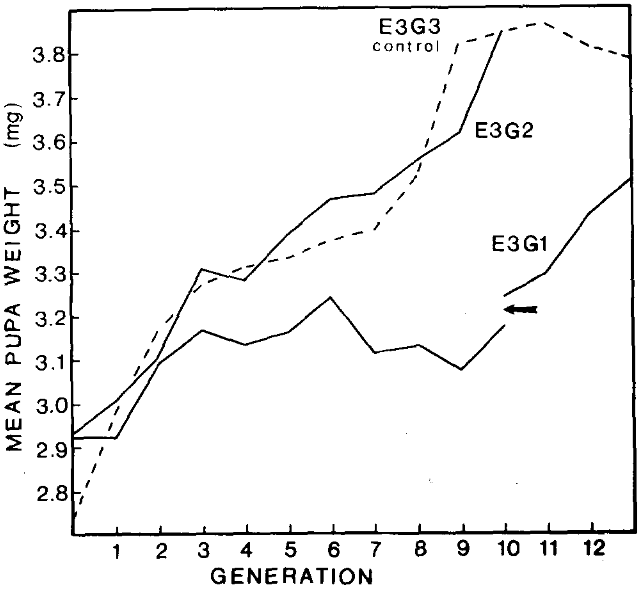

Fig. 4. Response to selection for 18-day pupa weight in experiment E3. Arrow indicates that natural selection was relaxed after generation 10

a consistently higher response per generation. This suggests that simulated natural selection was slowing down the progress in 18-days pupa weight of the treatment lines. Individual comparisons between lines or combinations of lines are shown in Table 2. No significant difference was found between the 3 control groups. The regression coefficients were similar to those obtained by Gall (1971) who selected for high 21-day pupa weight. Within each experiment however, a significant difference was found between the response in the control line EiG3 and the pooled treatment replicates (EiG1, EiG2).

But in both experiments E2 and E3 the two replicates responded very differently to the selective process: in each case one behaved somewhat like the control line while the other showed significantly less response. The effective population size was very close to 16 in all lines; random genetic drift and genetic differences in the founding parents might be partly responsible for the differences observed between replicates.

Drift alone does not satisfactorily explain the plateau reached by line E3G1. Expressed in standard deviations, the difference between the average pupa weight of lines E3G2 and E3G1 increased from about $\sigma/20$ at generation 0 to 2σ at generation 10, a 40-fold incrase. Moreover, after simulated natural selection was stopped at generation 10, line E3G1 responded quickly to artificial selection, with an average response of 0.090 mg per generation from generation 10 to 13. This suggests very strongly that the mode of natural selection imposed on line E3G1 was a main cause of the plateau.

Selection Differentials

For each control line, expected (E) and realized (R) selection differentials were compared to evaluate the possible effect of 'real' natural selection on 18-day pupa weight. Results are given in Table 3. The ratio (R/E) was always close to 1 indicating that natural selection did not seem to oppose artificial selection in the control lines.

The realized accumulated selection differentials in the

Table 2. Comparisons of the response to selection, estimated as the regression of generation mean (mg) on generation number

Comparison	df		Mean square	
	Between	Error	Between	Error
E1G3-E2G3-E3G3	2	27	0.008580 ns	0.006963
(E1G1, E1G2)-E1G3	1	29	0.045834 *	0.009379
(E2G1, E2G2)-E2G3	1	29	0.045834 *	0.007034
(E3G1, E3G2)-E3G3	1	29	0.141974 *	0.026759
E1G1-E1G2	1	18	0.017820 ns	0.008389
E2G1-E2G2	1	18	0.040095 **	0.004778
E3G1-E3G2	1	18	0.218295 ***	0.005278

ns = not significant; * = $0.01 < P < 0.05$; ** = $0.001 < P < 0.01$; *** = $P < 0.001$)

Table 3. Expected (E) and realized (R) accumulated selection differentials (mg) in the control lines

line	E	R	R/E
E1G3	3.460	3.403	0.984
E2G3	3.375	3.460	1.025
E3G3	3.206	3.157	0.985

Table 4. Average selection differential per generation, calculated as the regression of the accumulated selection deifferential (mg) on the generation number

Line	Regression Coefficient	Standard error
E1G1	$0.366^{\dagger}$	0.011
E1G2	0.295	0.006
E1G3	0.343	0.012
E2G1	0.347	0.010
E2G2	0.335	0.005
E2G3	0.347	0.005
E3G1	0.292	0.012
E3G2	0.307	0.006
E3G3	0.317	0.009

$\dagger$ = all regression coefficients greater than 0 at $P < 0.001$

Table 5. Realized heritability, calculated as the regression of the generation mean (mg), before artificial selection, on the accumulated selection differential for each line and for pooled treatment lines

Line	Realized heritability	Standard error	
E1G1	$0.212^{\dagger}$	0.029	
E1G2	0.207	0.032	
E1G3	0.276	0.017	(0.030)
E2G1	0.204	0.018	
E2G2	0.135	0.024	
E2G3	0.242	0.026	(0.032)
E3G1	0.053 ns	0.029	
E3G2	0.262	0.017	
E3G3	0.290	0.032	(0.041)
(E1G1, E1G2)	0.202	0.027	
(E2G1, E2G2)	0.170	0.016	
(E3G1, E3G2)	0.174	0.039	

$\dagger$ = All realized heritabilities greater than 0 at $P < 0.001$ except as indicated; ns = not significant. Standard errors according to Hill (1972) are given in parentheses

treatment lines E1G1, E1G2, E2G1, ..., E3G2 were respectively 3.534, 3.008, 3.418, 3.362, 2.926 and 3.065 mg. These values are very similar to those calculated for the control lines. The latter was not unexpected since selection differentials in the treatment lines were measured as the difference, averaged over sexes, between the mean weight of the pupa selected as future parents and the average weight of the pupa remaining after natural selection. Consequently, the effect of natural selection will be observed as an effect on the selection response rather than on the selection differential. The accumulated selection differentials were slightly smaller in the experiment E3 where selection pressure was the smallest.

Average selection differentials per generation, calculated in each line as the regression of the accumulated selection differential on the generation number, are shown in Table 4. No consistent difference was observed between control and treatment lines or between treatment lines that would explain the differences in response observed per generation.

Realized Heritabilities

Realized heritability was calculated as the regression of the generation mean pupa weight on the accumulated selection differential (Table 5). As expected, realized heritabilities were consistently larger in the control lines; their values were in good agreement with those obtained by

Gall (1971). Comparisons of regression coefficients are shown in Table 6. The latter results are qualitatively equivalent to those listed in Table 2 for the response per generation. They indicate that the lines under simulated natural selection showed less response to artificial selection than the control lines. These differences between treatments and control, however, could be partly due to systematic differences in the actual selection differentials obtained for artificial selection and/or differences in phenotypic variances between treatment and control lines. The average selection differentials per generation (Table 4) did not show any consistent systematic trend. The same observation can be made with regard to the average changes in phenotypic variance, estimated by linear regression on generation number (Table 7). The general increase in phenotypic variance with time was certainly a scale effect: the coefficients of variation calculated for each line at generation 0 and at generation 10 were similar (around 10%). Then, simulated natural selection appears indeed to be the main cause of the differential responses observed in treatment and control lines.

It should also be noted that the realized heritability for line E3G1 was not found to be significantly different from 0. However, both the selection differential and the phenotypic variance for this line behaved very much like those of the control line E3G3. These observations suggest strongly that the observed selection plateau in line E3G1 was mainly due to the antagonistic action of simulated natural selection. It was shown by computer simulation (Minvielle 1979) that the qualitative outcome of the selective process may depend critically on the original gene frequencies when natural selection is opposing artificial selection. Also, the effect of the initial gene frequency on the rate of response to selection is well known: progress is slower at extreme frequencies, faster at intermediate frequencies. Then, different starting gene frequencies at some of the loci which control pupa weight and random drift might be the primary causes of the very different behaviours of replicates E1G3 and E2G3.

Table 7. Regression of the phenotypic variance in each generation on the generation number

Line	Intercept	Regression	Standard error
E1G1	0.043	0.006 †	0.002
E1G2	0.036	0.004	0.001
E1G3	0.028	0.008	0.001
E2G1	0.036	0.006	0.001
E2G2	0.040	0.003	0.001
E2G3	0.049	0.004	0.001
E3G1	0.020	0.009	0.001
E3G2	0.043	0.007 ns	0.004
E3G3	0.029	0.009	0.003

† = all regression coefficients greater than 0 at $P < 0.01$ except as indicated; ns = not significant

Natural Selection

At each generation, the pressure of simulated natural selection was evaluated in each treatment line as the difference between the mean pupa weight after and before natural selection. These consistently negative selection differentials (Table 8) first demonstrate that our procedure of simulated natural selection was effective. They indicate also that the average intensity of (natural) selection was stronger in experiment E3. Then, the plateau observed for line E3G1 may have resulted from the combined effect of the stronger pressure of natural selection (Table 8) and of the weaker intensity of artificial selection (Table 4) achieved in this line.

Realized heritabilities, corrected for the simulated natural selection were calculated. As expected, the values obtained for the treatment lines (0.270, 0.301, 0.259, 0.176, 0.124 and 0.373, respectively for lines E1G1, E1G2, E2G1, ..., E3G2) were larger than before correction, and were not consistently smaller than the ones calculated for the corresponding control lines.

Table 6. Comparisons of realized heritabilities, calculated as the regression of the generation mean (mg) before artificial selection on the accumulated selection differential

Comparison	d.f.		Mean square	
	Between	Error	Between	Error
E1G3-E2G3-E3G3	2	27	0.007713 ns	0.008148
(E1G1, E1G2)-E1G3	1	29	0.046911 ††	0.013448
(E2G1, E2G2)-E2G3	1	29	0.045383 *	0.007448
(E3G1, E3G2)-E3G3	1	29	0.096441 ††	0.024276
E1G1-E1G2	1	18	0.000146 ns	0.011222
E2G1-E2G2	1	18	0.030566 *	0.005667

ns = not significant; †† = $0.05 < P < 0.10$; * = $0.01 < P < 0.05$

Table 8. Intensity of simulated natural selection, calculated as the difference (mg) between the mean pupa weights after and before simulated natural selection

Generation	Line					
	E1G1	E1G2	E2G1	E2G2	E3G1	E3G2
1	– 0.075	– 0.132	– 0.080	– 0.088	– 0.067	– 0.030
2	– 0.058	– 0.037	– 0.120	– 0.051	– 0.058	– 0.183
3	– 0.064	– 0.093	– 0.057	– 0.086	– 0.134	– 0.050
4	– 0.066	– 0.047	– 0.085	– 0.108	– 0.138	– 0.156
5	– 0.046	– 0.059	– 0.063	– 0.079	– 0.082	– 0.085
6	– 0.017	– 0.072	– 0.113	– 0.022	– 0.063	– 0.154
7	– 0.098	– 0.061	– 0.060	– 0.099	– 0.129	– 0.050
8	– 0.066	– 0.107	– 0.080	– 0.102	– 0.084	– 0.048
9	– 0.105	– 0.146	– 0.061	– 0.067	– 0.171	– 0.086
10	– 0.050	– 0.082	– 0.033	– 0.014	– 0.126	– 0.015
Average	– 0.065	– 0.084	– 0.075	– 0.072	– 0.105	– 0.086

The 3 modes of simulated natural selection were chosen to mimick possible effects of opposing natural selection: 'linear' selection in E1, 'decelerating' selection in E2 and 'accelerating' selection in E3. In our experiment, however, the variation observed between replicated lines was large, therefore it was not possible to show statistically whether the 3 modes of simulated natural selection differentially altered the behaviour of the treatment lines.

Experimental natural selection was shown to be successful in slowing down genetic progress. However, it had to be relatively strong to have a noticeable effect in our short term experiment. In reality, after a large number of generations of artificial selection, a smaller intensity of natural selection would be able to stop genetic progress. This observation was also made by Enfield (1977) who found that natural selection eventually stopped the response to selection for high pupa weight in a long-term experiment on *Tribolium castaneum*.

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