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Simultaneous selection for two correlated traits in *Tribolium**

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SUMMARY

Simultaneous selection by *independent culling levels* of two correlated traits in all four combinations of directions was investigated with *Tribolium* in a replicated experiment extending over nine generations. In addition to the two primary traits, 13-day larval weight and pupal weight, four secondary traits (pupation time, adult emergence time, adult weight and larval number) were observed.

The observed responses for both selected and unselected traits agreed with theoretical expectations after the latter were adjusted for changes which occurred in genetic and phenotypic parameters. Phenotypic variances for the selected traits were correlated positively with population means, yet genetic variances and heritabilities declined in all selected populations. No change was detected in the genetic correlation between selected traits even though the divergent two-trait selection was designed especially to 'break' the positive correlation of $+0{\cdot}55 \pm 0{\cdot}12$ present in the base population.

Striking changes in growth and developmental patterns resulting from the divergent selection were discussed in terms of metamorphic limits and 'stabilizing' genetic correlations.

1. INTRODUCTION

Simultaneous change in two or more traits is frequently the goal of artificial selection. In fact, the desired direction of change for a particular trait may be reversed for different ages in the same animal. For instance, the beef cattle breeder's goal may be maximum weight at an immature or market age with a minimum weight at maturity in order to reduce the maintenance costs of breeding animals. Genetic correlations between traits of interest as well as their heritabilities are expected to determine the success of such selection.

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Expected genetic change resulting from simultaneous selection for two traits using selection indices or independent culling levels have been treated theoretically by Hazel & Lush (1942), Young & Weiler (1960) and Young (1961) and experimentally by Rasmuson (1964) and Sen & Robertson (1964). These experimental studies did not agree in certain important aspects with theoretical expectations. Another aspect of theoretical interest which has not been verified experimentally relates to the correlated responses in unselected traits.

The present study with *Tribolium* was designed to test experimentally the genetic theory relating to the simultaneous selection of two correlated traits based on *independent culling levels*. The experiment was replicated and included eight generations of two-trait divergent selection as represented by the four diverse combinations of high–low selection based on 13-day larval weight and on pupal weight. Other traits related to growth and development were observed for correlated responses. Possible changes in the genetic correlation between traits under divergent selection was of special interest. A preliminary report of the findings was made earlier (Burris & Bell, 1965).

2. MATERIALS AND METHODS

Each replication of this study was initiated from an independent random sample of 60 single pair matings from the Purdue '+' Foundation Population of the flour beetle, *Tribolium castaneum*. This heterogeneous population had been reproduced without selection since its origin in 1954 (see Bell & Moore (1972) for details). The 60 single pair matings were assigned randomly in groups of 12 to form base populations for five different selection schemes to be compared over eight generations of selection. Under each selection scheme, 96 individuals (eight from each of 12 single pair matings) were observed each generation. A second replication of this comparison followed the first each generation after a delay of one week.

Culturing techniques. All populations were cultured on standard medium (whole wheat flour enriched with 5 % dried brewers' yeast) in an environmental chamber at approximately 33 °C and 70 % relative humidity. For the initial generation, a 24 h egg collection from each mating (taken at the age of peak fecundity, 7–12 days after emergence) was cultured for 13 days in a $\frac{3}{4}$ oz. creamer containing 2 g of medium. All larvae were then screened from each creamer and counted. Eight larvae were taken at random from each of the 12 matings within each population, weighed individually on an analytical balance, and placed in individual creamers containing 0·1 g of medium to be observed periodically for additional growth manifestations. In all generations after the first, two 12 h egg collections were taken from each mating to provide a more precise measurement of developmental rate. Four larvae were then sampled from each 12 h collection to provide the prescribed eight progeny per mating.

Traits observed. Six quantitative traits were observed. In addition to *Larval numbers* (LN), defined as the number of progeny per mating for the uniform collec-

tion period, the following five traits were observed on each of the eight progeny sampled from each mating:

(1) *Larval weight* (LW) – weight in decamicrograms (dμg = 10 μg) 13 days after egg collection.

(2) *Pupation time* (PT) – days from egg collection to pupation measured in 12 h intervals.

(3) *Pupal weight* (PW) – weight in decamicrograms taken within 24 h of pupation.

(4) *Adult emergence time* (AT) – days from egg collection to adult emergence measured in 12 h intervals.

(5) *Adult weight* (AW) – weight in decamicrograms taken between 24 and 72 h after adult emergence.

Only viable individuals with recorded information on every trait were included in subsequent analyses.

Selection methods. Four of the five methods being compared featured the four diverse combinations of positive and negative selection based on individual merit for larval weight and pupal weight. The fifth method was a randomly selected control. Selected males and females under each method were mated at random except for the avoidance of sib matings.

The five methods of selection were designated as follows:

L^+P^+, high 13-day larval weight and high pupal weight;
L^+P^-, high 13-day larval weight and low pupal weight;
L^-P^+, low 13-day larval weight and high pupal weight;
L^-P^-, low 13-day larval weight and low pupal weight and
L^cP^c, unselected control.

Selection under all methods excepting L^cP^c featured *independent culling levels.* All individuals observed each generation within each population were ranked within sex for larval weight and separately for pupal weight in accordance with the goals specified. Thereby, each individual had a separate ranking for each trait. Next these two rankings were truncated simultaneously at that point whereby twelve individuals of that sex would be included in the truncated or selected portion of *both* traits. Only those 12 individuals included in both truncations would be selected. Obviously, the proportion of the total population included within the truncated portion for each trait would be a function of the phenotypic correlation between larval weight and pupal weight. For example, consider the P^+P^+ method. If the correlation was positive and perfect, the individuals would have identical ranks for both traits and the truncation would simply include the twelve highest ranks for each trait. The above scheme deviates slightly from that originally developed by Hazel & Lush (1942) for the selection of n uncorrelated traits by the method of independent culling levels. However, this deviation does not affect the assumptions and the solutions provided by Young & Weiler (1960) and by Harvey & Bearden (1962) for computing the expected genetic changes when the selected traits are correlated.

Statistical analyses. The observations made each generation on pedigreed individuals within each population were analysed by standard analyses of variance and covariance for the estimation of appropriate parameters. Heritability was estimated as twice the 'mating' or full-sib family variance components as a ratio of the total phenotypic variance. The genetic correlation for any two traits was estimated as the ratio of the mating covariance component to the product of appropriate genetic standard deviations. This procedure yields unbiased estimates of genetic parameters provided the gene action is additive and maternal effects are negligible.

Predicted responses. The expected genetic changes for both selected and unselected traits were calculated following the approach of Harvey & Bearden (1962). For two traits (e.g. 1 and 2) selected simultaneously, the expected genetic change per generation (ΔG) for each expressed in standard units becomes

$$\Delta G_1 = a_1 h_1^2 + a_2 r_{G_1 G_2} h_1 h_2 \quad \text{and} \quad \Delta G_2 = a_2 h_2^2 + a_1 r_{G_1 G_2} h_1 h_2,$$

where

$$a_1 = \frac{i_1 - t_{P_1 P_2} i_2}{1 - r^2_{P_1 P_2}} \quad \text{and} \quad a_2 = \frac{i_2 - r_{P_1 P_2} i_1}{1 - r^2_{P_1 P_2}}.$$

Other designations include: h^2 as heritability, i as selection differential in standard units, r_{GG} as genetic correlation coefficient, and r_{PP} as phenotypic correlation coefficient with subscripts for appropriate traits. For any unselected trait, the expected genetic change or correlated response is

$$\Delta G_u = a_1 r_{G_1 G_u} h_1 h_u + a_2 r_{G_2 G_u} h_2 h_u,$$

where the notation is as given above and the subscript u stands for the unselected trait.

3. RESULTS

Initial population parameters. Observations made on pedigreed off-spring from the 60 random single pair matings initiating each replication of this study were analysed statistically to provide estimates of the initial genetic and phenotypic parameters. Included in the analyses were 426 and 469 individuals for replications 1 and 2, respectively. The two analyses were not pooled so that independent comparisons of predicted and observed responses could be made for the two replications. Initial means and their standard errors for the various traits are given in Table 1. The number of larvae per mating (LN) was observed as representing a major component of fitness. The other traits represent different manifestations of growth and development. Pupation time (PT) and adult emergence time (AT) are functions of the rate of development, while 13-day larval weight (LW) represents body mass at a fixed age when growth is normally at a more or less exponential rate. Body weights at the pupal (PW) and adult (AW) stages are equivalent to mature body size.

Initial estimates of h^2 plus genetic and phenotypic correlation coefficients for

the various traits together with their sampling errors are given by replication in Table 2. Since they were obtained from analyses involving full-sib groups only, the estimates for additive genetic variance will be inflated by $1/2\sigma^2_D + 1/2\sigma^2_{AA} + 1/4\sigma^2_{AD} + 1/8\sigma^2_{DD} + 1/4\sigma^2_{AAA}$, etc., in addition to twice the variance due to maternal effects, and a fraction due to sex-linked genes. These non-additive effects are most noticeable for LW parameters.

Table 1. *Estimated means and their standard errors for various traits in the base populations*

(Weight traits are reported in decamicrograms with development rates in days.)

Trait	Means by replication 1	2
Larval number per mating	25·4 ± 0·48	25·2 ± 0·60
13-day larval weight	170·5 ± 1·87	184·9 ± 2·10
Pupation time	17·0 ± 0·03	16·7 ± 0·03
Pupal weight	216·5 ± 0·84	210·5 ± 0·82
Adult emergence time	21·9 ± 0·02	21·4 ± 0·03
Adult weight	187·5 ± 1·37	167·2 ± 0·81

Table 2. *Genetic and phenotypic correlation coefficients and heritability estimates from full-sib variance-covariance components in the base populations*

(Genetic correlations above the diagonal and phenotypic correlations below. The diagonal elements in bold are heritability estimates. At each point the upper value is replication 1.)

	Larval weight	Pupal weight	Pupation time	Adult weight	Emergence time
Larval weight	**0.41 ± 0.10**	0·54 ± 0·13	−0·63 ± 0·13	0·11 ± 0·16	−0·41 ± 0·19
	0·61 ± 0·11	0·55 ± 0·12	−0·78 ± 0·08	0·60 ± 0·11	−0·75 ± 0·08
Pupal weight	0·36 ± 0·05	**0·69 ± 0·11**	−0·08 ± 0·19	0·36 ± 0·13	0·06 ± 0·21
	0·29 ± 0·05	**0·67 ± 0·11**	−0·31 ± 0·16	0·92 ± 0·05	−0·28 ± 0·16
Pupation time	−0·68 ± 0·03	−0·19 ± 0·05	**0·30 ± 0·09**	−0·32 ± 0·18	0·93 ± 0·06
	−0·69 ± 0·03	−0·13 ± 0·06	**0·46 ± 0·10**	−0·47 ± 0·15	1·00 ± 0·01
Adult weight	0·20 ± 0·06	0·45 ± 0·06	−0·12 ± 0·06	**1·36 ± 0·09**	−0·21 ± 0·19
	0·46 ± 0·04	0·68 ± 0·03	−0·29 ± 0·05	**0·56 ± 0·10**	−0·44 ± 0·15
Emergence time	−0·50 ± 0·04	−0·13 ± 0·05	0·79 ± 0·02	−0·15 ± 0·06	**0·22 ± 0·08**
	−0·68 ± 0·03	−0·14 ± 0·06	0·94 ± 0·01	−0·31 ± 0·05	**0·47 ± 0·10**

Other studies of these traits in the same foundation population (Bell, 1969; Bell & Moore, 1972; Englert & Bell, 1969, 1970) have found LW and PW to be some 10–20 % less heritable than the estimates in Table 2, but the estimates for genetic and phenotypic correlations are in close agreement with previous studies. In addition, the estimated parameters for traits associated with developmental rate, PT and AT, are in close agreement with our earlier findings. PT and AT as observed here are so highly correlated, genetically and phenotypically, that for all practical purposes they represent the same underlying phenomenon.

The replicate estimates for various traits in Table 2 agree within the limits of sampling error with the obvious exception of adult weight (AW). The AW values for replication 2 agree closely with our previous studies which consistently found body weights at the pupal and adult stages to be highly correlated, both genetically and phenotypically. An examination of the original data for the present study revealed some families of replication 1 with adult weights unexpectedly larger than their pupal weights, to suggest the possibility that these families had by accident been fed previously to weighing while others had not. This unexpected 'common environment' effect not only inflated the h^2 estimate, but biased other AW parameters for the initial generation of replication 1.

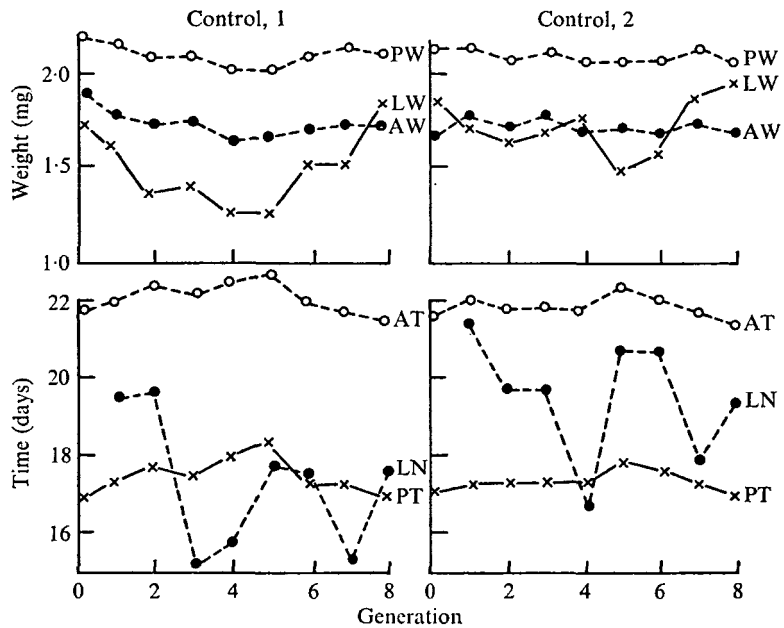

Fig. 1. Trends for various traits in the two control populations (see text for definition of traits). Ordinate scale for larval number (LN) is number of larvae per mating.

Controls. The performance of an unselected control population, L^cP^c, was measured simultaneously with the experimental populations of each replication. Genetic change for both selected and unselected traits in the experimental populations will be presented in later sections as deviations from control.

Trends for the various traits in the two control populations are presented in Fig. 1. Even though both replications were cultured in the same incubator with humidity, light and temperature controlled within narrow ranges, sizable fluctuations are seen for LN and LW. Developmental rates, PT and AT, were affected to a smaller degree with PW and AW least affected. The trends observed are assumed to be environmental in origin rather than genetic. The major points of interest in Fig. 1 relate to the association between traits. These are: (1) AW consistently follows the trends in PW with a 16–18 % loss in weight during this final

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metamorphosis; (2) environmental trends for LW had little influence on PW or AW; (3) trends in PT and LW were closely related and suggest some common environmental influence acting inversely on these two traits; (4) AT consistently parallelled the trends in PT with the time between pupation and adult emergence being a fairly uniform 4·5 days; and (5) environmental fluctuations for LN were unrelated to fluctuations in the other traits.

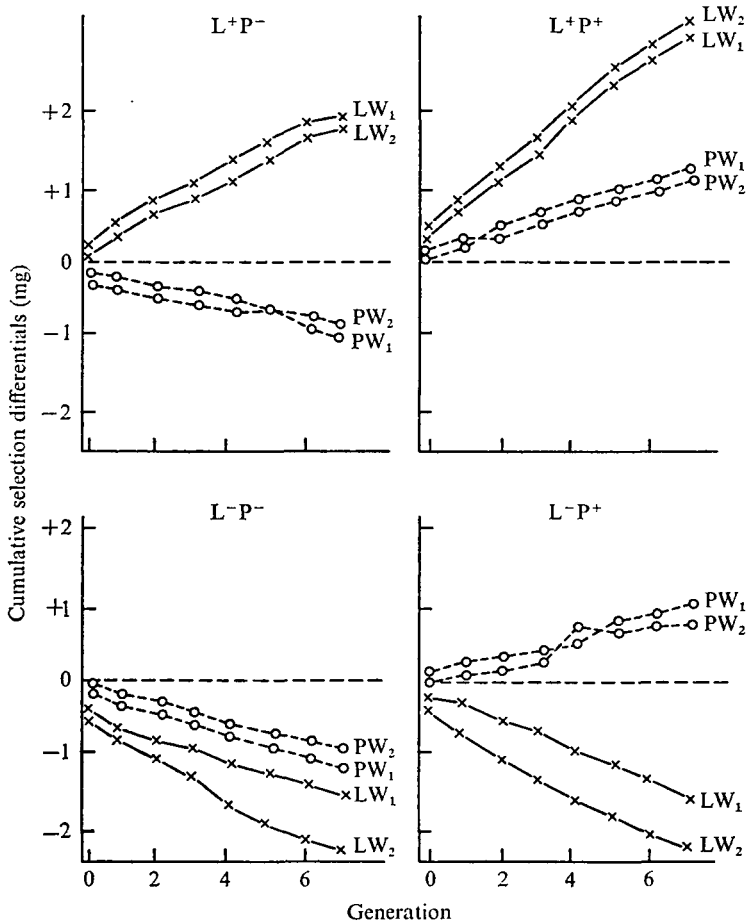

Fig. 2. Cumulative selection differentials for selected traits, larval weight (LW) and pupal weight (PW), by replication and selection method.

Selection differentials. The proportion of parents selected each generation under each method was designed for 25 % (24 parents selected from 96 individuals observed); however, the proportion realized was closer to 30 % due to fewer observations on the average than expected (93·3 versus 96) and to the replacement of sterile matings (averaged at 9 % over all populations).

Using the nomogram given by Young & Weiler (1960), selection differentials of 0·68 standard units were predicted for each of the selected traits under L^+P^+ and L^-P^- with differentials of 0·56 standard units predicted for L^+P^- and L^-P^+.

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These values when multiplied by appropriate phenotypic standard deviations predicted selection differentials in absolute units.

The cumulative selection differentials observed for each method are shown in Fig. 2. The observed trends in Fig. 2 in terms of the regression of cumulative selection differentials on generations are compared in Table 2 with predicted values based on the theoretical nomogram of Young & Weiler (1960). It is readily apparent that the selection differentials for each trait were smaller when the two traits were selected in opposite directions, i.e. L^+P^- and $^-P^+$. The observed selection differentials agreed closely with those predicted from initial parameters with the exception of LW under L^-P^- selection and PW under L^-P^+. The nature of these discrepancies will be discussed in a later section.

Table 3. *Predicted and observed selection differentials for selected traits*

(At each point the upper value is replication 1.)

Method of selection	Selection differentials per generation (dμg)			
	Larval weight		Pupal weight	
	Predicted	Observed*	Predicted	Observed*
L^+P^+	+34·6	+38·7	+15·6	+16·0
	+40·7	+40·6	+15·2	+15·5
L^+P^-	+22·6	+24·9	−10·2	−10·3
	+26·6	+22·8	−9·9	−9·6
L^-P^+	−22·6	−19·0	+10·2	+14·2
	−26·6	−26·7	+9·9	+12·2
L^-P^-	−34·6	−15·0	−15·6	−14·9
	−40·7	−27·5	−15·2	−14·3

* Standard errors for LW and PW values ranged from 0·59 to 1·07 and from 0·27 to 0·65, respectively.

Genetic changes in selected traits. Observed responses for the two selected traits, LW and PW, are presented as deviations from control in Fig. 3 by method of selection and replication. It is evident that the primary goals of selection were realized in every case. As expected from the known positive genetic correlation between the two traits, the largest changes occurred under L^+P^+ and L^-P^- selection. To assist in interpreting these response curves, the genetic changes each generation (defined as the difference between the mean performance in any particular generation and the mean in the previous generation) were subjected to analyses of variance with 'Generations' and 'Methods' considered as fixed variables and 'Replications' as random. These analyses as summarized in Table 4 reveal for the selected traits that among the main effects, methods of selection were the only significant influence. The significant interactions, 'G × R' and 'G × M', are of little concern to the overall interpretation. It is important to note that the 'R × M' interactions were non-significant to indicate that the methods of selection responded similarly over both replications.

Table 4. *Analyses of variance among differences between successive generations in population means for both selected and unselected traits under diverse methods of selection.*

		Mean squares by trait					
		Selected traits		Unselected traits			
Source	d.f.	Larval weight	Pupal weight	Pupation time	Emergence time	Adult weight	Larval numbers
Generations (G)	7	297·1	76·2	0·0212	0·0200	31·4	34·95**
Replications (R)	1	1·2	15·0	0·0015	0·0014	10·8	4·74
G × R	7	292·1**	82·4*	0·1504*	0·1475	32·7	3·25
Methods (M)	3	1396·1**	588·7**	0·2683**	0·2689*	384·1**	1·54
G × M	21	135·8*	20·9	0·0436	0·0481	17·3	4·15
R × M	3	26·4	8·8	0·0114	0·0130	8·2	0·93
G × R × M	21	63·1	16·1	0·0574	0·0566	14·7	3·73

* Statistically significant ($P < 0·05$). ** Statistically significant ($P < 0·01$).

Table 5. *Selected traits, observed genetic change in population means compared with those predicted from initial parameters and those expected from parameters estimated each generation.*

(At each point the upper value is replication 1.)

Method of selection by trait	Genetic change per generation		
	Predicted	Expected*	Observed*
	Larval weight (dμg)		
L^+P^+	+17·7	+15·5	+10·4
	+30·3	+10·3	+10·3
L^+P^-	+4·3	+2·2	+2·2
	+9·6	+4·9	+0·1
L^-P^+	−4·3	−5·1	−5·4
	−9·6	−8·5	−4·8
L^-P^-	−17·7	−9·1	−9·8
	−30·3	−14·5	−9·7
	Pupal weight (dμg)		
L^+P^+	+11·1	+5·8	+8·1
	+12·0	+6·3	+7·0
L^+P^-	−6·4	−2·3	−2·5
	−4·4	−2·5	−2·8
L^-P^+	+6·4	+2·9	+1·8
	+4·4	+3·7	+3·9
L^-P^-	−11·1	−5·3	−6·2
	−12·0	−8·4	−5·1

* Standard errors for LW and PW values ranged from 0·41 to 1·68 and from 0·24 to 0·97, respectively.

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These observed responses when measured as the regression of cumulated response on generation of selection are compared in Table 5 with genetic changes calculated by prediction equations given earlier. 'Predicted' values were based on initial parameters. The 'Expected' genetic changes were based on new parameters calculated each generation during the selection phase. These values are regression coefficients obtained by regressing the cumulated 'Expected' responses on generation of selection.

In contrasting the observed changes with those expected from genetic theory it is worth noting that the direction or goal of change was correctly predicted in every case with the initial parameters; however, with a single exception (LW in L^-P^+, Rep. 1), these 'predicted' changes were consistently greater than those observed. On the other hand, the Type 2 predictions calculated from new parameters each generation were much closer to reality. Apparently some of the predictive parameters were estimated poorly in the base populations or they changed substantially during the selection phase.

Parameter trends. Selection differentials predicted from initial parameters agreed well, with two notable exceptions (LW in both replications of L^-P^- and PW in both replications of L^-P^+), with those actually observed (Table 3). Phenotypic correlations between the selected traits, LW and PW, calculated each generation revealed no trends associated with these exceptions. However, trends for the phenotypic variances were revealing. In the first case, sharp declines in σ^2_P for LW were observed under L^-P^- selection (averaged 526 and 916 over the last four generations for replications 1 and 2, respectively, versus 2024 for L^cP^c). The second case, larger than predicted PW selection differentials under L^-P^+, resulted from an increased σ^2_P for PW (690 and 462 for replications 1 and 2 versus 382 for L^cP^c). In general, the phenotypic variances tended to be correlated positively with population means, but to a lesser degree than noted above.

An attempt was made to detect changes in genetic parameters by regressing the estimates of genetic variances and covariances calculated each generation on generation number. Only 12 matings were represented at each point and the resulting estimates were highly variable. More reliable estimates were obtained by pooling data within each population for the four terminal generations. This provided 48 full-sib families for each method of selection and replication combination. These are contrasted in Table 6 with 'initial' estimates from the unselected base populations. It should be noted that the estimates for the unselected control, L^cP^c, were based on the same generations (5–8) as were pooled for the other methods of selection. Yet the L^cP^c parameters, other than for genetic drift, would have the same expected values as those found initially. In view of the large sampling variance associated with such estimates, the 'initial' estimates and those from the terminal generations of L^cP^c agree remarkably well.

In regards to the genetic variances listed in Table 6, it appears that a decline occurred for both traits under all methods of selection except L^cP^c. These declines in the genetic variance for LW when taken relative to trends for phenotypic variances signified reductions in h^2 values by about 20% in 5/8 of the selected

populations. For PW the declines in h^2 were more obvious with 7/8 of the selected populations having terminal values near one-half of the initial estimates (i.e. 0·35 versus 0·68). Standard errors for the h^2 estimates for both traits ranged from 0·07 to 0·12.

Table 6. *Genetic variance and covariance estimates for selected traits pooled over the last four generations for different selection methods in comparison with initial estimates.*

	Parameters by trait and replication								
	Genetic variance						LW-PW genetic covariance		
	LW			PW					
Selection method	1	2	Pooled	1	2	Pooled	1	2	Pooled
Initial	641	1422	1032	221	193	207	185*	287	236
L^cP^c	902	1015	958	174	241	208	246	357	301
L^+P^+	819	580	700	148	133	140	265	189	227
L^-P^-	164	409	287	133	129	131	78	210	144
L^+P^-	680	765	722	140	125	133	252	193	222
L^-P^-	746	527	637	210	166	189	326	94	210

* All covariances are positive

The genetic covariances between LW and PW listed in Table 6 are all positive as was the case initially, but somewhat surprisingly no obvious trend is associated with any selection method. While the estimates for some selected populations are smaller than those for L^cP^c, the suggested declines for the genetic covariance seem to be associated with reduced genetic variances rather than with any particular method of selection. Consequently, and of particular interest in this study, when these values are translated into genetic correlations, one finds no evidence for any change in this parameter (average values over replications for L^cP^c, L^+P^+, L^-P^-, L^+P^- and L^-P^+ were positive 0·67, 0·72, 0·75, 0·72 and 0·61, respectively, with sampling errors of 0·06 to 0·09).

While declines in h^2 for both selected traits were clearly evidenced, the possibility of an upward bias in these estimates from non-additive gene effects and/or common environmental influences was pointed out earlier. Calculations of realized heritabilities separately for LW and PW confirmed this point.

Secondary selection among correlated traits can bias estimates of realized h^2 for any one selection method, with the direction of this bias depending on whether the two traits are selected in the same or in opposite directions. An overall realized h^2 was calculated as the regression of response for any trait on the cumulated selection differential for that trait averaged over the four diverse methods of selection practiced here. The resulting realized h^2s were: $0{\cdot}25 \pm 0{\cdot}07$ for LW and $0{\cdot}30 \pm 0{\cdot}06$ for PW. These values agree with other studies on these traits originating from the same foundation population.

Genetic changes in unselected traits. The unselected traits of pupation time (PT), adult emergence time (AT), adult weight (AW) and larval number (LN), were

measured each generation. Genetic changes for each trait, calculated as deviations from control, were first examined by analyses of variance over generations, replications and selection methods. (The resulting mean squares are listed under 'Unselected traits' in Table 4.) The fact that 'Methods' was the most consistent significant influence for all traits, other than LN, indicated that these changes were correlated with genetic changes for one or both of the selected traits. Also, the non-significant 'R × M' interactions for all traits suggest that genetic drift was unimportant and, in general, add confidence to the overall findings.

Table 7. *Unselected traits, observed genetic change in population means compared with those predicted from initial parameters and those expected from parameters estimated each generation*

(At each point the upper value is replication 1.)

Method of selection by trait	Genetic change per generation		
	Predicted	Expected*	Observed*
	Pupation time (days)		
L^+P^+	−0·10	−0·19	−0·06
	−0·29	−0·04	−0·05
L^+P^-	−0·09	−0·04	−0·07
	−0·14	−0·06	−0·01
L^-P^+	+0·09	+0·27	+0·17
	+0·14	+0·17	+0·14
L^-P^-	+0·10	+0·27	+0·21
	+0·29	+0·21	+0·15
	Adult weight (dμg)		
L^+P^+	+8·75	+6·67	+6·66
	+10·66	+3·96	+5·16
L^+P^-	−7·34	−2·17	−1·89
	−2·96	−2·21	−2·28
L^-P^+	+7·34	+1·20	+0·78
	+2·96	0·00	+2·80
L^-P^-	−8·75	−3·99	−5·25
	−10·66	−6·55	−4·41

* Standard errors for PT and AW values ranged from 0·01 to 0·03 and from 0·26 and 0·95, respectively.

The fitness trait, LN, apparently was unaffected by the genetic changes in the selected traits in view of its non-significant mean square for 'Methods' in Table 4. The significant influence of 'Generations' could arise from either (1) an unreliable control or (2) all experimental populations were deviating from their control in the same direction, e.g. a general decline in fitness. Trends within populations were examined by regressing mean LN on generations of selection. Three of the four populations selected for small PW yielded negative regression coefficients but only one was significant ($P < 0·01$). The other populations showed no over-all

change or a small but non-significant increase. Yet these trends were not of sufficient magnitude to be identified significantly with 'Methods' in Table 4.

Theoretically, the genetic changes for unselected traits are predictable from appropriate parameters. Comparisons are made in Table 7 between observed and predicted changes in a manner similar to those made for selected traits. LN is excluded since the necessary parameters for predicting change were not available. Also, AT is not listed because this trait (measured as deviation from control) had values nearly identical with those for PT in Table 7. That is to say, that the difference between AT and PT (time spent as pupae) as measured in this study was essentially invariant.

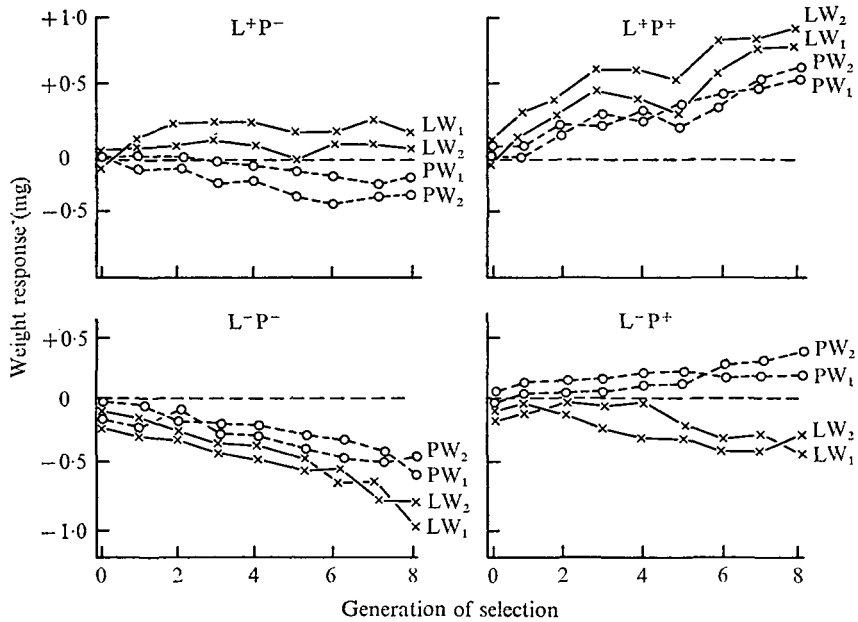

Fig. 3. Observed responses for selected traits, larval weight (LW) and pupal weight (PW) by replication and selection method as deviations from control.

Points of major interest from Table 7 are the following: (1) Significant changes were observed for PT, AT and AW, and these changes were consistent in direction and generally in amount for any particular selection method. (2) The direction of these changes were correctly predicted from initial parameters, but the selection phase parameters were more accurate in predicting the magnitude of change. (3) PT and AT changed inversely with LW selection (they increased in L^-P^- and L^-P^+ while decreasing in L^+P^+ and L^+P^-). Change in AW was influenced by both selected traits, but to a greater degree by PW (i.e. $L^+P^+ > L^-P^+ > L^+P^- > L^-P^-$). (4) Changes in the unselected traits were in general agreement with selection theory.

Overall changes in growth patterns. The overall changes resulting from divergent two-trait selection can best be shown by contrasting the relative growth and

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development patterns of the selected populations in the terminal generation with the control. Since the two replications of each method had similar overall responses, they were pooled in generation 8 for the graphic presentation in Fig. 4. The observed points on the developmental continuum depicting each method of selection are connected for discussion purposes and are not intended to represent the intermediate points.

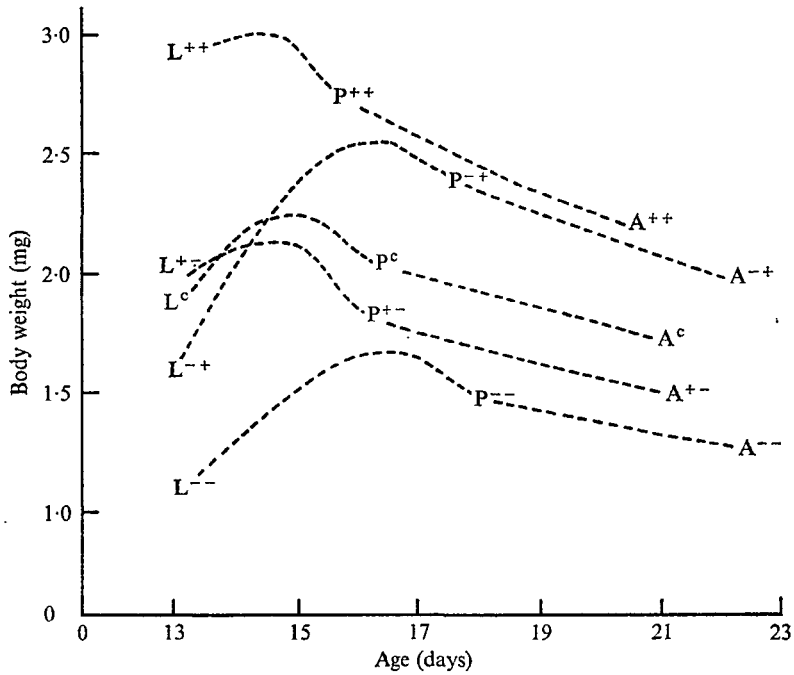

Fig. 4. Relative growth patterns after eight generations of divergent two-trait selection. Different methods of selection are identified by superscripts to the various metamorphic stages (i.e. L^c refers to 13-day control larvae, P^{+-} to pupae from L^+P^- selection, and A^{-+} to adults from L^-P^+ selection). Developmental time is plotted from the abscissa with body weight from the ordinate scale.

In examining Fig. 4, note first that the primary goals of selection were achieved. That is, the populations selected for either large or small LW and PW deviated from control as expected. Direct responses for LW and PW under L^+P^+ and L^-P^- selection were of the order of two and three phenotypic standard deviations, respectively. However, in achieving these primary goals, numerous accommodations or changes in developmental rate were required. For example, LW selection resulted in significant changes in days to pupation which were inversely related to the LW changes. Yet selection for PW scarcely affected the rate of development.

The compensatory growth which occurred after day 13 illustrates the animal's inherent flexibility or potential to adapt. For example, the control, which grew more slowly than L^+P^- during the first 13 days, had significantly more growth during the second observational period (13th day to day of pupation). Growth for

L^-P^+ during this second period was even more striking in that it surpassed both control and L^+P^-. But such was the precise goal of L^-P^+ selection. If one expresses growth during this period as *gain* in body weight, the moderate *positive gain* for control can be considered 'normal' for these environmental conditions. However, two selection methods were characterized by *positive gains* which were significantly larger than control and the other two methods developed significantly *negative gains*.

Adult emergence time as plotted on the horizontal scale reflected differences in developmental rates already established at pupation. Also, differences between selection methods for body weight at the pupal stage continued through metamorphosis and were reflected to a large degree in adult weights. Losses in body weight during this final metamorphosis differed significantly between selection methods and were not simply proportional to body weight. This result suggests the possibility of genetic differences in metabolic efficiency arising as a correlated response. Since the total metabolic resources required for the complex transformation from mature larva to adult beetle must be encompassed in the pupa, selection limits for antagonistic changes in growth patterns (e.g. PT^+AT^-, LW^+PW^- or PW^-AW^+) would likely be a function of metabolic efficiency in the utilization of these fixed pupal resources.

5. DISCUSSION

While single trait selection has been investigated extensively, the simultaneous selection of two or more traits has had a limited experimental examination even though the theoretical expectations are well known (Hazel & Lush, 1942; Young, 1961). Two selection studies with *Drosophila* have compared different methods of selection for the simultaneous improvement of two traits. Rasmuson (1964) found, contrary to theoretical expectations, that selection based on independent culling levels was superior in two experiments to index selection for increasing the total number of bristles. Yet index selection was more effective in an unreplicated test for decreasing the total number. The two traits, sternopleural and abdominal bristle numbers, initially were estimated to have a high positive genetic correlation, but the results suggested little or no genetic correlation. Declines in heritabilities were evidenced under both methods of selection, but changes in the genetic and phenotypic correlations were undetected. On the other hand, Sen & Robertson (1964) compared index, independent culling levels, and tandem methods for increasing both abdominal and sternopleural bristles in two experiments involving several replicate lines and concluded from their results that the index method was superior as predicted by selection theory. While they assumed the two traits were uncorrelated for theoretical calculations, mildly positive genetic and phenotypic correlations were consistently found. No changes in h^2 or genetic correlations were detected over 12 generations of combined selection. Neither of the above studies attempted to select the positively correlated traits in opposite directions.

The present study with *Tribolium* was undertaken in order to provide a more detailed experimental examination of the consequences of two-trait selection. In a general sense, the observed responses from the four diverse combinations of two-trait selection (L^+P^+, L^+P^-, L^-P^+ and L^-P^-) were in agreement with the theoretical predictions based on initial parameters. This statement is supported by the fact that in every case, the direction of changes was correctly predicted for both selected and unselected traits. However, the actual magnitude of these changes was not accurately predicted from initial parameters in many cases.

Evidence from three sources indicated that the discrepancies between predicted and observed genetic changes arose in part from inflated initial h^2 estimates and in part from declines in h^2 during the selection phase. Evidence for the former came from previous studies in this laboratory which reported h^2 values (obtained by less biased procedures) some 50 % smaller than our initial estimates. Secondly, realized h^2 calculated over the selection phase for LW and PW were about one-half of the initial estimates to suggest either an initial upward bias or a subsequent decline or both. Further evidence for actual declines came from h^2 values calculated for the terminal generations of selection by the same procedures as used initially. These h^2 estimates for both LW and PW in the selected populations were significantly smaller than comparable estimates in the unselected controls, while the latter values were in agreement with the initial estimates. Changes in the phenotypic variances occurred in a few cases, but these declines in h^2 resulted primarily from declines in the genetic variances.

The validity of the genetic correlation in predicting correlated responses has been demonstrated amply in single trait selection experiments. Yet reported changes in genetic correlations are not so prevalent, even though the genetic covariance between two traits is supposed to be more sensitive to changes in gene frequencies than the genetic variance of the single traits (Bohren, Hill & Robertson, 1966).

From an intuitive point of view, the simultaneous selection of two correlated traits should change the genetic correlation more quickly than single trait selection. In fact, if pleiotropic genes with '+ +' or '− −' effects on the two traits were equally frequent as those with '+ −' or '− +' effects, changes in the genetic correlation would not necessarily follow single trait selection. On the other hand, the genetic correlation would be expected to change toward negative when two traits are selected simultaneously in the same direction as in the extensive experiments of Rasmuson (1964) and Sen & Robertson (1964). Yet neither of these studies were able to detect changes in the genetic correlation even though Rasmuson observed a decline in heritabilities.

The present experiment was designed especially to 'break' a genetic correlation in a population where linkage equilibrium was a reasonable assumption in view of the population's long history of random mating without artificial selection. Consequently, genetic correlations are assumed to result from genes with pleiotropic effects or from tightly linked genes which segregate as a single unit. In regards to the various combinations of plus and minus pleiotropic effects, the L^+P^+

and L^-P^- selection methods should eventually move all genes acting on either trait toward fixation or elimination except those with antagonistic effects ($+-$ or $-+$). With the latter effects predominating, the genetic correlation would become negative. In contrast, the L^+P^- and L^-P^+ methods should capitalize on all genes except those acting synergistically ($++$ or $--$) on both traits with the genetic correlation tending to become more positive.

Even though this study reflects only short-term trends, the selection intensities were such that sizeable changes resulted in population means, phenotypic and genetic variances. Yet the genetic correlation between the two selected traits apparently remained unchanged. This apparent stability of the genetic correlation was not anticipated. One measure of reproductive fitness, number of offspring per mating, indicated that natural selection was not a significant counter force in this study.

In view of the large sampling errors associated with estimates of genetic variance and covariance, one could assume that the above trends for h^2 and r_G are rare chance events with no general significance. On the other hand, it should be pointed out that the current theory of genetic correlation relates solely to additive gene effects and assumes a linear relationship between them. In reality, when genes affecting such complex processes as growth and development are changed in their frequencies by diverse selection pressures, their pleiotropic effects will undoubtedly be modified in a non-linear manner by switch genes or modifiers to maintain some 'norm' within the developmental or physiological limits of the organism. In the simplest case, this might involve no more than a scaling effect. This concept of developmental balance via non-linear gene action seems to be a more plausible hypothesis for the 'stabilized' genetic correlations than to assume that the observed declines in genetic variances resulted solely from changes in the frequency of genes without pleiotropic effects. In order to resolve this problem, additional experimental evidence from a broad spectrum of genetic material is needed.

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