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Certain Growth Characters of *Tribolium castaneum* as Modified by Long Term Selection for 21-day Pupa Weight[1]

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Summary. Seven related populations of *Tribolium castaneum* were compared to determine the effect that selection for increased 21-day pupa weight had on certain other aspects of growth.

It was found that the response to selection for increased 21-day pupa weight had been accompanied by an increase in weight at all ages from day 6 to day 30, an increase in the growth rate during the larval period, and an increase in weight loss during the pupal stage. As a consequence, the shape of the growth curve was altered. The removal of selection pressure for six generations resulted in a decline in all of these parameters. The decline in weight was most pronounced at 21 days. Overall, it appeared that the shape of the growth curve was reverting to the shape found in the unselected populations.

Comparisons between the select line and a reconstructed base population indicated no significant difference in egg weights or weight on day of hatch. The select line had significantly fewer larval molts and less variability in time of molting.

Selection experiments have been used extensively by geneticists to study genetic contributions to growth. Most of this work has focused upon specific aspects of growth such as developmental time, growth rate during a particular period of the life cycle or weight at some specific age. Relatively little work has been done to examine the impact that such selection may have on the entire pattern of growth. It was found by Timon and Eisen (1969) that selection for postweaning growth rate in mice increased the mean absolute growth rate over the entire period from age 5 days to age 98 days but had no effect on the relative growth rate or the shape of the growth curve. However, Englert and Bell (1969) found for *Tribolium castaneum* that selection for 13-day larval weight, pupation time, and pupal weight had profound effects on the total growth and development complex.

The present study was designed to gain additional experimental evidence concerning the consequences of long-term selection for increased 21-day pupa weight upon the total pattern of growth in *Tribolium castaneum*. To accomplish this, a selected population and variously related populations were used and growth curves were estimated by measuring weights periodically throughout all developmental stages. In addition, egg weights, weight on day of hatch, and the occurrence of larval molts were examined. Preliminary results have been published previously (Walker and Goodwill, 1971).

Materials and Methods

In these experiments, the following seven lines of *Tribolium castaneum* were used: select, random, relaxed, CSI-5, CSI-10, F, and G. CSI-5 and CSI-10 were two inbred lines which had been crossed to produce the base population for the random and select lines. These inbred lines had been maintained for more than 60 generations and as a consequence both lines have a theoretical inbreeding coefficient greatly in excess of 0.99. These are described more fully by Goodwill and Enfield, 1971. These four lines (CSI-5, CSI-10, random, and select) were obtained from F. D. Enfield of the University of Minnesota. The select line had undergone 55 generations of selection for heavier 21-day pupa weight, while the random line had passed through 27 generations of random mating (Enfield, Comstock, and Braskerud, 1966 and Enfield, 1972).

After receiving the select line, it was divided into three samples. One sample established a population to be maintained under selection pressure for increased 21-day pupa weight, while selection was relaxed in the populations established from the other two samples. Both the select line and relaxed lines were maintained by mass mating and allowing a 3-day egg laying period. On day 21, 60 male and 60 female pupa from the select line were weighed. The heaviest 20 males and 20 females were selected and mated in a half pint milk bottle to produce the next generation. In the relaxed line, 30 males and 30 females chosen at random were weighed on day 21. All 60 were mass mated in a half pint milk bottle to produce the next generation. These lines were sampled for this study in the contemporary fifth and sixth generations for the select and relaxed lines, respectively.

The foundation population of the random and select lines was recreated by making the reciprocal crosses between the inbred lines and permitting a generation of random mating. These two new lines were designated F, (CSI-5 males × CSI-10 females) and G, (CSI-10 males × CSI-5 females).

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[2] The data have been taken from the masters thesis presented to the faculty of the University of Kentucky, Lexington, by the senior author.

All populations were maintained in a growth chamber at 31° ± 0.5 °C and 65 ± 5% relative humidity. Each of the seven populations were reared in half pint milk bottles containing an ample supply of the standard medium (95% whole wheat flour and 5% dried brewer's yeast). Individual matings were set up in 3/4 oz. coffee creamers (20 ml glass bottles with cardboard pull-caps), containing 1.2 g of the standard medium. Offspring were then isolated either in petri dishes or compartmentalized trays, which contained 0.3 g and 0.1 g of the standard medium in each dish and tray compartment, respectively. The sex of each individual was determined at the pupal stage. All weighings were performed on a Cahn electrogram balance.

Pilot Study. Two generations of the random line were used to determine which days in the life cycle were critical to plotting the growth curve. This was desirable since it has been shown that excessive handling and prolonged exposure to less than optimum environmental conditions may retard growth (Sokoloff, 1966).

The individuals to be weighed were produced by mating 10 males each to 3 females. After a 24-hour mating period, the females were isolated in separate creamers for three consecutive 24-hour egg laying periods and then discarded. Nine days after egg laying, 10 progeny were randomly selected from each of the full sib families and raised in individual creamers containing 0.6 g of the standard medium. In generation 0, each individual was weighed every other day, beginning 10 days after day of egg lay and ending on day 24. In generation 1, each individual was weighed every other day, beginning on day 11 and ending on day 25.

The growth curve for each generation was then plotted using the average weight for each weigh day. It was decided, by observation, that a good approximation of the growth curve could be obtained by weighing the individuals on days 9, 11, 13, 19, 21, 27, and 29.

Experiment 1. In the first experiment, the growth curves of the CSI-5, CSI-10, random, and select lines were compared. Males from each line were mated to a single female from the same line and after a 24-hour mating period females were transferred to fresh media for three consecutive 24-hour periods of egg laying. Six progeny were selected at random from each full sib family on the ninth day following egg laying, weighed, and placed in an individual creamer. Each individual was weighed again at age 11, 13, 19, 21, 27, and 29 days. This procedure was replicated twice using 10 full sib families per line in each replication.

Experiment 2. The procedures described previously for experiment 1 were followed in both generations of experiment 2. In generation 0, the select, random, and the two F_2 (F and G) lines were compared. In generation 1, comparisons were made among the select, relaxed, F and G lines. The F and G lines in generation 2 were really the F_3's from the cross CSI-5 × CSI-10.

Egg and Early Larval Weight. Egg and early larval weights of the F and G lines were compared with those of the select line. Ten eggs were randomly selected from each of seven full sib families per line. Each group of 10 were weighed *en masse* on day 0, day of hatch, and day 6. Three replications were performed.

Molting Frequency. The F and G lines were compared with the select line with respect to molting frequencies. Samples of approximately 200 eggs per line were obtained by mass mating 20 males and 20 females and allowing three 24-hour egg laying periods. The majority of these eggs hatched on the third day after egg laying. One hundred of these day three larvae were randomly selected in each line. The larvae were isolated in compartmentalized trays (dispo trays purchased from Scientific Products) containing 0.1 g of the standard media per compartment. Extra media was added from time to time as needed. Each tray was covered with a transparent glass which permitted observation but prevented cross contamination. Following isolation, the larvae were checked daily for the occurrence of larval molts, and the age of each molt was recorded for each individual.

Statistical Procedures. All weights were analyzed for each sex separately, using least squares procedures (Harvey, 1960). The model used in the analysis of variance for experiments 1 and 2 was;

$$Y_{ijkl} = U + L_i + R_j + (LR)_{ij} + F_{ijk} + E_{ijkl}\,,$$

where, U is the overall mean, L_i is the effect of the i^{th} line ($i = 1, 2, 3, 4$), R_j is the effect of the j^{th} replication ($j = 1, 2$), $(LR)_{ij}$ is the interaction between the i^{th} line and the j^{th} replication; F_{ijk} is the effect of the k^{th} full sib family within the i^{th} line and the j_{th} replication ($k = 1, 2, 3, \ldots, 10$), and E_{ijkl} is the random error associated with the i^{th} individual. The effect of lines was considered fixed, while all other effects were assumed to be random.

The model used in the analysis of variance of egg and early larval weights was identical to the model used in experiments 1 and 2 except the F_{ijk} term was omitted.

Results and Statistical Analysis

Pilot Study. The growth curves for both generations of the random line are presented in fig. 1. Areas of approximate linear weight change were noted from day 13 to day 19, and from day 19 to day 26. Maximum weight occurred around day 19, two to four days prior to pupation. A decline in weight was observed after pupation, and remained until the adult stage was reached. This decline probably resulted from the lack of nutrition during the metamorphic changes of the pupal stage.

It was decided that a good approximation of the growth curve could be obtained by eliminating weighings during the two linear periods of growth. Consequently, individuals in subsequent experiments were weighed only on days 9, 11, 13, 19, 21, 27, and 29.

Experiment 1. The mean squares and F values from the analysis of variance on the weights of males and females in experiment 1 are presented in tables 1 and 2. Significant differences existed among weights for the select, random, and the two inbred lines, on all days measured. Significant differences

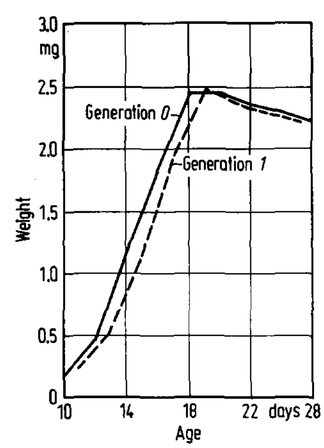

Fig. 1. Growth curves for generations 0 and 1 in the pilot study

Table 1. *Mean squares on all weigh days for males of the random, select, CSI-5, and CSI-10 lines in experiment 1*

Source[a]	Weigh Day						
	9	11	13	19	21	27	29
Line	212,945**	2,003,463**	9,970,593**	60,819,859**	53,651,601**	31,391,691**	28,675,700**
Rep	1,078	20,937	2,588,746**	171,388	179,939	373,062*	352,001*
Line × Rep	2,253	8,011	60,883	27,069	44,604	23,880	51,226
FS/LR	2,092*	32,904**	196,375	93,999	102,469	90,162	68,964
Off/FS	1,500	18,864	145,755	104,752	131,617	112,593	77,501

* Denotes significance at .05 level.
** Denotes significance at .01 level.
[a]Degrees of freedom for the sources of variation are 3, 1, 3, 69, and 122 respectively, on all weigh days.
FS/LR = Full sib family within line and replication.
Off/FS = Offspring within full sib family.

Table 2. *Mean squares on all weigh days for females of the random, select, SCI-5 and SCI-10, lines in experiment 1*

Source[a]	Weigh Day						
	9	11	13	19	21	27	29
Line	162,873*	1,290,918*	6,617,472**	46,776,867**	42,170,865**	25,528,777**	21,373,510**
Rep	7,075	214,572*	3,467,443**	98,088	70,222	40,643	135,340
Line × Rep	7,618	60,682	202,356	283,003	404,683	699,320*	169,382
FS/LR	3,481**	34,451**	194,035**	207,872*	203,364**	208,271	111,813*
Off/FS	2,104	19,113	107,305	146,133	95,142	88,100	63,052

* Denotes significance at .05 level.
** Denotes significance at .01 level.
[a] Degrees of freedom for the sources of variation are 3, 1, 3, 67, and 127, respectively, on all weigh days.
FS/LR = Full sib family within line and replication.
Off/FS = Offspring within full sib family.

were also observed among full sib families within lines on all weigh days for females and on days 9 and 11 for males. The variance among full sib families within lines contains progeny variance as well as full sib family variance. The F value which tests full sib family variance within lines is equivalent to:

$$\frac{\sigma^2 + k_1\sigma^2_{FS/LR}}{\sigma^2},$$

where σ^2 is the error variance and $\sigma^2_{FS/LR}$ is the full sib family variance within lines and replications. The significant F value obtained means that $k_1\sigma^2_{FS/LR} > 0$. According to Falconer (1960), $\sigma^2_{FS/LR}$ estimates $^1/_2\, \sigma^2_A + {}^1/_4\, \sigma^2_D + \sigma^2_{ec}$, where σ^2_A is additive genetic variance, σ^2_D is dominance genetic variance, and σ^2_{ec} is the variance due to the effects of environment, common to the individual full sib families. This common environment effect is probably nil because all full sib families were reared in the same incubator under the same rigidly controlled environment. Also individuals within full sib families were isolated from one another nine days from egg laying and were, therefore, subject to a common environment for only nine days. Therefore, the significant variation among full sib families within lines is probably due to the presence of either additive genetic variance or dominance genetic variance within these lines.

Orthogonal comparisons were made between the lines on each weigh day. The F values for these comparisons are given in table 3. There were no observed differences in weights between the CSI-5 and CSI-10 inbred lines throughout the growth period, except for

Table 3. *Orthogonal comparisons and F values on all weigh days for males and females of the random, select, CSI-5, and CSI-10 lines in experiment 1*

Day		Select vs All other lines	Random vs Inbreds	CSI-5 vs CSI-10
9	Males	372.64**	0.65	0.02
	Females	189.26**	0.20	0.31
11	Males	249.49**	1.01	2.15
	Females	157.43**	1.43	1.09
13	Males	182.23**	0.60	0.00
	Females	142.21**	0.76	4.43*
19	Males	1,796.84**	0.00	3.63
	Females	832.73**	3.97	1.04
21	Males	1,320.71**	0.13	3.53
	Females	951.91**	1.83	1.88
27	Males	895.70**	0.76	3.30
	Females	588.94**	0.86	0.19
29	Males	1,155.37**	4.53*	0.12
	Females	796.43**	4.37	0.42

* Denotes significance at .05 level.
** Denotes significance at .01 level.

Table 4. *Mean squares on all weigh days for males of the random, select, F, and G lines, respectively, in generation 0 of experiment 2*

Source[a]	Weigh Day						
	9	11	13	19	21	27	29
Line	63,916*	454,724*	2,040,594*	38,344,124**	46,818,660**	34,554,124**	27,245,826**
Rep	3,084	72,429	29,513	178,189	42,241	310,045	8,470
Line × Rep	3,349	39,982	144,440	77,101	46,113	640,260	355,865
FS/LR	2,342*	23,924**	96,228*	544,113**	178,251*	233,440*	151,181*
Off/FS	1,484	11,097	60,955	105,303	116,585	144,015	109,755

* Denotes significance at .05 level.
** Denotes significance at .01 level.
[a] Degrees of freedom for the sources of variation are 3, 1, 3, 60, and 120, respectively, on all weigh days.
FS/LR = Full sib family within line and replication.
Off/FS = Offspring within full sib family.

Table 5. *Mean squares on all weigh days for females of the random, select, F, and G lines, respectively, in generation 0 of experiment 2*

Source[a]	Weigh Day						
	9	11	13	19	21	27	29
Line	102,674**	1,073,553**	5,326,667**	47,301,998**	39,628,983**	21,867,897**	19,469,280**
Rep	1,985	222,309**	147,666	78,761	114,569	453,594	332,233
Line × Rep	3,401	103,085**	151,781	188,569	99,916	129,819	138,715
FS/LR	2,336	20,006**	86,622	167,123*	194,946*	157,055	102,122**
Off/FS	1,571	11,863	65,480	121,401	111,376	117,163	61,314

* Denotes significance at .05 level.
** Denotes significance at .01 level.
[a] Degrees of freedom for the sources of variation are 3, 1, 3, 66, and 125, respectively, on all weigh days.
FS/LR = Full sib families within line and replication.
Off/FS = Offspring within full sib family.

Table 6. *Mean squares on all weigh days for males of the relaxed, select, F, and G lines, respectively, in generation 1 of experiment 2*

Source[a]	Weigh Day						
	9	11	13	19	21	27	29
Line	71,420**	430,205**	2,388,083**	50,255,381**	43,665,419**	29,746,176**	19,599,697**
Rep	4,637	30,504	9,707	519,699*	353,561	296,225	434,210*
Line × Rep	185	1,918	10,379	190,359	196,569	122,863	186,284
FS/LR	2,153**	15,099**	51,160**	127,120	139,006	161,234*	95,994*
Off/FS	1,085	5,909	22,183	115,090	105,834	94,204	57,245

* Denotes significance at .05 level.
** Denotes significance at .01 level.
[a] Degrees of freedom for the sources of variation are 3, 1, 3, 62 and 88, respectively, on all weigh days.
FS/LR = Full sib families within line and replication.
Off/FS = Offspring within full sib family.

Table 7. *Mean squares on all weigh days for females of the relaxed, select, F, and G lines, respectively, in generation 1 of experiment 2*

Source[a]	Weigh Day						
	9	11	13	19	21	27	29
Line	60,039**	423,441**	2,387,326*	52,136,807**	41,854,892**	30,712,150**	19,005,866**
Rep	13	154,979**	320,757**	762,741*	17,240	10,370	341,562
Line × Rep	1,890	32,192	143,626*	139,172	376,746	817,906*	347,670*
FS/LR	2,172**	11,412**	39,026*	155,278**	164,825*	206,954*	115,785*
Off/FS	1,036	6,207	22,386	73,189	97,421	135,339	72,795

* Denotes significance at .05 level.
** Denotes significance at .01 level.
[a] Degrees of freedom for the sources of variation are 3, 1, 3, 65, and 80, respectively, on all weigh days.
FS/LR = Full sib families within line and replication.
Off/FS = Offspring within full sib family.

females on day 13. The only significant differences observed between the random line and the average of the inbred lines was on day 19 for females and day 29 for males and females. Overall, the random, CSI-5 and CSI-10 lines differed only slightly, and when the average of these three lines was compared with the select line, only a slight loss of variation resulted. The comparison between the select line and an average of the other three lines showed highly significant differences at all ages weighed. This indicated that the significant differences between lines in tables 1 and 2 were apparently due to differences between the select line and the other lines. This became more apparent when the growth curves were examined. The growth curves for the select, random, CSI-5, and CSI-10 lines are presented in fig. 2 and 3 for males and females, respectively. The select line was heaviest at all ages weighed. However, the difference in weight between the select line and the other lines was not uniform throughout all growth periods. Maximum differences occurred on approximately day 21. This might be expected since direct selection pressure had been applied to 21-day weight.

The slopes (growth rates), in mg./day, for the linear portion of the growth curves (days 13 to 19) where 0.24 ± .01, 0.46 ± .01, 0.25 ± .01 and 0.26 ± ± .01 for the random, select, CSI-5, and CSI-10 lines, respectively. The larval growth rate then was significantly larger in the select line. However, the select line exhibited the largest weight loss during the pupal stage. The growth rates, in mg./day, for this period were −0.07 ± .01, −0.15 ± .01, −0.07 ± .01, and −0.08 ± .01 for the random, select, CSI-5, and CSI-10 lines, respectively. Therefore, it appears that selection for increased 21-day pupa weight has actually changed the shape of the growth curve.

Overall, the random line growth curve did not differ significantly from the growth curves of the inbred lines. There were slight upward or downward shifts of the growth curves but no change in shape. In contrast to selection for heavier 21-day pupa weight, it appears then that the long period of random mating has had no effect upon the shape of the growth curve.

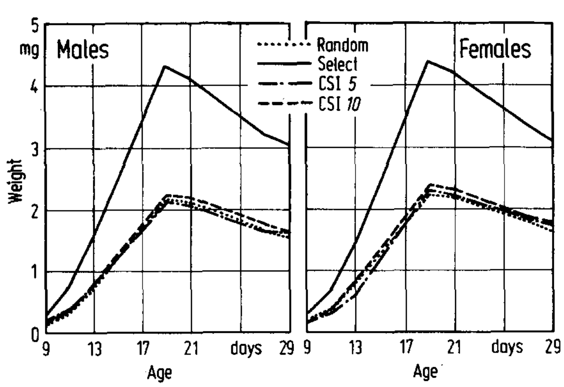

Fig. 2. and 3. Growth curves for males and females of the random, select, CSI-5, and CSI-10 lines

Experiment 2. The mean square and F values (from the analysis of variance on weights) are listed for males and females for each generation in tables 4 through 7. Variation among lines was significant at either the .05 level or the .01 level on all days. As in experiment 1, there was much variation present among full sib families within lines. The F values for this source of variation were significant at either the .05 level or .01 level on all days except for day 13 and day 27 for the females in generation 0. This can be considered, as in experiment 1, evidence for the presence of genetic variability in these lines.

The orthogonal comparisons in table 8 provide a closer examination of the variation between lines. In generation 0, the F_2 lines differed significantly on day 9 for males and females, days 11 and 13 for males, and day 27 for females. The comparison of the random versus the F_2 line average shows a highly significant difference in weights for 9-day-old males and females, and a significant difference for weights on day 11 males, day 13 males and females, and day 27 males and females. When the select line was compared with an average of the other three lines, highly significant differences were observed for all ages measured. While some of the observed variation among lines at the early ages was undoubtedly due to differences between the F_2 lines and the random line, it is evident that the majority of this variation was due to the differences between the select line and the other lines.

The random and F_2 lines were expected to be quite similar genetically since both were established by crossing CSI-5 and CSI-10 (both highly inbred lines).

Table 8. *Orthogonal comparisons and F values on all weigh days for males and females of the random, relaxed, select, F, and G lines in generations 0 and 1 of experiment 2*

Day		Generation 0			Generation 1
		Select vs All other lines	Random vs F_2's	F vs G	Select vs Relaxed
9	Males	94.00**	14.77**	9.97**	0.41
	Females	156.12**	8.33**	4.60*	2.35
11	Males	78.29**	5.03*	12.57*	1.02
	Females	215.19**	3.83	1.34	0.45
13	Males	70.59**	4.45**	15.93**	1.81
	Females	213.59**	4.72*	2.23	0.61
19	Males	466.86**	0.06	0.02	17.81**
	Females	1,033.84**	0.61	0.15	12.19**
21	Males	1,033.42**	2.87	0.55	12.92**
	Females	844.25**	1.05	1.07	4.59*
27	Males	596.11**	3.17	1.28	5.32*
	Females	487.05**	6.75*	5.41*	0.82
29	Males	675.21**	0.26	1.10	1.67
	Females	771.58**	1.12	0.01	1.38

* Denotes significance at .05 level.
** Denotes significance at .01 level.

The slight variation observed at the early ages (days 9—13) could be attributed to several things (such as loss of genes in the random line through sampling over 28 generations, or the presence of linkage disequilibrium and epistatic blocks of genes in the F_2 lines). The available data provide no basis for examining the cause of these differences, which are certainly relatively small compared with the differences among the three lines and the select line.

The only meaningful orthogonal comparison in generation 1 was between the select and the relaxed line. Highly significant differences existed between the select and relaxed lines for day 19 males and females and for day 21 males, and significant differences occurred for day 21 females and day 27 males.

Growth curves for the select, random, and F_2 lines from generation 0 are presented in fig. 4 and 5 for males and females, respectively. This is in effect, a comparison among the growth curves of the random and select lines and a reconstruction of their base population (i.e., the F_2 lines). The select line was heaviest at all ages.

The growth rates, in mg./day, during the larval period were 0.30 ± .01, 0.54 ± .01, 0.30 ± .01, and 0.28 ± .01 for the random, select, *F*, and *G* lines, respectively. The growth rates, in mg./day, for the pupal stage were −0.07 ± .01, −0.14 ± .01, −0.07 ± .01, and −0.08 ± .01 for the random, select, *F*, and *G* lines, respectively. These support the conclusion made earlier that selection for 21-day pupa weight has changed the shape of the growth curve from that of the original base population.

The growth curves of the select, relaxed, and F_2 lines from generation 1, are presented for males and females in fig. 6 and 7, respectively. The select line was again heaviest at all ages weighed. The growth rates, in mg./day, during the larval period were 0.55 ± 0.1, 0.60 ± .01, 0.31 ± .01, and 0.32 ± .01 for the relaxed, select, *F*, and *G* lines respectively. The growth rates, in mg./day, for the pupal stage were −0.12 ± .01, −0.14 ± .01, −0.07 ± .01 and −0.08 ± .01 for the relaxed, select, *F*, and *G* lines respectively. The select line had a higher larva growth rate than the other lines, but the pupal growth rates in the relaxed and select lines did not differ significantly. Evidently, the genotypes contributing to the heavier weight in the select line had less than optimum fitness, and when selection pressure was relaxed natural selection brought about a change towards a more fit weight. Although, it is possible that a breakdown of epistatic blocks of genes might also be responsible for the decrease in weight in the relaxed line (see Griffing, 1960). In any case, this is very convincing evidence that appreciable genetic variation remains in the select line.

Egg and Early Larval Weights. Average weights for eggs on day of laying and for larva on day of hatch and day 6, in the select and F_2 lines, are shown in table 9. Egg weights were larger than weights of larva on day of hatch (days 3 and 4). This was undoubtedly due to the loss of the egg membrane and extraneous material, and to the exhaustion of food supply prior to hatching. Individuals either hatched on day 3 or day 4 and weight gain began after hatching. The two lines appeared to have relatively similar weights until day 6, at which time the select line weights were significantly larger.

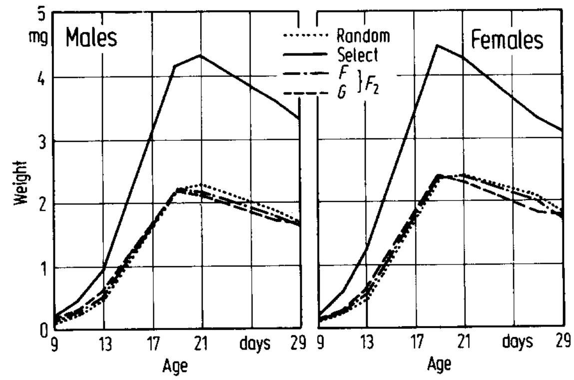

Fig. 4 and 5. Growth curves for males and females of the random, select, F, and G lines

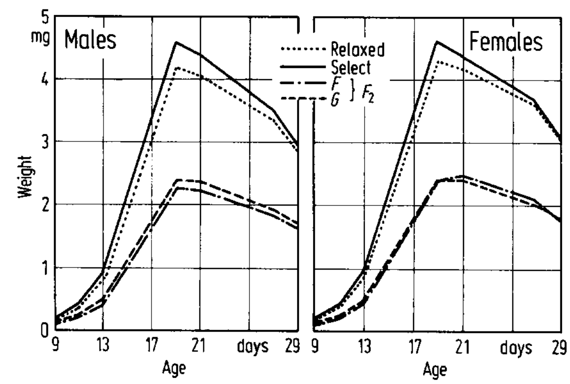

Fig. 6 and 7. Growth curves for males and females of the relaxed, select, F, and G lines

Table 9. *Average egg and early larval weight in the select and F_2 lines*

Stage	Age	Select Avg. Wt. (μg.)	F_2 Avg. Wt. (μg.)
Egg	0	36.0 ± 0.8[a]	37.0 ± 0.7[a]
Day of hatch	3	29.0 ± 1.8[b]	28.0 ± 1.0[b]
Day of hatch	4	26.0 ± 1.3[b]	22.0 ± 0.6[b]
Larva	6	52.0 ± 2.2[b]	38.0 ± 1.1[b]

[a] Average of seven sample averages from each of three replications, where each sample contained ten individuals.
[b] Due to death losses, larval numbers were somewhat less than egg numbers.

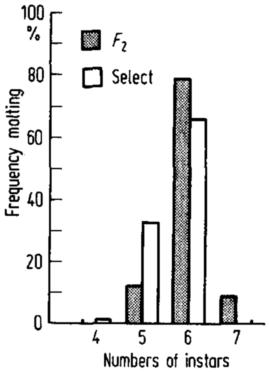

Fig. 8. Frequency of instar numbers in the select and F_2 lines

Table 10. *Analysis of variance for egg weights, day of hatch, and day 6 larval weights*

Source[a]	Egg		Day of Hatch		Day 6	
	MS	F	MS	F	MS	F
Line	2.76	0.26	38.71	1.02	1,887.81	94.34*
Rep	83.25	10.74**	38.06	3.11	63.97	1.15
Line × Rep	10.53	1.36	37.77	3.09	20.01	0.36
Residual	7.75		12.24		55.73	

[a] Source degrees of freedom are 1, 2, 2, and 36 respectively.
* Denotes significance at .05 level.
** Denotes significance at .01 level.

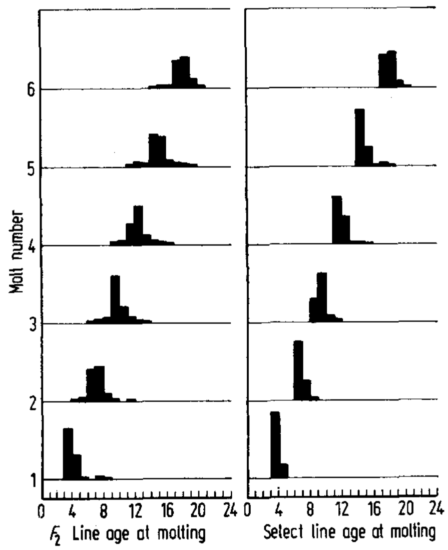

Fig. 9. Age distribution (days) for each larval molt in the select and F_2 lines

The analyses of variance for egg weights, weights on day of hatch, and weights on day 6 are presented in table 10. Line effects were not significant until day 6, at which time the select line was much heavier than the F_2 lines. It was expected that egg weights would differ significantly, since under the dissecting scope, the select line eggs appeared to be much larger than eggs from the F_2 lines. If the difference in egg size is real, then apparently it is not highly correlated with egg weight.

Molting Frequencies. A comparison of the number of larval molts in the select and F_2 lines is shown in fig. 8. *Tribolium* normally exhibit from five to eight instar events during the larval period (Sokoloff, 1966).

None of the F_2 line individuals had as few as four molts, while none of the select line individuals had as many as seven molts. There were significantly fewer molts in the select line (5.65 ± .05) than in the F_2 lines (6.00 ± .07).

Fig. 9 shows the distribution by day of occurrence of each larval molt in the select and F_2 lines. The distribution of larval molts in the F_2 line seems to follow a more normal distribution than those of the select line. The select line distributions tend to be skewed to the left. This may indicate that molting has to occur more uniformly and perhaps within a certain optimum time span in order for heavier pupa to develop. In any case, selection for 21 day pupa weight has apparently reduced the variability of developmental pathways.

Discussion

There was no significant difference between the average weight of eggs from the select line and the average weight of eggs from the unselected base population (F_2 lines). However, by day 6, the select line larvae were significantly heavier than larvae from the F_2 lines. At all subsequent ages observed, the select individuals were significantly heavier than the individuals of the F_2 lines. In contrast, very few differences were observed among the inbreds (CSI-5 and CSI-10), the F_2 lines and the random bred line. It is highly probable, therefore, that the significant differences observed between the select line and the other lines are a result of the select pressure that had been applied to 21-day pupa weight in the select line.

Similar arguments can be made for the increased rate of larval growth and increased rate of pupa weight loss observed among the select line individuals. The combined effect of these rate changes was a modification in the shape of the growth curve of the select line as compared with the growth curves of the other lines. Weight loss during the pupal stage is to be expected since there is no intake of nutrients during the complex developmental stage.

Six generations of random mating in the select line resulted in a reduction in weight at all ages, larval growth rate, and pupal weight loss. The reductions were greatest around the 21-day age. This suggests that natural selection is acting to restore more optimum growth patterns in this line. Moreover, it provides additional evidence that the selection pressure applied to 21-day pupa weight was responsible for the modification of the growth curve.

The select larvae had a more uniform pattern of growth than the F_2 larva. This was exhibited by a reduction in the average number of molts as well as a more uniform length of instar period among the select line larvae as compared to the F_2 larvae. This reduction in larval molt number is contradictory to what was reported by Englert and Bell (1969). However, this conflict can probably be attributed to slight differences in the type of selection pressure applied to pupa weight. The select lines observed by Englert and Bell had been formed by selecting the heaviest individuals after the majority of the larvae had pupated (Bell and Moore, 1958 and Bartlett et al., 1966). Since larval molt number and pupation time are highly correlated (Englert and Bell, 1969), this method of selection may have permitted the delay in pupation time and, therefore, the increase in larval molt number which was observed in the select lines. In contrast, the select line used in this study was formed by selecting the heaviest pupae at a specific age (day 21) and, therefore, pupation time was not permitted to lengthen. In fact, this method of selection actually selected against delayed pupation time by eliminating the late pupating individuals from consideration. This may have reduced the variability in pupation time and contributed to the occurrence of fewer larval molts.

The result of the increased uniformity of instar duration among the select larvae is the appearance of a threshold effect (i.e. the molt does not occur until a minimum duration is obtained). This causes the distribution in ages of each molt to be skewed to the left in the select line, whereas this distribution has an approximately normal shape in the F_2 populations. It is possible that the reduction of instar numbers along with this threshold effect permits a greater proportion of the metabolic energy to be utilized in growth. This may result in a more efficient pathway for obtaining greater 21-day pupa weight.

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