Theoretical and Applied Genetics 44, 100–105 (1974)

Crossbred Response from Purebred Selection, an Experimental Check on Selection Theory with *Tribolium*[1]

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Summary. Two unrelated populations of *Tribolium castaneum* were subjected to full-sib family selection for purebred 13-day larval weight. Over 30 selection generations, replicate lines became differentiated with respect to the trend of phenotypic variance and the path of response. All lines were responding in the latter generations indicating no tendency toward plateau. Crossbreds between the two populations responded over the total selection period. In the first ten generations, observed crossbred responses were much greater than those predicted by selection theory; it is postulated that additive maternal effects were responsible. In the remaining generations, the agreement between observed and expected response was reasonably good.

Introduction

Crossbred response from purebred selection is dependent upon the genetic covariance between additive effects of alleles in purebreds and crossbreds. Appropriate models and their description are well-presented by Griffing (1962). If either the purebred population or the crossbred population exhibits no additive genetic variation, the covariance must be zero. However, with a non-zero covariance, one would expect on theoretical grounds that purebred response from purebred selection would necessarily be accompanied by crossbred response (McNew and Bell, 1971).

The recent years have seen an intensified application of crossbreeding to commercially important animals. Much of this effort has been spent on evaluating crossbreds for their performance relative to purebreds. A review of such works on beef production is given by Cundiff (1970). If the crossbred proves to be superior, the next step is to determine if continued purebred selection will be accompanied by crossbred response. Efforts to predict response are being made; e. g., studies have been conducted in swine by Stanislaw, *et al.*, (1967) and in sheep by Salah, *et al.*, (1970). These indicate that crossbred response will result from purebred selection. However, Louca and Robison (1967) obtained evidence with swine that purebred selection for 154-day weight would not yield response in crossbreds because additive genetic variance in the crossbred was near zero.

Working with the laboratory organism *Tribolium castaneum*, Wong and Boylan (1970) examined crossbred response from purebred selection for pupal weight. They found an increase in both purebred and crossbred performance over 22 generations of selection. However, performance during the latter generations led them to predict that further purebred selection would result in continued purebred response but not in continued crossbred response. The magnitude of the purebred-crossbred genetic covariance in their latter generations was not presented. Vinson, Eisen and Robison (1969) found substantial purebred-crossbred genetic covariances for most traits studied in mice and thus predicted crossbred response from purebred selection.

The many examples available show that crossbred response is expected from purebred selection. A breeder needs only to determine the magnitude of the genetic covariance. A more critical question may then be the behavior over long term selection. The occurrence of the situation of Wong and Boylan, if their predictions hold true, would indicate that a continued evaluation of genetic parameters is required to assess the potential for continued crossbred response.

The purpose of this paper is to present information on the response of both purebreds and crossbreds to long term purebred selection for increased larval weight in *Tribolium castaneum*. Further, the predictor of crossbred response will be examined by comparison with observed response.

Experimental Procedures

The genetic material consisted of two unrelated populations of *Tribolium castaneum, black* Foundation (B) and *pearl* Foundation (P), (see Krause and Bell, 1972, for details of origin). Two replicates designated 1 and 2 were sampled from each population.

Each generation, a line was perpetuated by matings between 20 males and 40 females, each male being crossed to two females. In addition, each male was crossed to

[1] Journal Paper No. 4794 of Purdue University Agricultural Experiment Station with support from the USDA Cooperative Agreement 12-14-100-5448(48). This material is based in part on a dissertation by the senior author in partial fulfillment of the requirements for Doctor of Philosophy at Purdue University.

two females from a companion line originating from the other population. The purebred matings which were sterile were usually less than 10%; thus, the effective population size was not less than 43 per generation. Eggs were collected from each female in two consecutive 24-hour periods. Offspring were reared in a 3/4-ounce creamer containing 2 *g* of standard medium (95% whole wheat flour and 5% yeast). Creamers were maintained in a controlled chamber at 70% relative humidity and 33 °C. Five larvae from each damcollection were weighed in mass in decamicrograms ($d\,\mu g$) on a microanalytical balance at 13 days of age. If there were fewer than five larvae, all were weighed.

Selection of full-sib families for increased larval weight was conducted at an intensity of 25% where the criterion was the family mean. Individuals from all selected families were saved in mass except that weighed larvae were separated from unweighed larvae. Males for matings were taken only from the weighed-larvae group. Females from the weighed-larvae group were used in pure-line matings; females from the un-weighed group were used in cross-line matings. An unselected control line originating from each Foundation Population was observed with each replication for the first 10 generations of the study.

Prior to generation 12, *B*-1 and *P*-1 were processed on different days of the same week; *B*-2 and *P*-2 were processed on different days of the same week but in a different week than *B*-1 and *P*-1.

Beginning with generation 12, the four replicates were processed in four consecutive weeks. Between selection generations 12—13 and 24—25 there were 2 and 1 generations, respectively, of no selection. In the period 24—25, mating was between full sibs selected in generation 24 with their offspring becoming parents of generation 25. In the period 12—13, mating was random.

Statistical Procedures

The mean weight of the 5 offspring sampled from each 24-hr egg collection per dam was the variable analyzed each generation. Representing this mean by $\bar{Y}_{ijk}$, the assumed statistical model is

$$\bar{Y}_{ijk} = \mu + s_i + d_{j(i)} + c_k + \bar{e}_{ijk}$$

where s_i, $d_{j(i)}$ and c_k are the random effects of the i-th sire, the j-th dam mated to sire i, and the k-th collection day; $\bar{e}_{ijk}$ is the random error. The corresponding variance components are σ_s^2, σ_d^2, σ_c^2 and $\sigma_{\bar{e}}^2$. This model was applied to both purebred and crossbred offspring.

The generation mean was calculated as the average of the dam means. The selection criterion was the weighted average of the dam's two collection means with weights proportional to the number of weighed larvae in each collection. Phenotypic variance was estimated by

$$\sigma_s^2 + \sigma_d^2 + \sigma_c^2 + \bar{n}\,\sigma_{\bar{e}}^2$$

where $\bar{n}$ is the harmonic mean number of weighed larvae. The variance of the selection unit was estimated by

$$\hat{\sigma}_s^2 + \hat{\sigma}_d^2 + \frac{1}{2}\hat{\sigma}_c^2 + \frac{1}{2}\hat{\sigma}_{\bar{e}}^2$$

which ignores unequal numbers of weighed larvae per collection. Since σ_c^2 was usually insignificant, this creates no difficulty. The four variance components were obtained from analysis of variance.

Selection differentials were unadjusted for mortality prior to breeding or for sterility because identities of individuals were lost as a result of the holding procedure. Realized heritability of purebred response was calculated by regression of accumulated response on accumulated selection differential.

The crossbred response to be described now is patterned after Griffing (1962). When purebred selection of full-sib families is practiced in two lines, say *B* and *P*, the crossbred response when selected *B* males are mated to selected *P* females is

$$\frac{n_B + 1}{2\,n_B}\frac{Cov(B)}{V_{FB}}(SD)_B + \frac{1}{2}\frac{Cov(P)}{V_{FP}}(SD)_P$$

where n_B is the number of weighed larvae in a full-sib family in line *B*, V_{FB} is the variance of the family mean in line *B*, $(SD)_B$ is the selection differential for line *B*, and $Cov(B)$ is the covariance of additive effects of alleles in the purebreds of line *B* and the crossbreds; corresponding terms for line *P* are similarly defined. The reason the coefficients of the two terms in the response are not similar is that *P* females, crossed to *B* males, were not weighed; i.e. they do not contribute to the selection criterion. If *P* females used in crossing had been weighed, the coefficient $\frac{n_P + 1}{n_P}$ would be multiplied by the second term.

For the reciprocal mating (selected *P* males and selected *B* females), the response is obtained from the above expression by interchanging *B* and *P*.

The covariance component in the response expression is estimated by twice the covariance of a sire's purebred and crossbred family means. Estimates were made each generation and variation over generations used as a measure of variability of the estimate.

Results

The following results are described for purebred lines *B*-1, *B*-2, *P*-1, *P*-2 and reciprocal crossbreds of companion lines.

The major objective in maintaining control populations was an attempt to monitor possible environmental trends. However, by the tenth generation the controls were showing erratic responses. No environmental trends were evident in companion studies sharing the same environmental facilities, yet the *B*-2 and *P*-1 controls reflected significant ($P < .05$) negative trends while *B*-1 and *P*-2 indicated no trend. Earlier studies using these facilities had observed minor generation to generation environmental fluctuations, but no time trends. For example, the randombred lines of Hardin and Bell (1967) indicate no environmental trend; for the eight contemporary lines of similar origin, there are 5 positive slopes and 3 negative slopes for change in the mean. Randombred lines of Wilson, *et al.*, (1968) show a slight decline over eight generations but the effect on the estimates of realized heritabilities was unimportant. Bell and Moore (1972) observed pupal weight in 8 control populations over 20 generations without any detectable environmental time trend. In view of the above, the controls were discontinued and environmental trends were assumed to be negligible. It should be noted that critical comparisons, such as purebred vs crossbred performance, are always made on contemporary progenies.

The average phenotypic variance in the generation intervals 0—10, 11—20 and 21—30 are shown in Table 1. These means portray the trend over the total selection period. In purebreds, *B*-1 has the

Table 1. *Average phenotypic variance of purebreds and crossbreds in the generation intervals 0—10, 11—20, 21—30. Crossbreds are presented by sire line*

Line		Variance by generations		
		0—10	11—20	21—30
B-1	*P**	3124	4720	8320
	X	2779	2864	4170
B-2	*P*	2776	4213	3745
	X	3389	2162	2218
P-1	*P*	3550	3914	5417
	X	3179	4060	3890
P-2	*P*	3708	4017	3522
	X	2703	2290	1531

* *P* and *X* refer to purebred and crossbred respectively.

largest increase in phenotypic variance; *P*-1 also shows an increase but to a lesser extent; *B*-2 and *P*-2 show a tendency of a quadratic trend with the period 11—20 having the largest average. It is common to observe a positive relationship between mean and variance; however, linear regression analysis for trends over the 30 generation period resulted in statistical significance for only the *B*-1 purebred phenotypic variance. Considering the large sampling error associated with variance estimates, the other observed trends may reflect only chance deviations.

The crossbred phenotypic variances are generally smaller than corresponding purebred phenotypic variances. The Replication 1 crossbreds (X-1) show an increase in phenotypic variance over the 30 generations, but X-2 crossbreds show a decrease. None of the trends are statistically significant.

The observed selection differentials, as expected, follow the trend pattern of the purebred phenotypic variances. These are shown in Table 2.

Purebred response means are shown in Fig. 1 with response for each third of the selection period presented in Table 3. The general picture is one of continuing, albeit variable, response. There is no indication of plateau in these lines at generation 30. The *P*-lines initially have higher mean larval weights than the *B*-lines and this relation is consistent over the selection period. Also, Replication 1 lines become larger in mean weights than comparable 2-lines which is readily apparent by generation 30. The numerical values for generations 0 and 30 are presented in Table 3.

Table 2. *Accumulated selection differentials in each line for the generation intervals 1—10, 11—20, 21—30*

Line	S. D. ($d\mu g$) by generations		
	1—10	11—20	21—30
B-1	351.6	406.8	480.3
B-2	299.1	360.7	315.4
P-1	330.3	342.8	346.8
P-2	320.0	351.7	316.1

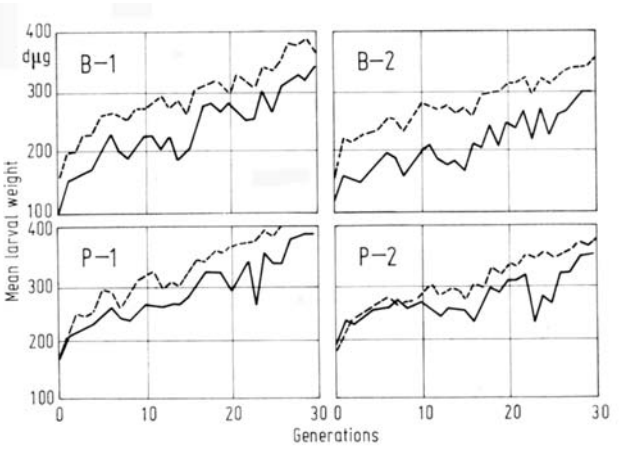

Fig. 1. Purebred and crossbred responses over 30 generations. The dash line in each case represents the crossbred of the same dam type as the purebred

Table 3. *Initial and terminal means and responses or genetic gains for the generation intervals 1—10, 11—20, and 21—30 in both purebreds and crossbreds. Crossbreds are shown by sire line*

Line		Means and genetic gains ($d\mu g$)				
		Means		Generation Interval		
		Gen. 0	Gen. 30	1—10	11—20	21—30
B-1	*P**	104	350	124	61	61
	X	175	414	140	49	50
B-2	*P*	121	304	81	49	53
	X	178	370	102	54	36
P-1	*P*	176	386	93	18	99
	X	158	378	120	23	77
P-2	*P*	190	347	77	37	43
	X	162	360	119	33	46

* *P* and *X* refer to purebred and crossbred, respectively.

An alternative view of purebred response is provided by Fig. 2 which shows the response relative to accumulated selection differential. *B*-1, which had a large increase in phenotypic variance, has a much larger accumulated selection differential than the other lines. *B*-1 and *B*-2 appear to follow the same response path with *B*-2 doing so at a slower rate. *P*-1 and *P*-2 which have about the same accumulation of selection differential, are following different response paths.

Realized heritability in purebreds is presented in Table 4. Obviously, the effective heritability declined during the early generations, but it remains relatively constant in the last 20 generations. This reiterates the graphical illustration of Figure 1 that there is no tendency toward exhaustion of additive genetic variance.

Crossbred response means are shown in Fig. 1. A particular crossbred response is shown with the purebred response of corresponding dam type; e. g.

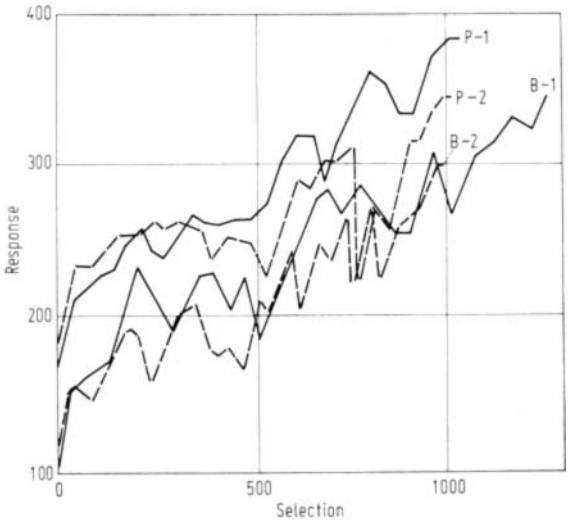

Fig. 2. Purebred responses ($d\,\mu g$) relative to accumulated selection differentials

Table 4. *Realized family heritabilities for the generations intervals 1—10, 11—20, 21—30*

Line	Heritabilities by generations		
	1—10	11—20	21—30
B-1	.26*	.19	.16
B-2	.18	.14	.18
P-1	.20	.17	.27
P-2	.18	.11	.19

* Standard error for each estimate is 0.05.

Table 5. *Reciprocal differences ($d\,\mu g$) for the generation intervals 0—10, 11—20, 21—30*

Cross		Generation Interval		
		0—10	11—20	21—30
P-1	*B*-1	+23.0±3.6	+30.1±6.0	+34.1±5.1
P-2	*B*-2	+15.1±3.6	+13.9±4.2	+20.2±3.4

crossbred means from mating *B*-1 females to *P*-1 males are shown on the *B*-1 graph. The reason for doing this is the large reciprocal difference. These differences are presented in Table 5. *B* females mated to *P* males produce smaller offspring than the reciprocal mating of *P* females to *B* males. At generation 0 the crossbred is not superior to both purebreds in either replicate. The crossbreds are about the same as the *P* purebreds: *P*-1 purebreds-176, crossbreds from *P*-1 females-175-, *P*-2 purebreds-190, crossbreds from *P*-2 females-178. This result would approximate directional dominance of the genes in the *P* lines. In Replication 1, the crossbred becomes superior at generation 2 and remains so thereafter. In the 2 lines, the crossbreds do not obtain a definitive superiority over both purebreds until generation 10. As with purebred response, there is no hint of a plateau of crossbred response. Calculations for heterosis are presented in Table 6. These data reflect the pattern described above and discernible in Fig. 1.

Table 6. *Purebred and crossbred performance summarized by 5-generation intervals with calculations of heterosis*

Generations	Larval weight, $d\,\mu g$			
	Purebred[1]	Crossbred[2]	$F_1 - MP$	% Heterosis
Replication 1				
0—4	184	217	33±3	18
5—9	228	276	48±6	21
10—14	242	297	55±5	23
15—19	282	325	43±6	15
20—24	295	348	53±9	18
25—30	342	389	47±5	14
Replication 2				
0—4	190	219	29±5	15
5—9	221	259	38±3	17
10—14	224	280	56±4	25
15—19	237	293	56±5	24
20—24	269	328	59±9	22
25—30	299	348	49±7	16

[1] Purebred = mid-parent or average of pure lines during the respective generation intervals.

[2] Crossbred = similar calculation on reciprocal F_1 or crossbreds.

Table 7. *Average estimates of the genetic regression of crossbred on purebred family means, $Cov\,(\cdot)/2V_\cdot$, for the generation intervals 1—10, 11—20, 21—30*

Sire Line	$Cov\,(\cdot)/2\,VF_\cdot$ by generations		
	1—10	11—20	21—30
B-1	+.11*	+.02	+.10
P-1	+.09	+.12	+.11
B-2	+.02	+.11	+.10
P-2	+.04	+.05	+.02

* Standard error of each estimate is 0.04.

Table 8. *Observed and expected crossbred response from purebred selection*

Cross	Responses ($d\,\mu g$) by generations					
	1—10		11—20		21—30	
	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
B-1 × *P*-1	72±18	130	50±23	36	93±30	60
B-2 × *P*-2	19±19	110	60±18	44	40±18	41

Crossbred response when purebred family selection is practiced has the predictive expression given in the procedures section. Table 7 contains the average estimates of $\frac{Cov(\cdot)}{2\,V_F}$ for each line. The responses expected using these estimates are compared in Table 8

with those observed. One finds a poor agreement over the first ten generations in that observed responses are considerably greater than those expected. The simplest explanation is the existence of additive maternal effects, which would not be contained in the covariance of sire family means but would contribute to response from full-sib family selection. The reasonable agreement for the last 20 generations shows that this maternal source has been exhausted. *P*-2, whose purebred response was smallest of the four lines, shows the smallest crossbred response rate over the total selection period; on the other hand, *B*-1 and *P*-1 have the largest crossbred response rates and produced the largest purebred responses. Finally, it is apparent that all lines are contributing to response of crossbreds.

Discussion

Larval weight at 13-days of age in *Tribolium castaneum* is a heterotic trait of medium heritability and has exhibited influence of maternal differences (Englert and Bell, 1963; Hardin and Bell, 1967). Heritability estimated from the dam component of variance is consistently and substantially larger than heritability obtained from sire component estimates (Hardin and Bell, 1967; Wilson, *et al.*, 1968; and Krause and Bell, 1972). The heterotic nature of the trait is reflected in our results by the crossbred being about the same as the superior purebred during the initial generations. The difference of reciprocal crossbreds could be explained by maternal differences between populations.

Differentiation of replicate lines is a common phenomenon of selection experiments. Clayton and Robertson (1957) found that repeatability for the mean number of abdominal bristles in Drosophila was not high, even in early generations. In later generations, differentiation was also manifested in the variance. Differentiation has occurred in our experiment; it has occurred in different ways for different lines. *B*-1 and *P*-1 showed increases of phenotypic variance in both purebreds and crossbreds; *B*-2 and *P*-2 showed a tendency toward a quadratic change of purebred phenotypic variance, increasing then decreasing, while their crossbreds's phenotypic variance declined. *B*-1 and *B*-2 followed the same response path but at different rates over generations. *P*-1 and *P*-2 followed different response paths. The preceding comments merely describe the results observed on the four lines. Except for *B*-1, trends of variance components were not statistically significant. Also, the differences among realized heritabilities of replicate lines were not statistically significant.

Crossbred means in the early generations were not superior to both parents. However, their superiority was soon established and maintained over the last 20 generations of selection. An accumulation of inbreeding would act to depress the mean of purebreds. However, this probably isn't the sole cause since crossbred superiority in Replication 1 was established by the second generation; any inbreeding effect should not be important in such a short period. Thus, at least in these lines, purebred selection was accumulating favorable genotypes in the crossbred, superior to those in the purebred.

A positive response in both purebreds and crossbreds continues over 30 generations of selection with no indication of cessation. This is in contrast to the results of Wong and Boylan (1970) where continued purebred response in pupal weight was predicted while crossbred response appeared to be ceasing after 22 generations of purebred selection.

There was reasonable agreement of expected response and observed response in crossbreds over the last 20 generations, although the expected response was somewhat larger than that observed. The crossbred response in the first 10 generations was larger than expected. Since selection was applied to fullsib families, this discrepancy could result from additive maternal effects which would not be included in the expected response estimated from sire family means.

If laboratory organisms are reasonable models for larger animals, these results show that crossbreds superior to both purebreds can be produced by purebred selection and that selection response can continue over a long period.

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Received June 20/December 29, 1972

Communicated by J. E. Legates

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