Correlated Response in Skeletal Traits and Replicate Variation in Selected Lines of Mice[1]

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Summary. Correlated responses in caudal vertebrae number (VN), lengths of eighth and ninth caudal vertebrae $(V8$ and $V9$, respectively), femur length (FL) and femur weight (FW) were evaluated in lines of mice which had been selected for six-week body weight $(WK6)$ and/or six-week tail length $(TAIL)$. Ten males and ten females were randomly sampled from each of ten selected lines (two replicates each of five selection treatments) after seven generations of selection. Sexes and lines were significant $(P < .01)$ sources of variation in all seven traits. Sex × line interactions were unimportant except for $V8$ and $V9$. Male mice of both replicate lines selected for increased $WK6$ and decreased $TAIL$ had shorter vertebrae than females, whereas the reverse was true for all other lines. Multiple regression and canonical correlation analyses indicated a high phenotypic relationship of FL with both $WK6$ and $TAIL$. Examination of the correlated responses indicated that FL was the only skeletal trait that showed a substantial correlated response to single trait selection for both $WK6$ and $TAIL$. Thus, the genetic relationships among the three traits also appeared to be high. Between replicate variation was not significant for randomly selected control lines. However, about one-third of the statistical tests between selected replicates were significant. This was taken to indicate a joint effect of selection and drift in causing variation between replicate lines. Replicate variation was further examined by canonical variate and generalized distance analyses. The first two canonical variates accounted for most of the generalized variance. Graphically, the first two canonical variables discriminated among selection treatments, whereas the replicates tended to cluster. Thus, although between replicate differences were significant for several traits, the differences were relatively small compared with the variation between lines having different selection criteria.

Introduction

The manifold effects of selection for quantitative characters have long been recognized. Pleiotropy of genes affecting such characters can be demonstrated by calculating genetic correlations or by assessing correlated responses to selection. The objective in the present study was to search for correlated responses in certain skeletal traits and to assess the magnitude of between replicate variability in lines of mice which had been selected for six-week body weight, six-week tail length or antagonistic indexes involving body weight and tail length.

Materials and Methods

Experimental stocks and traits: Ten males and ten females were randomly selected in generation eight from each of the ten selected lines developed by Rutledge, Eisen and Legates (1973). There were two replicates each of five selection treatments. Selection criteria used in developing the lines are shown in Table 1. The $W^+T_i^0$ and $W^0T_i^+$ lines were selected for six-week body weight and six-week tail length, respectively. The $W^+T_i^-$ and $W^-T_i^+$ lines were selected for indexes giving weighting factors of opposite sign to each trait. The unselected control lines were designated $W^0T_i^0$. Seven traits were studied: caudal vertebrae number (VN), lengths of the eighth and ninth caudal vertebrae $(V8$ and $V9$, respectively), femur length (FL), femur weight (FW), six-week body weight $(WK6)$

and six-week tail length $(TAIL)$. After sacrifice at about seven weeks of age, the mice were taped to a one-sixteenth inch thick Plexiglass sheet. An X-ray film packet was placed underneath the Plexiglass (about one meter from the X-ray source) and radiographs were taken. $V8$ and $V9$ were measured from the radiographs with a micrometer attached to a low-power light microscope. $V8$ and $V9$ are reported in micrometer units. VN was scored using an X-ray illuminator and a hand-held magnifying glass. Femurs were removed by dissection, freeze-dried and weighed. FL was obtained from the dried femurs using the micrometer and microscope.

Statistical analyses: Univariate analyses of variance were calculated for each of the seven traits. The model used was

$$Y_{ijk} = \mu + L_i + S_j + (LS)_{ij} + e_{ijk},$$

where Y_{ijk} is an attribute measured on the kth mouse of the ith line and jth sex, μ is the overall mean, L_i is the effect of the ith line, S_j is the effect of the jth sex, $(LS)_{ij}$ is the interaction effect and e_{ijk} is a residual. Lines and

Table 1. *Selection criteria and line designation*

Designation[a]	Selection criteria[b]	
	$WK6$	$TAIL$
$W^+T_i^0$	1	0
$W^0T_i^+$	0	1
$W^+T_i^-$	0.2079	−1.0437
$W^-T_i^+$	−.2079	1.0437
$W^0T_i^0$	0	0

[a] For each line, $i = 1, 2$.
[b] $WK6$ and $TAIL$, respectively, refer to six-week body weight and six-week tail length.

Table 2. *Means for each trait*

Sex[a]	Trait[c]						
	VN	$V8$	$V9$	FL	FW	$WK6$	$TAIL$
Male	28.81	576.98	577.41	15.56	60.18	31.26	8.70
Female	27.94	549.63	548.70	14.98	53.05	26.58	8.25
Line[b]							
$W^+T_1^0$	28.20	573.75	569.50	15.65	65.31	33.83	8.51
$W^+T_2^0$	28.85	560.95	559.85	15.49	61.81	33.77	8.36
$W^0T_1^+$	28.85	608.45	607.10	15.52	58.42	29.40	9.52
$W^0T_2^+$	29.80	585.10	584.55	15.49	57.02	29.51	9.55
$W^+T_1^-$	27.10	530.95	526.70	15.43	57.07	30.68	7.45
$W^+T_2^-$	26.70	515.35	528.60	14.97	54.84	28.61	6.91
$W^-T_1^+$	29.10	574.85	576.00	14.92	45.40	24.03	9.08
$W^-T_2^+$	28.85	559.00	559.65	14.78	51.77	24.17	8.84
$W^0T_1^0$	27.90	567.50	562.65	15.20	58.07	27.88	8.32
$W^0T_2^0$	28.40	557.15	555.95	15.20	56.43	27.33	8.21
Error Variance							
(d.f. = 180)	0.92	837.21	808.50	0.15	25.06	6.00	0.24
S.E. (Sex)	0.10	2.89	2.84	0.04	0.50	0.24	0.05
S.E. (Line)	0.21	6.47	6.36	0.09	1.12	0.55	0.11

[a] Marginal means of each sex.

[b] Marginal means of each line.

[c] VN = vertebrae number, $V8$ = length of 8th vertebra (micrometer units), $V9$ = length of 9th vertebra (micrometer units), FL = femur length (mm), FW = femur weight (mg), $WK6$ = 6-week body weight (g), $TAIL$ = 6-week tail length (cm).

sexes were considered fixed effects. The assumptions of normality and independence of the e_{ijk} were made. Comparison were made among line-sex means. The Bonferroni t-statistic (Kirk, 1968) was used to test two comparisons which were not mutually orthogonal. This was an attempt to keep the experiment-wise error rate constant. A correlation analysis indicated a high correlation between $V8$ and $V9$. This suggested only a slight gain in information from their joint consideration, and $V9$ was omitted in further analyses. Multiple regression analyses were calculated within sexes and lines, using $WK6$ and $TAIL$ as dependent variables and VN, $V8$, FL and FW as independent variables. Canonical correlation analyses were calculated between Set 1 ($WK6$, $TAIL$) and Set 2 (VN, $V8$, FL, FW). Canonical variate and generalized distance analyses were calculated using all six variables in an attempt to differentiate between lines. This was done separately for each sex since there were significant ($P < .01$) sex × line interactions in the univariate ($V8$ and $V9$) and multivariate analyses of variance. Normalized scores yielding variances of one were used in the canonical correlation and discriminant function analyses.

Results and Discussion

Marginal means for lines and sexes and error variances for the seven traits are shown in Table 2. Lines were a significant ($P < .01$) source of variation for all traits, indicating that seven generations of selection were successful in altering phenotypes. Selected linear contrasts among line-sex means are given in Table 3. Contrasts 1 through 17 are mutually orthogonal. Contrasts 18 and 19 were tested by the modified t-test cited earlier.

Males had significantly ($P < .01$) larger scores than females (contrast 1). Sexual dimorphism in body weight and linear dimensions is common in mammals. The result for VN conflicts with the finding of Barnett (1965) who found no sex difference for caudal vertebrae number in sixteen-week old mice of several inbred lines and their crosses. Contrasts 2 through 6 test replicate line differences. Contrast 3

Table 3. *Linear contrasts for each trait*

Contrast[a]	VN	$V8$	$V9$	FL	FW	$WK6$	$TAIL$
1. Sex	0.87**	27.35**	28.71**	0.58**	7.13**	4.68**	0.45**
2. $W^+T_1^0$ vs $W^+T_2^0$	-.65*	12.80	9.65	0.16	3.50*	0.06	0.15
3. $W^0T_1^+$ vs $W^0T_2^+$	-.95**	23.35*	22.55*	0.02	1.40	-.10	-.03
4. $W^+T_1^-$ vs $W^+T_2^-$	0.40	15.60	-1.80	0.46**	2.22	2.06**	0.54**
5. $W^-T_1^+$ vs $W^-T_2^+$	0.25	15.85	16.35	0.14	-6.36**	-.13	0.24
6. $W^0T_1^0$ vs $W^0T_2^0$	-.50	10.35	6.70	0.00	1.64	0.55	0.11
7. W^+T^- vs W^-T^+	-2.08**	-43.78**	-40.17**	0.34**	7.37**	5.54**	-1.78**
8. $(W^+T^- + W^-T^+)$ vs W^0T^0	-.21	-17.29**	-11.56**	-.18*	-3.32**	-.19	-.74*
9. W^+T^0 vs W^0T^+	-.80**	-29.42**	-31.15**	-.06	5.84**	4.34**	-1.10**
10. Sex × 2	0.45	2.80	0.95	0.11	-.83	0.13	0.10
11. Sex × 3	0.45	3.35	-10.75	0.23	1.80	1.44	0.06
12. Sex × 4	0.50	-8.30	-13.50	0.12	-.49	1.01	0.21
13. Sex × 5	-.25	1.45	-3.95	-.19	-1.21	-.70	-.22
14. Sex × 6	-.80**	4.75	2.40	0.00	-1.02	0.02	0.03
15. Sex × 7	0.22	23.57**	19.87**	0.07	1.02	0.58	0.16
16. Sex × 8	0.22	-16.62**	-16.83**	-.03	-1.53	-.27	-.01
17. Sex × 9	0.05	3.67	1.55	-.12	-2.04	-.50	0.00
18. W^+T^0 vs W^0T^0 [b]	0.37	5.02	5.38	0.37*	6.31**	6.19**	0.18
19. W^0T^+ vs W^0T^0 [b]	1.17*	34.45*	36.52**	0.30	0.47	1.85	1.27**

[a] First 17 contrasts are mutually orthogonal.   [b] Last two contrasts tested by Bonferroni t-statistic.

* $P < .05$ ** $P < .01$.

is of particular interest. The two replicates selected solely for increased tail length did not differ in $TAIL$; however they did differ in the components of tail length. The replicate having fewer $(P < .01)$ vertebrae had longer $(P < .05)$ vertebrae. The responses for replicate one infer that tail length was increased primarily by elongation of vertebrae, whereas the responses for replicate two infer that tail length was increased primarily by an increase in vertebrae number (Table 2). Contrast 4 appears to indicate considerable between replicate variation. However, these results must be interpreted with caution since $W^+T_1^-$ had less cumulative selection pressure than $W^+T_2^-$ (Rutledge et al., 1973). Contrast 6 measures the effects of drift on these traits in the absence of intended selection; none of the seven tests revealed a significant difference between replicates. Ignoring Contrast 4 in which replicates could have differed due to markedly unequal selection intensities, six of twenty-one tests of replicate difference in the selected lines were significant. This is too large a fraction to dismiss as due to chance alone. These populations were founded with an effective number of parents of about 26 and thus Mayr's "founder principle" might be invoked. However, drift seems unlikely as the sole reason for these results since the control lines did not differ. Selection, too, can be discounted since replicate lines had exactly the same selection criteria. Rather, there seems to be evidence for an important joint effect of random sampling and selection as predicted by Wright (1948).

Contrast 7 which tests for divergence between the index selected lines was significant $(P < .01)$ for all traits. Asymmetry in index response (Contrast 8) was noted for several traits. Contrast 9 is a comparison of the lines selected for increased $WK6$ with the lines selected for increased $TAIL$. The former lines were heavier $(P < .01)$ in $WK6$ and FW, but they were significantly $(P < .01)$ smaller in $TAIL$ and its components. There was no difference in FL. Thus seven generations of selection for increased $TAIL$ did not lead to increased linear growth of the femur relative to selection for increased $WK6$.

Contrasts 10 through 14 are tests of differential responses for sexes with Contrasts 2 through 6; only one of the thirty-five tests was significant. The analyses of variance indicated that sex × line interactions were significant $(P < .05)$ only for vertebrae length. The nature of this interaction is given by Contrast 15. Male mice of both replicates of W^+T^- had significantly shorter vertebrae than females while the reverse was true for all other lines.

The lines selected for increased $WK6$ had positive correlated responses in all traits. However, except for the selection criterion, only the femur measures were significant (Contrast 18). Similarly, the lines selected for increased $TAIL$ responded positively for all traits, but correlated responses in $WK6$, FL and FW were not significant (Contrast 19). These two contrasts should not be taken to infer a lack of genetic correlation between $WK6$ and $TAIL$. An analysis of generation means has shown significant correlated responses in these traits (Rutledge et al., 1973). These results indicate that the distributions of the W^+T^0 and W^0T^+ lines still overlap considerably for the correlated traits.

Phenotypically, all traits were positively correlated, indicating that all were measures of body size (Table 4). The correlation between $V8$ and $V9$ was 0.92, suggesting only a slight gain of information from their joint consideration. Thus, $V9$ was omitted in further analyses.

Table 4. *Correlations among traits pooled within line-sex subclasses* [a]

Trait	$V8$	$V9$	FL	FW	$WK6$	$TAIL$
VN	0.21	0.20	0.21	0.21	0.16	0.36
$V8$		0.92	0.56	0.33	0.31	0.59
$V9$			0.56	0.34	0.29	0.57
FL				0.57	0.58	0.54
FW					0.56	0.40
$WK6$						0.45

[a] All correlations are significant at $P < .01$ except between VN and $WK6$ which is significant at $P < .05$ (d.f. = 179).

Table 5 gives the multiple regression analyses of $WK6$ and $TAIL$, respectively, on VN, $V8$, FL and FW. Also given are standard partial regression coefficients which can be used to judge the utility of the independent variates in predicting the dependent variates. FL, followed closely by FW, was most important in predicting $WK6$. FL as well as VN and $V8$ were important in predicting $TAIL$.

Table 5. *Multiple regression analyses using the skeletal traits to predict $WK6$ and $TAIL$, respectively* [a]

Independent Variable	Dependent Variable			
	$WK6$		$TAIL$	
	b ± s.e.	b'	b ± s.e.	b'
VN	0.043 ± .153	0.017	0.109 ± .029 **	0.213
$V8$	−.002 ± .006	−.024	0.007 ± .001 **	0.414
FL	2.551 ± .503 **	0.403	0.283 ± .095 **	0.224
FW	0.164 ± .034 **	0.335	0.010 ± .007	0.102
R^2	0.415 **		0.470 **	

[a] Adjusted for sex, line and sex × line effects (d.f. = 176). b = partial regression coefficients, s.e. = standard error and b' = standardized partial regression coefficients.
** $P < .01$.

Table 6. *Canonical correlation analysis between set 1 (WK6, TAIL) and set 2 (VN, V8, FL, FW) traits*

Canonical Variable	Characteristic Root	Set 1		Set 2			
		WK6	TAIL	VN	V8	FL	FW
		Correlations between Canonical Variables and Traits[a]					
1	0.55**	0.81	0.89	0.44	0.75	0.89	0.74
2	0.18**	0.59	−.45	−.38	−.53	0.21	0.46
		Characteristic Vector[b]					
1		0.77	1.00	0.42	0.68	1.00	0.66
2		1.00	−.91	−.46	−1.00	0.20	0.64

** $P < .01$.

[a] All correlations are significant at $P < .01$.

[b] In standard units with largest coefficient scaled to unity within each vector.

The canonical correlation analysis is summarized in Table 6. Set 1 traits ($WK6$ and $TAIL$) were those subject to selection and the remaining traits (Set 2) were those used to measure changes in the skeletal growth. The canonical variate transformation creates linear combinations which have a maximum between set correlation, and successive canonical variates are orthogonal. The first two canonical correlations were significant ($P < .01$), accounting for 54.5 and 18.0 percent of the variance, respectively. The first canonical correlation (0.74) was higher than any correlation between individual variables of the two sets (Table 4). FL had the largest weighting factor in the first characteristic vector and thus was the most important variate in explaining the joint distribution of the Set 1 traits. This was consistent with the multiple regression analyses in that FL was the only trait

which yielded a significant contribution to the regression sum of squares of both dependent variates. This result is also consistent with Contrasts 18 and 19 of Table 3. Of the skeletal traits, FL was the only trait which had a sizable correlated response for both single trait selection treatments. This correlated response was significant ($P < .05$) for W^+T^0 and approached significance for W^0T^+, even when tested by the conservative Bonferronit-statistic.

Table 7 summarizes the canonical variate analysis. For both sexes, differences among mean vectors of lines were signifcant ($P < .01$) as tested by Wilk's criterion (Overall and Klett, 1972). The characteristic vectors are the coefficients for that linear combination of the traits (the canonical variate) which maximizes the sum of squared differences among line means relative to the pooled within line variance for

Table 7. *Largest two characteristic roots (λ_1, λ_2) and their percent of the sum of the roots ($\sum_{i=1}^{6} \lambda_i$), correlations between canonical variables and traits, and characteristic vectors [a]*

Characteristic Root	Percent of $\sum_{i=1}^{6} \lambda_i$	Correlations between Canonical Variables and Traits					
		VN	V8	FL	FW	WK6	TAIL
		Males					
4.40**	60.4	0.41**	0.17	−.11	−.26*	−.41**	0.65**
1.59	21.9	0.44**	0.35**	0.57**	0.54**	0.90**	0.66**
$\sum_{i=1}^{6} \lambda_i = 7.29$**							
		Females					
9.39**	81.1	0.25*	0.28*	−.08	−.24*	−.30**	0.52**
1.37	11.8	0.40**	0.53**	0.52**	0.82**	0.79**	0.73**
$\sum_{i=1}^{6} \lambda_i = 11.57$**							
		Characteristic Vector[a]					
Canonical variable		VN	V8	FL	FW	WK6	TAIL
		Males					
1		0.30	−.25	0.07	−.17	−.70	1.00
2		0.36	−.30	0.04	−.02	1.00	0.51
		Females					
1		0.06	0.03	−.33	−.38	−.41	1.00
2		0.26	0.41	−.45	1.00	0.85	0.41

[a] In standard units with largest coefficient scaled to unity

* $p < .05$ ** $p > .01$

Tabl 8. *Summary of classification based on generalized squared distance*[a]

Line	Number of Mice Classified into Line									
	$W^+T_1^0$	$W^+T_2^0$	$W^0T_1^+$	$W^0T_2^+$	$W^+T_1^-$	$W^+T_2^-$	$W^-T_1^+$	$W^-T_2^+$	$W^0T_1^0$	$W^0T_2^0$
$W^+T_1^0$	13	4	0	0	1	0	0	0	0	2
$W^+T_2^0$	4	15	0	0	0	0	0	0	1	0
$W^0T_1^+$	0	0	13	2	0	0	1	3	1	0
$W^0T_2^+$	0	0	2	15	0	0	1	1	0	1
$W^+T_1^-$	2	2	0	1	8	6	0	0	1	0
$W^+T_2^-$	0	1	0	0	6	12	0	0	0	1
$W^-T_1^+$	0	0	1	0	0	0	18	0	0	1
$W^-T_2^+$	0	0	2	0	0	0	2	16	0	0
$W^0T_1^0$	0	0	0	0	1	1	0	0	12	6
$W^0T_2^0$	0	1	0	0	0	0	0	1	3	15

[a] Calculated within sex and then pooled.

that canonical variate. Within sexes, the canonical variates are uncorrelated. Only the first two canonical variates are shown as their associated characteristic roots accounted for 82.3 and 92.9 percent of the sum of the characteristic roots for males and females, respectively. Comparing characteristic vectors for each sex suggests considerable differences in the utility of some traits to discriminate. For example, the coefficients for FL for males were small and positive, whereas the coefficients for females were moderate and negative. However, linear correlations between the traits and the canonical variates were remarkably consistent across sexes. In no case did analogous correlations differ in sign. In addition, each trait was significantly correlated with at least one of the canonical variates. This was taken as an indication that all traits were useful in discrimination.

Since most of the discriminatory power of the six traits is contained in the first two characteristic vectors, the line-sex mean values for the first two characteristic vectors were used as Cartesian coordinates to plot the locations of the line-sex means in two-dimensional space. These plots are shown in Fig. 1a and 1b for males and females, respectively. With the exception of the W^+T^- males, replicates tended to cluster in the space defined by the first two characteristic vectors.

Results of the generalized distance analysis using all six traits are given in Table 8. The discriminant function classified 86% of the mice correctly into treatment group. This result confirms the earlier observation that although the lines are differentiated on a mean basis, there is still an overlap of distributions. However, it is apparent that the generalized distance is a powerful tool in distinguishing genetic groups as demonstrated by Festing (1973) for mandible bone measurements in inbred lines of mice.

General Discussion

In general, these results tend to point towards relatively little variation between replicate selected lines compared to variation between selection treatments and could be interpreted to indicate that repli-
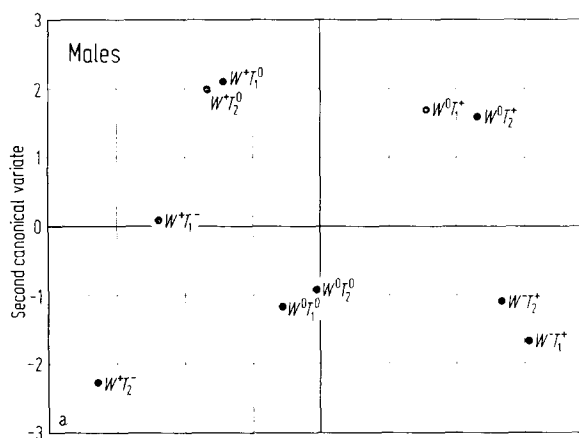

Fig. 1a. Plot of the line means of canonical variables 1 and 2 for males (Standard errors of line means for canonical variables 1 and 2 are 0.39 and 0.43)
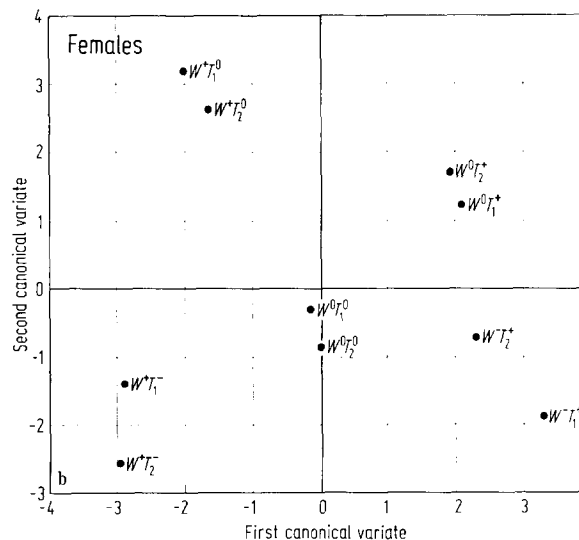

Fig. 1b. Plot of the line means of canonical variables 1 and 2 for females (Standard errors of line means for canonical variables 1 and 2 are 0.32 and 0.56)

cation is unnecessary in the design of selection experiments. The authors, however, take the opposing viewpoint from two lines of reasoning. First, replication is basic to the science of statistical inference and functions to provide estimates of experimental error. Second, although replicates did respond similarly in most correlated traits and hence would give confidence in predicting correlated responses in further experiments, this was not always the case. For example, consider VN and $V8$ responses in the two lines selected for increased tail length. The two lines yielded almost exactly opposite inferences in correlated responses of the components of tail length. Clearly, from this experiment it is impossible to predict which component of tail length will be increased more relative to the other when selection is practiced solely for tail length.

The results of the multivariate analyses indicate that different selection criteria have led to morphologically distinct lines in terms of linear functions of body size and skeletal measurements. Hanrahan, Hooper and McCarthy (1973) and Byrne, Hooper and McCarthy (1973) have demonstrated marked changes in muscle fiber number and diameter in lines of mice selected for body weight. Further research would be desirable in order to relate the very marked changes in morphological traits to genetic changes in hormonal and enzyme levels and the ability of tissue to respond to given doses of hormones.

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