Alterning Developmental Trajectories in Mice by Restricted Index Selection

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ABSTRACT

A restricted index selection experiment on mice was carried out for 14 generations on rate of early postnatal development (growth rate from birth to 10 days of age) vs. rate of development much later in ontogeny (growth rate from 28 to 56 days of age). Early rate of development (E) approximates hyperplasia (changes in cell number) and later rate (L) reflects hypertropy (changes in cell size). The selection criteria were as follows: E+L.0 was selected to increase early body weight gain while holding late body weight gain constant; E−L.0 was selected to decrease early body gain while holding late gain constant; E0L+ was selected to increase late gain holding early gain constant; and E0L− was selected to decrease late gain holding early gain constant. After 14 generations of selection, significant divergence among lines has occurred and the changes in the growth trajectories are very close to expectation. The genetic and developmental bases of complex traits are discussed as well as the concept of developmental homoplasy.

Complex morphological traits are generally assumed to be composite entities whose various component parts may have different embryological origins, underlying controlling factors, and patterns of growth and morphogenesis (Atchley 1987; Atchley and Hall 1991). Each individual component follows a characteristic pattern of ontogenetic change called a developmental trajectory (Alberchi et al. 1979; Atchley 1987). The dynamics of the component trajectories, their coordination during ontogeny and final integration into a complex morphological structure are assumed to have been the focus of stringent natural selection. Indeed, coordination and integration of the various components into the final composite morphology has been described as a developmental choreography (Atchley et al. 1991). For these reasons, the evolution of complex morphologies results from heritable changes in the underlying developmental components that produce the relevant morphologies (Atchley 1987; Atchley and Hall 1991).

The developmental quantitative genetic model describing the evolution of complex morphological traits is a powerful paradigm. It assumes that systematic evolutionary change occurs by selection (1) acting on the various components that comprise the morphological structure, (2) operating at many levels of biological organization, (3) occurring at different time periods in ontogeny when separate genes are being expressed and separate patterns of tissue and organ differentiation are occurring and (4) acting to refine the coordination and integration of the various components during ontogeny.

Herein, we describe a restricted index selection experiment in mice that explores several assumptions of this model. For a series of replicated mouse lines, selection was focused on rates of early and late postnatal development. Changes in growth rate during early mouse postnatal development could arise from variation in hyperplasia (changes in cell number) in many organs (Riska and Atchley 1985) and changes later in ontogeny could stem from variation in hypertrophy (changes in cell size). Indeed, this hypothesis has now been experimentally verified and we have shown that by focusing selection on developmental rates at disparate times during ontogeny, morphological change can arise as the result of alteration in different cellular processes during development (W. R. Atchley, unpublished data).

A number of authors have recently proposed models for evolutionary change in morphology that focus on events at the cellular level (e.g., Wolpert 1990; Atchley and Hall 1991; Hall 1992). At the cellular level, changes in hyperplasia and hypertrophy present two distinct mechanisms to alter the size and patterns of morphogenesis of a morphological structure (Katz 1980; Atchley et al. 1984; Risika and Atchley 1985; Atchley 1987). Thus

$$\log (\text{size of structure}) = \log (\text{number of cells}) + \log (\text{size of cells}).$$

The underlying control of these two mechanisms differ considerably (Baserga 1981; Corradino 1982; Venezi-
A cellular model involving hyperplasia and hypertrophy has a significant temporal component because the relative contribution made to the overall size of a given structure by cell number and cell size varies considerably during ontogeny. Mammalian growth, for example, occurs in three phases: (1) an early phase of cell multiplication with little cell enlargement, (2) a later phase reflecting a combination of cell multiplication and enlargement and (3) a final stage of growth almost entirely by cell enlargement (Enesco and Leblond 1962; Winick and Noble 1965; Goss 1966; Falconer et al. 1978). Different tissues and organs may initiate and terminate growth at different times during ontogeny and therefore may exhibit asynchronous periods of hyperplasia and hypertrophy. Consequently, the time during ontogeny at which selection occurs can have a significant impact on the developmental mechanisms involved and their morphological consequences.

Because of this mosaic of tissue-specific patterns of cellular development, age-specific selection on traits like body weight or rate of development might produce quite distinct results. First of all, age-specific selection could produce different patterns of growth and morphogenesis, depending upon when selection occurred during ontogeny and whether a given tissue or organ was undergoing cell multiplication, cell enlargement or a combination of both processes (Riska and Atchley 1985; Atchley 1987). Second, superficially similar mature phenotypes (e.g., body size, bone length or organ size) can be obtained by different developmental mechanisms (i.e., hyperplasia vs. hypertrophy). Thus, altering the patterns of cell multiplication and/or cell enlargement during ontogeny could have a significant impact on many different developmental systems (Widdowson and McCance 1960; Enesco and Leblond 1962; Goss 1966; Hall 1978; Katz 1980; Burgess and Nicola 1983, Dixon and Saranat 1985). From a quantitative genetic perspective, the patterns of genetic covariance among traits arising from pleiotropy and epigenetic effects might be quite different depending upon when during ontogeny selection occurred and by which developmental mechanism the mature phenotype was achieved (Atchley 1984, 1987).

Several authors have incorporated hypertrophy and hyperplasia into explanations of evolutionary events (e.g., Katz 1980; Riska and Atchley 1985; Atchley 1987; Atchley and Hall 1991). Unfortunately, there are very few experimental studies where deliberate attempts have been made to explore the impact of selection at the cellular level on complex morphological traits. This paper describes the changes in selection criteria and the extent of divergence among the selection lines. Other aspects of this experiment are described by P. D. Crenshaw, W. R. Atchley and S. Xu (unpublished results).

**MATERIALS AND METHODS**

**Mouse stocks:** The founding stock for these selection lines was the random bred ICR (Institute for Cancer Research) mouse strain. A total of 271 females and 256 males obtained from Sprague-Dawley in 1988 were allowed to acclimate to the laboratory for several days before mating. One hundred and eighty litters from this foundation stock were assigned randomly to a selection line and two replicates, with each replicate containing 12 litters.

**Husbandry:** At each generation, individual mice were weighed at birth and at 10, 28 and 56 days of age. At birth, all pups in the litter were weighed together and the average pup weight for that litter was recorded as the individual mouse weights. Mice were assigned unique identification numbers by toe-clipping before 10 days of age. At 10 days of age and all subsequent ages, body weights were recorded for individual mice. At the end of each generation, an index score was computed for each mouse in each selection line and the best male and female from each litter was selected as parents for the next generation. In replicate lines with fewer than 12 successful litters, the second best males and females from those mice not previously selected within each family were chosen to produce replacement litters. Because matings sometimes fail to produce a litter, an extra litter (litter 13) was also set up for each generation. All selection and mating design procedures were carried out using automatic computer programs. For the control line, a pseudo index score for each individual mouse in the control line was generated by a random number generator that mimics a uniform distribution between 0 and 1. Selection of parents for the next generation was based on the pseudo index scores for the control line, which guaranteed randomization of selection.

Following pairing with a male, females were checked daily for presence of a copulatory plug. Several days before parturition, the males were removed from the cage and sacrificed. Because of the well-documented effect of litter size on development, each litter was standardized to eight pups with an equal sex ratio. Litters with fewer than eight pups were augmented using excess pups from other litters. The substitute pups were tail-clipped to distinguish them from the original members of the litter. Weights of these substitute mice were not included in any calculation.

**Selection regimens:** It is difficult to use cell size or number as a selection criterion because it would involve sacrificing the animals and quantifying the phenotype through laborious cytological analyses of various tissues. Further, the stages of the life cycle where relevant developmental events are occurring are sometimes relatively inaccessible (fetal development) for measurement of selection criteria. Hence, an indirect approach is necessary.

Five selection lines are represented in this experiment, of which four were selected on the basis of a restricted index (Kempton and Nordisco 1959) and one served as a randomly selected control. Each line was replicated three times giving a total of 15 populations. Selection was practiced within families to minimize maternal effects. The selection lines were closed at the beginning of the experiment and no new animals were added and no mating between replicate lines was permitted. Consequently, each replicate can be considered an independent line. This paper describes the results of selection through generation 14.

The selection criteria were body weight gain between birth and 10 days of age (E) and between 28 and 56 days of age (L). E relates to the phase of postnatal growth most influenced by changes in cell number in relevant organs and tissues, while L is a phase of postnatal growth more influenced by changes in cell size. We have experimentally verified this model with these mice. We have shown that significant differ-
ences in brain size between the E+L0 and E−L0 lines are the result of changes in the number of brain cells (W. R. Atchley, unpublished data).

The selection regimens were as follows: E+L0 was selected to increase early body weight gain while holding late body weight gain constant; E−L0 was selected to decrease early body gain while holding late gain constant; EOL+ was selected to increase late gain holding early gain constant; and EOL− was selected to decrease late gain holding early gain constant.

The estimates of genetic parameters used to obtain these restricted index weights are described in the next section. The control line replicates were randomly selected. Within each line and replicate, matings were made in a circular scheme (Kimura and Crow 1963) to minimize the rate of inbreeding.

Construction of selection indices: The selection indices have the following form:

\[ I = b_1(\text{early gain}) + b_2(\text{late gain}) \]

For a restricted index, genetic gain is maximized for one subset of traits subject to zero gain in another subset of traits. This is accomplished by solving for the index weights, \( b \), based on the equation given by Cunningham et al. (1970).

\[ b = Q^{-1}C{\alpha} \]

where \( Q \) is a matrix containing phenotypic variances and covariances, as well as some genetic variances and covariances; \( C \) contains genetic variances, covariances and zeros; and \( \alpha \) is a vector of economic weights. For example, to maximize the genetic change in early gain while holding change in late gain constant, \( b \) was obtained by

\[
\begin{bmatrix}
    b_1 \\
    b_2 \\
    \lambda
\end{bmatrix} = \begin{bmatrix}
    V_{11} & C_{12} & C_{12} \\
    C_{21} & V_{22} & V_{22} \\
    C_{21} & V_{22} & V_{22}
\end{bmatrix}^{-1} \begin{bmatrix}
    V_{11} & C_{12} & 0 \\
    C_{21} & V_{22} & 0 \\
    C_{21} & V_{22} & 0
\end{bmatrix} \begin{bmatrix}
    1 \\
    0 \\
    0
\end{bmatrix},
\]

where, \( \lambda \) is a Lagrange multiplier, \( V \)'s refer to variances, \( C \)'s to covariances, subscripts \( p \) and \( a \) refer to phenotypic and additive genetic effects, respectively, and subscripts 1 and 2 refer to early and late weight gains, respectively. Note that vector

\[ \alpha = \begin{bmatrix}
    a_1 \\
    a_2 \\
    1
\end{bmatrix} \]

in this example. If selection is in the opposite direction, the element 1 in vector \( \alpha \) is replaced by -1.

To compute the selection index during the initial generations of the selection experiment, we used estimates of heritabilities for early and late gains and their genetic correlation from data of a large quantitative genetic experiment on ICR mice carried out at the University of Wisconsin (Cheverud et al. 1983; Riska et al. 1984; Atchley et al. 1985a,b). These particular mice were obtained from a Sprague-Dawley population in Wisconsin. The heritability of early gain (approximated by the heritability of 14 day weight) was 0.19 whereas the heritability of late gain was 0.29. The estimated genetic correlation between early and late gain was 0.26. The new estimates of heritabilities for early and late gains and their genetic correlation in Wisconsin were based on data of a large quantitative genetic experiment on ICR mice

\[
\begin{array}{l}
\text{Early gain} \\
\text{Late gain}
\end{array}
\]

The least squares means were subtracted from the means in the experiment to account for environmental effects. Since selection index coefficients were changed for selection of par-

\[
\begin{array}{l}
\text{Line} \\
\text{Generation 0–4} \\
\text{Generation 5–14}
\end{array}
\]

\[
\begin{array}{l}
 \text{E+L0} \\
 \text{E−L0} \\
 \text{EOL+} \\
 \text{EOL−}
\end{array}
\]

\[
\begin{array}{llll}
 b_1 & b_2 & b_1 & b_2
\end{array}
\]

\[
\begin{array}{l}
 \text{0.169} & \text{0.039} & \text{0.226} & \text{−0.019} \\
 \text{−0.169} & \text{−0.039} & \text{−0.226} & \text{0.019} \\
 \text{0.302} & \text{0.275} & \text{−0.788} & \text{0.345} \\
 \text{−0.302} & \text{−0.275} & \text{0.788} & \text{−0.345}
\end{array}
\]

\* Primary selection differentials and predicted responses to selection were based on indices with the weights used in generation 5–14.

\[
\begin{array}{ll}
 P = \begin{bmatrix}
 0.56 & 0.64 \\
 0.64 & 9.04
\end{bmatrix} \\
 G = \begin{bmatrix}
 0.14 & 0.33 \\
 0.33 & 3.87
\end{bmatrix}
\]

respectively. Thus, the new estimates of heritabilities for early gain and late gain were 0.26 and 0.43, respectively. The new estimate of the genetic correlation between early and late gain was 0.14. The above matrices were used to construct the new selection index weights (also listed in Table 1), which were used for selection at generation 5 and thereafter.

The index weights were constructed using phenotypic and genetic statistics on an individual basis, whereas, index selection was conducted within families. Thus, conventional formulas of predicting direct and correlated responses to individual index selection (per generation) were adjusted by the following factor (Falconer 1981)

\[
\epsilon = (1 - r) \frac{n - 1}{\sqrt{n(1 - t)}},
\]

where \( r = 1/2 \), the coefficient of relationship for full-sibs; \( t \) is the phenotypic intraclass correlation of full-sibs for the index (the index itself is considered as a phenotypic trait); and \( n \) is the number of individuals of the same sex in a family, which, in our case, was about four.

The expected response to selection for the index unit was obtained by

\[
R_{\text{index}} = \frac{b^T G b}{\sqrt{b^T P b}}
\]

and the correlated responses of the component traits were predicted by (see Eisen 1993)

\[
C R_{\text{trait gain}} = \frac{C R_{\text{early gain}}}{\sqrt{b^T P b}}
\]

where \( i \) is the selection intensity. The proportion selected in our experiment was one out of four for each sex. The corresponding selection intensity obtained from Falconer's (1981) Appendix Table B was 1.029.

Statistical analysis: As will be shown in the results, the means of the original base population (see Table 2) showed significant sexual dimorphism for all traits except birth weight. Consequently, statistical analyses were carried out using sex as a fixed effect in the linear model. This procedure adjusted the data for any sexual differences in trait values. The least squares means were subtracted from the means of the control to account for environmental effects. Since selection index coefficients were changed for selection of par-
ents after generation 4, population means for selection index unit in generations 0–4 were computed based upon the index used in generations 5–14.

Direct response to selection on the index values and correlated responses of the component traits were estimated from regression of the deviation of selection line means from the control on generation number. Standard errors of the estimated response for each selection line are estimated by the standard deviation among the three replicates.

Selection differentials for each family were calculated as the mean differential for males and females, with the selection differential for each sex defined as the deviation of the selected individual from the mean of like-sexed fullsibs. The mean selection differential within replicates was computed from the within family selection differentials at every generation. Primary selection differentials were computed for index units while secondary selection differentials were computed for the weight and gain traits. Although age-specific weight traits were not directly included in the index, they were included in the weight gain traits that were the components of the index. Therefore, their selection differentials were still considered as secondary selection differentials. Cumulative selection differentials for the jth generation were defined as the sum of all individual generation selection differentials up to the jth generation.

Realized heritability for a selection index was estimated by regressing population means of an index (deviations from the control) on cumulative selection differentials of the index (Hill 1972a,b). To adjust for within family selection, this estimate of realized heritability was divided by c (defined earlier) to convert it into heritability on an individual basis. Only primary selection differentials were useful for estimation of realized genetic parameters of the component traits. With index selection, realized heritabilities and genetic correlation for component traits may not be estimated directly. Instead, one should assume a known phenotypic variance-covariance matrix. This is justified because the phenotypic variance-covariance matrix can be easily and accurately estimated with simple statistical methods. The least squares methods of Harvey (1972) and Berger and Harvey (1975) were used for estimating realized genetic parameters for two traits. However, the dependent variables in the Harvey's least squares method are now replaced by estimated regression coefficients of selection responses on primary cumulative selection differentials across generations, whereas, the design matrix was still the index weights used in the selection (see Gunsett et al. 1982). These estimates were adjusted by factor c to account for within-family selection.

Recall that four selection lines were used in this experiment. To use Harvey's method, at least two lines are required for two traits and the two lines have to be selected using different indices. However, the index of E+L0 line was identical to that of E−L0 line except they had opposite signs. Similarly, the index of E0L+ line was identical to that of E0L− line. Therefore, realized genetic variances and covariance were estimated from four pairs of lines: (E+L0 and E0L+), (E+L0 and E0L−), (E−L0 and E0L+) and (E−L0 and E0L−). Pooled estimates were also obtained by using all four lines. Data collected from generations 0–4 were not used for estimating realized genetic parameters due to changes in the index weights.

RESULTS

The base population: Means and standard deviations for body weights and weight gains are listed in Table 2 for the base population before selection. Body weights at 28 days (WT28), 56 days (WT56) and late gain (G2856) show significant sexual dimorphism. Average body weights and weight gains of males were significantly greater than those of the females. Least squares

### TABLE 2
Population means and SDs of mice in the base population (generation 0)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Code for trait</th>
<th>Male</th>
<th>Female</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight</td>
<td>BWT</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>10-day weight</td>
<td>WT10</td>
<td>8.08 (0.81)</td>
<td>7.89 (0.78)</td>
<td>7.98 (0.80)</td>
</tr>
<tr>
<td>28-day weight</td>
<td>WT28</td>
<td>22.69 (2.03)</td>
<td>31.64 (3.96)</td>
<td>35.93 (6.14)</td>
</tr>
<tr>
<td>56-day weight</td>
<td>WT56</td>
<td>31.64 (3.96)</td>
<td>35.93 (6.14)</td>
<td>35.93 (6.14)</td>
</tr>
<tr>
<td>Early gain (0–10)</td>
<td>G010</td>
<td>8.14 (0.77)</td>
<td>6.28 (0.70)</td>
<td>6.58 (0.74)</td>
</tr>
<tr>
<td>Late gain (28–56)</td>
<td>G2856</td>
<td>11.69 (3.96)</td>
<td>8.96 (2.80)</td>
<td>10.33 (3.29)</td>
</tr>
<tr>
<td>10day weight</td>
<td></td>
<td>6.28 (0.70)</td>
<td>6.38 (0.74)</td>
<td>6.38 (0.74)</td>
</tr>
<tr>
<td>WT10</td>
<td></td>
<td>7.89 (0.78)</td>
<td>7.98 (0.80)</td>
<td>7.98 (0.80)</td>
</tr>
<tr>
<td>WT28</td>
<td></td>
<td>22.69 (2.03)</td>
<td>31.64 (3.96)</td>
<td>35.93 (6.14)</td>
</tr>
<tr>
<td>WT56</td>
<td></td>
<td>31.64 (3.96)</td>
<td>35.93 (6.14)</td>
<td>35.93 (6.14)</td>
</tr>
<tr>
<td>G010</td>
<td></td>
<td>8.14 (0.77)</td>
<td>6.28 (0.70)</td>
<td>6.58 (0.74)</td>
</tr>
<tr>
<td>G2856</td>
<td></td>
<td>11.69 (3.96)</td>
<td>8.96 (2.80)</td>
<td>10.33 (3.29)</td>
</tr>
</tbody>
</table>

### TABLE 3
Expected responses per generation to selection of a selection index and correlated response to selection of a component trait

<table>
<thead>
<tr>
<th>Line</th>
<th>t</th>
<th>c</th>
<th>V(t) = b'Pb</th>
<th>R&lt;sub&gt;index&lt;/sub&gt;</th>
<th>CR&lt;sub&gt;2010&lt;/sub&gt;</th>
<th>CR&lt;sub&gt;2856&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>E+L0</td>
<td>0.609</td>
<td>0.629</td>
<td>0.026</td>
<td>0.026</td>
<td>0.116</td>
<td>0.000</td>
</tr>
<tr>
<td>E−L0</td>
<td>0.609</td>
<td>0.629</td>
<td>0.026</td>
<td>0.026</td>
<td>−0.116</td>
<td>0.000</td>
</tr>
<tr>
<td>E0L+</td>
<td>0.527</td>
<td>0.528</td>
<td>0.1078</td>
<td>0.195</td>
<td>0.000</td>
<td>5.564</td>
</tr>
<tr>
<td>E0L−</td>
<td>0.527</td>
<td>0.528</td>
<td>0.1078</td>
<td>0.195</td>
<td>0.000</td>
<td>−5.564</td>
</tr>
</tbody>
</table>

<sup>t</sup>, the phenotypic intraclass correlation of full-sibs for an index estimated from the base population; <sup>c</sup>, the adjusted factor for within-family selection; V(t) = b'Pb, the variance of the selection index; R<sub>index</sub>, selection index; CR<sub>rate</sub>, component trait.
Restricted Index Selection of Mice

FIGURE 1.—Variation in index scores among three replicates within each selected line of mice. E+L0, selection to increase early weight gain while holding late weight gain constant; E−L0, selection to decrease early weight gain while holding late weight gain constant; E0L+, selection to increase late weight gain while holding early weight gain constant; E0L−, selection to decrease late weight gain while holding early weight gain constant.

...means with sex effect removed of these weight and weight gain traits are used in subsequent analysis. Least squares means in the base population are similar to those for other experiments utilizing unselected populations of the ICR strain (e.g., Riska et al. 1984). Index weights, which were used for selection from generations 5–14, were calculated from the phenotypic (P) and genetic (G) variance-covariance matrices estimated from the base population. These index weights, as well as the weights used from generations 1–4, are given in Table 1.

Expected direct and correlated responses to selection: The expected values of response per generation for direct and correlated responses to selection are given in Table 3. The index scores of individuals in the base population were also analyzed to estimate the phenotypic intraclass correlation of full-sibs (see the t values in Table 3).

The responses of the index scores are expected to be 0.026 in lines E+L0 and E−L0, while correlated responses to selection for early gain and late gain are expected to be 0.116 and 0, respectively, in these lines. In the E0L+ and E0L− lines, the expected response in index scores is 0.195. The expected response for late gain trait is 0.564, and that for early gain should be 0.

Divergence among replicates: The three replicates within each line showed variation for all traits. Plots of generation means of traits INDEX, G010 and G26836 on generation numbers for individual line replicates are given in Figures 1–3, respectively. The variation among replicates within each line was primarily due to genetic drift.

Selection responses, correlated responses and general trends: The pooled generation means over three replicates for all selected lines were calculated and bidirectional plots are given in Figure 4 for selection index scores. Figure 4a shows the bidirectional plots for early gain selection (E+L0 vs. E−L0). The two lines diverged significantly as selection progressed. Response to selection for lower index scores (E−L0) is significantly different from zero (P < 0.05). However, selection for higher index scores (E+L0) does not show a significant response (P > 0.05), although the general trends qualitatively agree with theoretical expectations. There may be several explanations for the asymmetrical responses of high-low bidirectional selection (Falconer 1981). In this particular case, however, the most appropriate reason may be that a different index was used in the earlier stage of selection (generations 1–4). It is obvious from Figure 4a that E+L0 line did not respond to selection before generation 4. If data from generations 1–4 were deleted from the analysis, the
regression coefficient of generation means on generation number would be significantly different from zero.

Figure 4b gives the bidirectional plots of pooled mean index scores on generation numbers when late gain was selected while early gain held constant. Both upper and lower selection lines show significant responses to selection and there is no obvious asymmetry.

Correlated selection response for the early and late gain traits is important in this project because the goal of selection in the early gain lines (E+L0 and E−L0) is to alter early gain while holding late gain constant. The goal of selection in late gain lines (EOL+ and EOL−) is to change late gain while holding early gain constant. The general trends of change for these weight gain traits are shown in Figure 5, which includes four subfigures arranged in a 2 × 2 fashion. The two columns represent selection methods (early gain vs. late gain selection), while the two rows stand for early and late gain traits, respectively. Figure 5a shows the trends of G010 when selection is to alter this trait. The two lines diverged in the appropriate directions as expected, although asymmetry was observed. G2856 is not supposed to change for early gain selection, but it changed slightly as shown in Figure 5c, in which both lines have positive and statistically significant nonzero slopes. However, the magnitude of change of G2856 in Figure 5c is trivial compared with the change of the same trait in late gain selection lines (Figure 5d), where the purpose of selection is to change late gain. Figure 5b gives the trends of G010 in late gain selection lines. As expected, G010 did not show significant change.

Growth trajectories of mice: Figure 6 gives the plot of body weight against ages. In early gain selection lines (Figure 6a), mean body weight of the high line (E+L0) significantly diverged ($P < 0.01$) from the low line (E−L0) at age of 10 days and thereafter. In late gain selection lines (Figure 6b), growth trajectories in the high line (EOL+) are the same as the low line (EOL−) up to age of 28 days. However, the two lines diverged significantly ($P < 0.01$) at age of 56 days. These observed trends are exactly as we expected.

Cumulative selection differentials and realized genetic parameters: For the purpose of estimating realized genetic parameters, only primary selection differentials (selection differentials of index scores) are
useful. The regression coefficients of line replicate means of index score, early gain and late gain on cumulative primary selection differentials are listed in Table 4. These regression coefficients were used to estimate the heritabilities of index scores and the genetic variance-covariance matrix. The estimates of realized heritability of early gain index show a great difference between the two lines, 0.09 for E+L0 and 0.33 for E–L0. But, the average is 0.21, which is close to the expected value, 0.23. The realized heritabilities of index score estimated from the late gain selection lines had little variation between the high and low lines, the average of which is 0.28. This average value, however, is less than the expected value, 0.35 (Table 5). The realized genetic variances and covariance of early and late gain traits severely underestimate the true values (see Table

![Figure 3](image1.png)

**Figure 3.**—Variation in late body weight gains (G2856) among three replicates within each selected line of mice (see Figure 1 for explanations of the symbols E+L0, E–L0, E0L+ and E0L–).

![Figure 4](image2.png)

**Figure 4.**—Divergence in index scores between means of the high and low selected lines (see Figure 1 for explanations of the symbols E+L0, E–L0, E0L+ and E0L–). (a) Divergence between E+L0 and E–L0. (b) Divergence between E0L+ and E0L–.
Selection for Early Gain

Selection for Late Gain

(a) E+LO vs E-LO

(b) EOL+ vs EOL-

(c) E+LO vs E-LO

(d) EOL+ vs EOL-

**Figure 5.**-Responses of early and late body weight gains to restricted selection. The four subfigures are arranged in a 2 × 2 fashion where the first column (a and c) represents selection for early gain while holding late gain constant, and the second column (b and d) represents selection for late gain while holding early gain constant. The first row (a and b) represents changes of the early body weight gain, and the second row (c and d) represents changes of the late body weight gain.

6). Especially, the realized genetic covariance is negative whereas the expected value is positive. These discrepancies are most likely due to changes in variances and covariance caused by selection or genetic drift or both.

**DISCUSSION**

This selection experiment was initiated to produce strains of mice that were strongly differentiated due to changes in fundamental developmental processes (i.e., hypertrophy and hyperplasia). After 14 generations of selection, it is evident from these analyses that this goal of significant differentiation among lines is being met in a wide variety of traits. P. D. Crenshaw, W. R. Atchley and S. Xu (unpublished results) have shown significant divergence among these selected lines in various organ weights, skeletal structures and postnatal maternal effects.

Component nature of complex morphologies: These results provide additional documentation about the component nature of a complex morphology (body weight) and that changes in the various components bring about significant divergence in the final phenotype. We have shown experimentally (W. R. Atchley, unpublished data) that changes in early postnatal ontogeny (between birth and 10 days of age) are primarily the result of changes in the number of cells in various
tissues and organs. Further, later changes in postnatal ontogeny are occurring primarily by changes in cell size. Thus, these results clearly demonstrate that one can alter the final phenotype by either increasing or decreasing the number of cells (E+ or E−) or the size of cells (L+ or L−).

**Index selection**: Some restricted index selection experiments have been successful (e.g., D’OLIVIER 1980; SHARP et al. 1984). A number of others have not been so successful and selection response generally has not been as expected. In these latter cases restricted index selection typically has shown response to selection occurring in the restricted traits when none is expected (e.g., EISEN 1992) and observed responses less than expected for the selected traits (RUTLEDGE et al. 1973; BERGER and HARVEY 1975; EISEN 1977, 1978; MCCARTHY and DOOLITTLE 1977). These failures appear to mainly be due to genetic drift and changes in genetic parameters with selection. Restricted index selection seems to be more sensitive to parameter changes than the conventional index selection (EISEN 1993).

In contrast to some of the previous reported shortcomings of restricted index selection, our experiment was well behaved in terms of response to altering age specific growth rates; significant gains were achieved as expected, and there was little response to selection by the restricted trait in any of the lines.

**Temporal nature of selection**: The experimental results reported here demonstrate experimentally the ramifications of temporally varying selection on complex traits. It is evident in Figure 6a that early selection produced divergent body weight phenotypes very early in ontogeny. However, the late selected lines show no significant divergence in body weight until much later in postnatal ontogeny. Consequently, we have produced a series of mouse lines exhibiting significant divergence in body weight at different ontogenetic stages.

An important realization in dynamic models of morphological evolution is that selection is a multistage phenomenon in that it can occur at different points during ontogeny with differing consequences (XU and MUIR 1991; XU et al. 1994). Indeed, when selection operates at different points during ontogeny on a complex trait, the selection may be affecting different component parts or processes.

Development is often referred to as a hierarchical, sequential and epigenetic phenomenon characterized by cascades of actions and interactions. If selection is a multistage phenomenon operating at different times during ontogeny, then it may be occurring when different genes are being expressed, when different genetic backgrounds are present because of age- and tissue-specific patterns of gene expression, and when different pleiotropic and epigenetic interactions are occurring. We have shown previously that the genetic covariance structure within a single trait and among traits can vary significantly during ontogeny (ATCHLEY et al. 1984; ATCHLEY 1987; RISKA and ATCHLEY 1985). Consequently, as shown here the time during ontogeny when selection occurs is quite important in understanding the overall multitrait response.

COWLEY and ATCHLEY (1992) proposed an epigenetic model for the evolution of morphology. Epigenetic effects arise when the heritable processes or behavior of a given cell or tissue extrinsically controls the phenotype of a separate cell or tissue (ATCHLEY and HALL 1991). Epigenetic effects often involve inductive effects involving events in a hierarchical and sequential process so that the effect is unidirectional. That is, one population of cells influences another and the effect occurs slightly later in time. Because of the temporal separation, the effect is not reciprocal. Heritable events occurring early in development stages that influence traits expressed in later stages are another example of epigenetic effects.

This temporal ordering of events and their epigenetic...
consequences later in ontogeny can have a significant impact on the developmental of complex morphological structures. In working with artificially deformed human skulls, Kohn et al. (1993) found that modifying the cranial vault caused characteristic shape changes in the cranial base and face. Since the brain (and cranial vault) develops earlier in ontogeny than the face, an artificial widening or narrowing of the cranial base results in a consequent shortening or lengthening of the face. Cranial base width serves as a scaffold upon which future facial growth takes place so that the shape of the face is a consequence of the pre-existing shape of the anterior cranial base.

**Developmental and genetic basis of identical complex phenotypes**: Do similar phenotypes imply the existence of the same developmental mechanisms or similar evolutionary histories? A problem in evolutionary biology is to classify organisms into groups reflecting common origins. Decisions about common origins are often made based on phenotypic similarity in complex morphological traits. A significant complication occurs when homoplasy is present, i.e., structural resemblance is due to convergent evolution rather than common ancestry (Lincoln et al. 1982). In such cases, phenotypic similarity is not based on genotypic similarity. While discussions about convergent evolution are often based upon phylogenetic analyses, the possibility is that developmental homoplasy may play a significant role in these discussions.

From a developmental perspective, it is possible to achieve seemingly identical adult morphological phenotypes in different lineages through changes in different developmental pathways and processes. Thus, the same organ size in divergent lineages can be achieved by changes in the number of progenitor cells, the rate of cell division, changes in the patterns of programmed

### TABLE 4

Regression coefficients of population means on cumulative selection differentials of indices

<table>
<thead>
<tr>
<th>Line</th>
<th>Rep</th>
<th>Index</th>
<th>G016</th>
<th>G2856</th>
</tr>
</thead>
<tbody>
<tr>
<td>E+L0</td>
<td>1</td>
<td>0.025 ± 0.071</td>
<td>0.074 ± 0.319</td>
<td>-0.420 ± 0.542</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.158 ± 0.047</td>
<td>0.638 ± 0.226</td>
<td>-0.487 ± 0.513</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-0.001 ± 0.089</td>
<td>-0.056 ± 0.399</td>
<td>-0.580 ± 0.559</td>
</tr>
<tr>
<td></td>
<td>Pooled</td>
<td>0.060 ± 0.085</td>
<td>0.225 ± 0.380</td>
<td>-0.496 ± 0.079</td>
</tr>
<tr>
<td>E-LO</td>
<td>1</td>
<td>0.167 ± 0.034</td>
<td>-0.482 ± 0.127</td>
<td>3.007 ± 1.023</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.242 ± 0.072</td>
<td>-0.936 ± 0.306</td>
<td>1.582 ± 0.506</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.272 ± 0.054</td>
<td>-1.011 ± 0.237</td>
<td>2.256 ± 0.258</td>
</tr>
<tr>
<td></td>
<td>Pooled</td>
<td>0.227 ± 0.054</td>
<td>-0.810 ± 0.286</td>
<td>2.283 ± 0.712</td>
</tr>
<tr>
<td>EOL+</td>
<td>1</td>
<td>0.154 ± 0.076</td>
<td>-0.094 ± 0.035</td>
<td>0.233 ± 0.144</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.053 ± 0.053</td>
<td>-0.015 ± 0.049</td>
<td>0.120 ± 0.150</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.191 ± 0.048</td>
<td>-0.056 ± 0.036</td>
<td>0.423 ± 0.089</td>
</tr>
<tr>
<td></td>
<td>Pooled</td>
<td>0.133 ± 0.071</td>
<td>-0.055 ± 0.039</td>
<td>0.259 ± 0.153</td>
</tr>
<tr>
<td>EOL-</td>
<td>1</td>
<td>0.182 ± 0.042</td>
<td>0.049 ± 0.038</td>
<td>-0.414 ± 0.123</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.116 ± 0.047</td>
<td>0.054 ± 0.062</td>
<td>-0.211 ± 0.054</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.172 ± 0.052</td>
<td>0.053 ± 0.034</td>
<td>-0.377 ± 0.125</td>
</tr>
<tr>
<td></td>
<td>Pooled</td>
<td>0.157 ± 0.035</td>
<td>0.052 ± 0.002</td>
<td>-0.334 ± 0.108</td>
</tr>
</tbody>
</table>

Values are means ± SE. Standard error for the pooled average was calculated from the variation among three replicates.

### TABLE 5

Realized heritabilities of indices

<table>
<thead>
<tr>
<th>Rep</th>
<th>$h^2_{E+L0}$</th>
<th>$h^2_{E-LO}$</th>
<th>$h^2_{EOL+}$</th>
<th>$h^2_{EOL-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.036</td>
<td>0.241</td>
<td>0.293</td>
<td>0.346</td>
</tr>
<tr>
<td>2</td>
<td>0.228</td>
<td>0.349</td>
<td>0.101</td>
<td>0.220</td>
</tr>
<tr>
<td>3</td>
<td>-0.002</td>
<td>0.393</td>
<td>0.361</td>
<td>0.326</td>
</tr>
<tr>
<td>Pooled over replicates</td>
<td>0.087 ± 0.123</td>
<td>0.328 ± 0.078</td>
<td>0.252 ± 0.135</td>
<td>0.297 ± 0.067</td>
</tr>
<tr>
<td>Pooled over lines</td>
<td>0.207 ± 0.170</td>
<td>0.275 ± 0.032</td>
<td>0.345</td>
<td></td>
</tr>
</tbody>
</table>

$h^2_{E+L0}$, the heritability of index for early gain (lines E+LO and E-LO); $h^2_{EOL}$, the heritability of index for late gain (lines EOL+ and EOL-). Values are means ± SE over replicates or over lines. Expected $h^2 = b'Gb/(b'Pb)$. 

\[ h^2_{E+L0} \]
\[ h^2_{E-LO} \]
\[ h^2_{EOL+} \]
\[ h^2_{EOL-} \]
TABLE 6

Realized genetic variance for early gain, late gain, and covariance between early and late gains

<table>
<thead>
<tr>
<th>Lines used</th>
<th>$V_{a1}$</th>
<th>$V_{a2}$</th>
<th>$C_{a12}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>E+L0 and E0L+</td>
<td>0.137 ± 0.213 (0.245 ± 0.380)</td>
<td>1.538 ± 1.066 (0.170 ± 0.118)</td>
<td>0.004 ± 0.448 (0.009 ± 0.976)</td>
</tr>
<tr>
<td>E+L0 and E0L-</td>
<td>0.148 ± 0.147 (0.264 ± 0.263)</td>
<td>2.086 ± 0.734 (0.231 ± 0.081)</td>
<td>0.047 ± 0.508 (0.085 ± 0.554)</td>
</tr>
<tr>
<td>E-L0 and E0L+</td>
<td>0.031 ± 0.214 (0.055 ± 0.382)</td>
<td>0.914 ± 1.068 (0.101 ± 0.118)</td>
<td>-0.271 ± 0.449 (1.610 ± 2.667)</td>
</tr>
<tr>
<td>E-L0 and E0L-</td>
<td>0.042 ± 0.147 (0.075 ± 0.263)</td>
<td>1.461 ± 0.734 (0.162 ± 0.081)</td>
<td>-0.227 ± 0.308 (0.916 ± 1.243)</td>
</tr>
<tr>
<td>All</td>
<td>0.089 ± 0.127 (0.159 ± 0.227)</td>
<td>1.500 ± 0.637 (0.166 ± 0.070)</td>
<td>-0.111 ± 0.268 (0.304 ± 0.735)</td>
</tr>
</tbody>
</table>

$V_{a1}$ early gain; $V_{a2}$ late gain; $C_{a12}$ covariance between early and late gains. Realized heritabilities and genetic correlations are given in parentheses. Values are means ± SE.

cell death and related phenomena (ATCHLEY 1987).

Similarly, the same organ size in different lineages can be achieved by changes in either the number of cells or the size of the cells. These various mechanisms involve different genetically controlled developmental processes.

Similarly, it is well known in humans that genetic heterogeneity exists in disease phenotypes so that the same syndrome can be achieved by different underlying genetic and developmental mechanisms in different families. Examples of such developmental homoplasy in disease phenotypes include cancer, heart disease, obesity and many other syndromes.

An example of developmental homoplasy is found in replicate 2 of E+L0 and replicate 2 of E0L+, which have identical body weight phenotypes (35.24 g) at 56 days of age. Further, P. D. CRENSHAW, W. R. ATCHLEY and S. Xu (unpublished results) have shown that identical kidney weights and 91 day tail lengths for E-L0 and E0L- lines. In spite of phenotypic identity, the genetic basis of the E+L0 and E0L+ phenotypes are probably quite different since one was produced by selection for early rate of development and the other by changing late rate of development.

Developmental homoplasy has been reported in other experiments. For example, RUTLEDGE et al. (1973) found in a replicated selection experiment on tail length that in one replicate tail length was increased by changing the number of bones in the tail while the other replicate changed the size of the bones.

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LITERATURE CITED


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