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Body Weight and Tail Length Divergence in Mice Selected for Rate of Development

BRIAN K. RHEES* AND WILLIAM R. ATCHLEY
Department of Genetics, North Carolina State University, Raleigh, North Carolina, 27695-7614

ABSTRACT A series of mouse lines has been produced by 19 generations of restricted index selection for rate of development during early and late ontogeny. The selection program was based on an index with the following four replicated selection treatments: E^+ and E^- were selected to alter birth to 10-day body weight gain while holding late gain for both selection lines constant; correspondingly, L^+ and L^- were selected to alter 28- to 56-day body weight gain holding early gain for both lines constant. Herein, we characterize response to selection for growth rate by analyzing age-specific mouse body weight and tail lengths and for growth curves using a logistics model. Selection on developmental rate has resulted in divergence in both age-specific and growth curve traits. E^+ and L^+ lines reached identical weights during the late selection interval, then diverged to unique mature weights. E^- and L^- lines similarly achieved identical weights during late selection and diverged to unique mature weights. However, the shapes of early and late growth curves were significantly divergent, and at least two distinct growth patterns are shown to result from selection. Response in body weight gain was accompanied by similar, though less pronounced, change in tail length traits. Significant response during intervals of restricted growth was also found, especially in lines selected for late gain. The evolution of the growth trajectory under restricted index selection is discussed in terms of drift and available additive genetic variation and covariation. *J. Exp. Zool. (Mol. Dev. Evol.) 288:151–164, 2000.*

The history of quantitative genetics is replete with experiments where artificial selection has been used to systematically alter some phenotype(s) (Falconer and Mackay, '96; Lynch and Walsh, '98). The goals of such studies vary from efforts to improve economic aspects of agricultural organisms, such as conformation in cattle or litter size in swine, to resolution of fundamental questions in basic biological sciences. Further, selection experiments vary widely in the way they were carried out. For example, some focus on single age-specific traits, such as six-week weight in mice (Nagai et al., '76; Eisen and Roberts, '81) or on rate of change in traits over specific intervals during ontogeny (Swartz and Famula, '94; Moura et al., '97). Others involve efforts to change growth curves involving multiple measurements taken over time during ontogeny (Abplanalp, '63; McCarthy and Doolittle, '77).

Extensive documentation exists regarding the morphological consequences of selection on the specific traits. Unfortunately, less information is available about the developmental mechanisms underlying response to selection. Consequently, a "developmental quantitative genetics" approach has been practiced by some to explore questions about the interplay of development and quantitative genetics (e.g., Atchley et al., '84, '94; Riska and Atchley, '85; Atchley and Hall, '91; Cowley and Atchley, '92).

For selection studies, a developmental quantitative genetics approach explores questions about the underlying controlling mechanisms involved in response to selection. Indeed, it is important to understand which developmental mechanisms were altered at different levels of organization to achieve the final desired phenotypes. Fundamental questions to be resolved include which quantitative trait loci are involved in response to selection, when are they expressed during ontogeny, and in what developmental patterns of expression. Other important questions included whether changes in complex traits like body weight stem from changes in cell number or cell size in relevant organs and tissues? Are changes

Grant sponsor: National Institutes of Health; Grant number: GM-45344.
*Correspondence to: Brian K. Rhees, Hoffman–La Roche, 340 Kingsland Street, Nutley, NJ 07110–1199.
E-mail: Brian.Rhees@roche.com
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through selection achieved by different rates of programmed cell death or different temporal and spatial patterns of expression of growth factors or growth hormone? Has selection altered other important regulatory mechanisms, such as maternal-fetal interactions? Indeed, these are but a few of the important questions to be resolved if we are to understand the mechanisms for how selection operates in biological organisms.

Herein, we describe some results from an ongoing long-term restricted index selection on mice being carried out to explore the developmental quantitative genetics of response to selection. The particular selection regimen was designed to use quite different underlying genetic and developmental mechanisms to produce mice with the same adult phenotypes. Five replicated selection lines have been produced by selecting for increased or decreased body weight gain early (birth to ten days of age) or later (28–56 days of age) during postnatal ontogeny (Atchley et al., '97). It is well documented that growth in body weight during separate intervals in ontogeny is achieved by different cellular phenomena (e.g., Enesco and Leblond, '62). Early mammalian growth is primarily by increasing cell number (hyperplasia) while later growth emphasizes changes in cell size (hypertrophy). Consequently, selecting for rate of change in body weight during these critical intervals potentially provides an indirect mechanism for changing hyperplasia and hypertrophy. Indeed, experimental verification of this model has been obtained by cellular analyses in these selection lines of mice (Atchley et al., 2000).

The current study examines correlated response in body weight and tail length to selection for differential rate of growth in body weight. We provide detailed information about changes in age-specific body weight and tail length among these various selection lines and their replicates. Further, we examine the impact of restricted index selection on growth curves for these traits. Specifically, we test the null hypotheses that early and late selection has no effect on growth curve parameters or traits (i.e., mature weight, age and rate of growth). In addition, we examine the effects of selection on age and rate of maximum growth, and consider what relationship might exist between these traits and reproductive onset. These results are part of a comprehensive study in our laboratory where the impact of this selection regimen is examined on cell parameters, organ size, reproductive onset (Ernst et al., '99), uterine and post-natal maternal effects (Rhees et al., '99; Ernst et al., 2000), and skeletal morphology.

MATERIALS AND METHODS

Selection lines

Mouse lines used in these analyses were taken from generation 19 of an ongoing restricted index selection experiment. The founding stock was the random-bred ICR mouse strain obtained from Harlan Sprague-Dawley (Indianapolis, IN). Five selection treatments are represented: (1) $E^{+}L^{0}$ [E^{+}], selected for increased gain in early body weight (birth to 10 days), holding late gain (28–56 days) constant; (2) $E^{-}L^{0}$ [E^{-}], selected for decreased early gain, holding late gain constant; (3) $E^{0}L^{+}$ [L^{+}], selected for increased late gain while holding early gain constant; (4) $E^{0}L^{-}$ [L^{-}], selected for decreased late gain, while holding early gain constant; and (5) a control line, $E^{0}L^{0}$ [C], randomly selected. Each line was replicated three times, for a total of 15 independent lines. Selection was performed within litter to reduce maternal effects. (A more detailed description of these lines and their direct response to selection can be found in Atchley et al., '97).

Husbandry

Mice were housed in opaque cages on hardwood chips and supplied with food and water ad libitum. Room temperature was maintained between 23°C and 26°C with a controlled 14-hour light:10-hour dark cycle. Replicates were maintained in 12 litters standardized at birth to eight pups and, where possible, a balanced sex ratio. Litters with fewer than eight pups were augmented with excess pups from other litters and these mice were tail-clipped to distinguish them from their littermates. At 21 days, mice were weaned and separately caged by sex. Otherwise, litter integrity was maintained. Measurements on substituted pups are not included in analyses.

Age-specific growth traits

Mice from generation 19 of the selection experiment were measured for body weight and tail length. Average pup weight at birth was recorded as litter weight divided by the number of pups born. Individual body weights were taken at 10, 21, 28, 35, 42, 49, and 56 days of age using a computer driven analytical balance programmed to provide means for five repeated measurements over a short time interval. Tail lengths were measured at days 10, 28, 35, 42, 49 and 56.

Growth curve traits

Growth curves have been used to model functional relationships between treatments and effects as continuous phenomena, as opposed to using purely statistical models such as repeated measures (Kshirsagar and Smith, '95). Therefore, we have used growth curves to transform age-specific trait information into a dynamic and continuous description of ontogenetic change. Previous rodent growth studies have emphasized sigmoidal models belonging to the Richards family of growth curves, e.g., the logistic (Pahl, '69; Eisen, '76; Kasser et al., '83; Bailey et al., '88), Gompertz (Laird and Howard, '67; Kidwell et al., '69), and Bertalanffy (Di Masso et al., '90). These models provide a reasonable fit of growth data, relate model parameters to growth curve traits (e.g., asymptotic weight, age of maximum gain, and maximum growth rate), and permit estimation of age-specific traits and growth rate.

Initially, we tested the fit of the Bertalanffy, the logistic and Gompertz models on body weight. For our data, a three parameter logistic model produced the best fit. Therefore, we used a logistic growth model to summarize growth trends and to estimate growth curve traits for body weights and tail lengths. The equation was of the form

$$Y_i(t) = \frac{A_i}{1 + e^{b_i - k_i t}}$$

where $Y(t)$ = response (body weight/tail length) at age t days, A = asymptotic or mature body weight/tail length, k = intrinsic growth rate parameter (rate of change in logarithmic weight/length per unit of time) for individual i. Shape parameter b, by itself, lacks an explicit biological interpretation. However, individuals obtain a maximum growth rate at postnatal age $t' = b/k$, indicating that for fixed values of k, b determines the age of maximum growth rate ($mgr = Ak/4$).

Growth curve parameters may be estimated either by assuming homogeneous variances among age-specific traits, or by adjusting for heterogeneous variances by weighted non-linear regression (Rawlings et al., '98). Because individual birth weights were estimated from average pup weight at birth, weighted regression in the current study resulted in inappropriately high weighting of birth weights. This in turn led to a large prediction bias at day 10 and day 21 (e.g., –20% at day 10 and +14% at day 21 for E^+ mice). Therefore, we have chosen to assume a homogeneous variance structure in curve fitting. Growth curve parameters were estimated with SAS procedure NLIN (SAS Institute, '88) using the Marquardt search method. Of 1,204 regressions performed on individuals in generation 19, only ten failed to converge. In all instances, these ten individuals had aberrant growth data, with moderate or sharp decreases in weight during the interval of observation and these ten individuals were excluded from further analysis.

Growth curve residuals were found to be normally distributed by Shapiro-Wilk tests (Shapiro and Wilk, '65). However, there was a small bias in residual means. Individual growth curves in general over-predicted body weight at 0, 21, 42 and 49 days, and tail lengths at 35 and 42 days, while under predicting remaining age-specific traits. These effects may be partially due to increased number of age-specific observations fit in the latter part of the curve and moderate heteroscedacity due to greater variability in mid and late observations. While residual means were statistically non-zero, they were small in proportion to both absolute body weight and tail length (Figs. 1, 2). As such, they should not pose serious problems in hypothesis testing.

Population growth curves

Ideally, we might describe the evolution of growth trajectories over several generations of selection by fitting growth curves to the same age-specific trait measurements for each generation. Unfortunately, longitudinal data recorded at weekly intervals are not available prior to generation 19. Nevertheless, we can estimate growth curve selection response by fitting curves to (within-litter) birth weight and weights on days 10, 28, and 56. These four measurements are used to construct the selection index scores and are available each generation. We therefore report population growth curves on birth, 10-, 28-, and 56-day weights for generations 0–19 to describe selection response in growth curves and growth rate across these generations.

Statistical methods

Analysis of variance (ANOVA) was performed to test for effects of selection and drift on age-specific and growth curve traits. The data were fit to a model which included selection line, replicate within line, and sex as fixed effects (including all possible combinations of line, replicate and sex effects). Litter was included as a random effect to account for genetic and environmental covariation

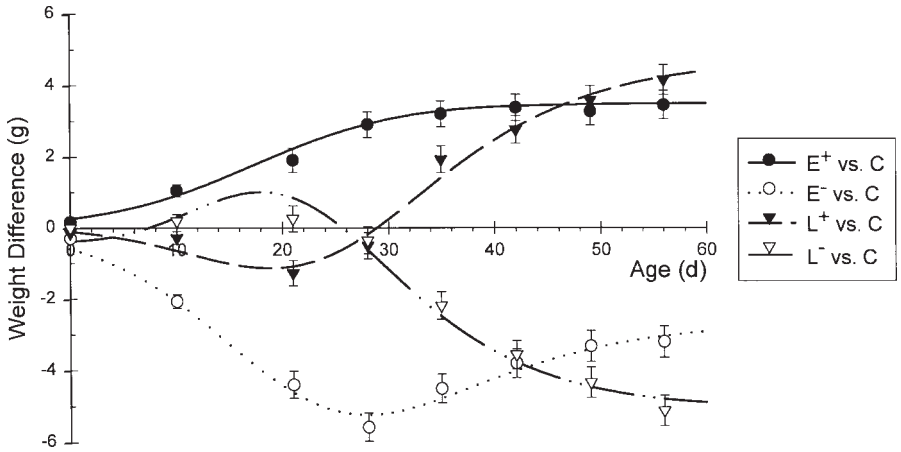

Fig. 1. Postnatal growth trajectories for selection line body weights. Weights are shown relative to unselected control. Mean growth curves for body weight and age-specific least squares weight means and standard errors are shown.

between observations within full-sib families. A litter-size covariate was added to explicitly test for litter size effects on postnatal growth. In addition, age-specific tail lengths were analyzed with and without body weight covariates to identify total divergence in tail lengths, as well as divergence after correcting for the effect of weight.

Statistical contrasts were performed—as indicated by the ANOVA—to detect differences between selection lines that arise from early and late selection. To do this, confidence intervals were estimated on least squares means and were corrected for multiple comparison effects. Models were fit using SAS procedure MIXED with a Bonferroni correction (SAS Institute Inc., '92).

RESULTS

Litter size effects

Litter size, measured as the sum of live and dead pups at birth had a non-significant effect on body weights, tail lengths and growth curve traits (Tables 1 and 2). In part, this is due to the statistical model, which first accounts for covariation among full sibs due to both genetic and environmental factors prior to testing for significance of the litter size covariate. Also, while litter size effects were not seen on body weights, growth curve traits or tail lengths, they nevertheless had significant effects on early weight gains. For this reason, litter size was included in the model as a covariate for all analyses.

Sex effects on growth trajectories

Analysis of variance (ANOVA) shows that males and females were significantly different ($P < 0.001$) for body weights from day 20 to day 56 (Table 1). Sex effects on tail length were similar (Tables 3 and 4). Significant sex × line interactions for all traits indicate a heterogeneous response to selection between sexes. However, magnitude and direction of sex × line means did not indicate a consistent trend in sex-specific response within lines (data not shown). In general, response in age-specific and growth curve traits was greater in males, with considerable line-specific variation.

Effects of selection on body weight growth trajectories

Testing for differences between selection lines and across replicates demonstrates the extent of divergence over and beyond random drift. Effects of selection remained highly significant after accounting for replicate within line variation and sexual dimorphism, both within and across lines. Selection had a highly significant effect ($P < 0.001$) on both age-specific and growth curve traits for body weights.

Analyses of body weight traits demonstrate that the selection indices have produced lines with divergent body weights from birth to 56 days (Table 1). In addition, selection response was clearly accompanied by change in all growth curve traits. Trait means and least significant differences (adjusted for multiple comparisons) indicate that all lines achieved significantly different mature body weights (Table 5). Growth rates for selection lines appear variable both in magnitude and age of highest instantaneous growth rate (Table 5). L^- mice achieved absolute maximum growth rate earliest (day 16) followed by E^+ and control lines (day 19) and E^- and L^+ lines (day 23). The E^+ line, while

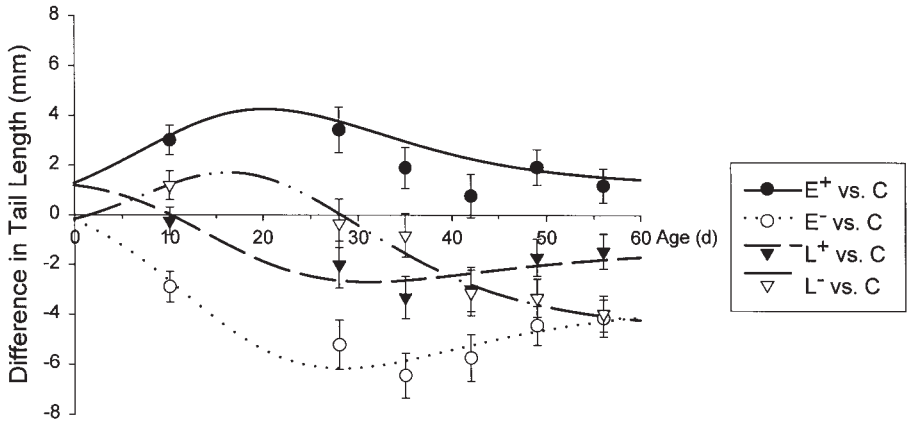

Fig. 2. Postnatal growth trajectories for tail lengths. Lengths are shown relative to the unselected control. Mean growth curves, age-specific least squares means and standard errors are shown. (Means are estimated from the analysis of covariance model including litter size, but not age-specific body weight, as a covariate.)

maximizing on the same day as the control, achieved a significantly greater growth rate. Similarly, while E^- and L^+ lines reached maximum growth rate on the same day, the L^+ mice achieve a much larger growth rate.

Mean growth curves for selection lines (as deviations from the control) are presented in Figure 1 with age-specific trait means and standard errors. Relative body weight for both early selection lines (E^+ and E^-) showed divergence from the control line immediately after birth, as would be expected considering the selection criterion. In the case of E^-, however, a late period of convergence back toward the control was seen during the constrained selection interval (28–56 days), beginning near day 30. Late selection lines similarly diverge from the control line, shortly before day 28. Between days 10 and 28, however, growth trajectories appear to have "anticipated" late selection response, and have also diverged from the control during this interval. In the L^- line this divergence may in part be due to a line specific biased fit in individual growth curves. Means for L^- age-specific weights at day 10 and day 20 were not significantly different from those of the control line. Lastly, body weights of E^+ and L^+ lines converged briefly during the late selection interval as did body weights for E^- and L^- mice.

Effects of selection on tail length growth trajectories

Response in body weight was accompanied by response in tail lengths from age 10 to 56 days (Table 2). Selection had a highly significant effect ($P < 0.001$) on age-specific tail lengths as well as growth curve traits for tail lengths, with the exception of shape parameter *b*. Analysis of covariance (ANCOVA) shows that body weight had a significant linear effect on tail lengths at all ages (Table 6). Therefore, to some extent tail length

TABLE 1. Analysis of variance for age-specific body weights and body weight growth curve traits from birth to 8 weeks of age in mouse selection lines[1]

		Age (days)								Growth curve traits				
Source	d.f.	0	10	21	28	35	42	49	56	*A*	*b*	*k*	*t′*	*mgr*
Line	4	***	***	***	***	***	***	***	***	***	***	***	***	***
Rep(line)	10	***	***	***	***	***	***	***	***	***	*	**		***
Sex	1	na	***	***	***	***	***	***	***	***	***	***	***	***
Line × Sex	4	na		***	***	***	***	***	***	***	***	***	***	***
Rep × Sex	10	na			**	***	***		**	**	***	**	***	**
Litter size[1]	1	na[2]												

[1]Litter size at birth covariate.
[2]Because birth weight is estimated from average pup weight within litter, the litter size covariate is nonestimable under the full statistical model (which includes litter as a random effect). However, litter size is highly significant on average pup weight in a simplified model excluding litter as a random effect ($P < 0.0001$).
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

TABLE 2. Analysis of variance for age-specific tail lengths and tail length growth curve traits from 10 days to 8 weeks of age in mouse selection lines[1]

		Age (days)						Growth curve traits				
Source	d.f.	10	28	35	42	49	56	*A*	*b*	*k*	*t′*	*mgr*
Line	4	***	***	***	***	***	***	***		***	***	***
Rep(line)	10	***	***	***	***	***	***	***				*
Sex	1			***	***	***	***	***	***	***	***	***
Line × Sex	4		*	*	**	*	**	**		***		**
Rep × Sex	10			*	**	***	***	***		***	**	
Litter size[2]	1											

[1]Empty cells represent values not significant at $P < 0.05$ level.
[2]Litter size at birth covariate.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

differences between lines (Fig. 2) are attributable to response in body weight.

Both early and late selection for increased growth rate resulted in longer tails compared to lines selected for decrease growth rate (Fig. 2). As with body weight, E^- and L^- mice achieve similar tail lengths during the late selection (28–56 day) interval. In contrast, E^+ and L^+ tail lengths do not converge.

Effects of selection on tail length growth curve traits were also observed, though to a lesser degree than for body weight growth curve traits (Table 2). While E^+ and E^- lines achieved significantly different mature tail lengths (*A*), L^+ and L^- lines did not (Table 7). Furthermore, no significant differences in shape parameter *b* were observed. Lines E^- vs. L^+ and E^+ vs. L^- were not significantly different for intrinsic growth rate *k*, indicating these selection lines exhibit similar patterns of growth rate.

Tail lengths reach maximum growth rate earlier than body weights. L^- and E^+ mice maximized earliest (day 12) with not significantly different growth rates. Control and L^+ lines reached maximum growth rate next (day 14), though with significantly different growth rates. E^- maximized the latest (day 15) and achieved the lowest maximum growth rate.

Within-line variation in selection response

Replicate within line [Rep(line)] was found to have a statistically significant effect ($P < 0.05$) on body weight growth curve traits *A, b, k,* and *mgr*. However, few significant differences in trait means were observed for body weights traits (Table 8). Early selection lines (E^+ and E^-) had the most homogeneous within-line replicate response to selection. While significant differences in age-specific body weights were found, no significant differences in

TABLE 3. Least squares means for body weight (g) from birth to 8 weeks of age by sex and line

	Line				
Age (days)	E^+	E^-	L^+	L^-	C
Females					
0[1]	1.8 ± 0.03	1.3 ± 0.03	1.5 ± 0.03	1.6 ± 0.03	1.6 ± 0.03
10	9.3 ± 0.13	6.1 ± 0.15	8.0 ± 0.14	8.5 ± 0.14	8.2 ± 0.12
21	17.4 ± 0.26	11.2 ± 0.29	14.4 ± 0.27	15.7 ± 0.28	15.3 ± 0.25
28	23.8 ± 0.28	16.8 ± 0.32	21.7 ± 0.30	20.7 ± 0.30	21.4 ± 0.26
35	27.8 ± 0.29	20.9 ± 0.33	27.2 ± 0.31	22.6 ± 0.31	25.2 ± 0.27
42	28.8 ± 0.29	22.5 ± 0.33	28.6 ± 0.31	22.9 ± 0.32	26.0 ± 0.28
49	29.7 ± 0.31	23.9 ± 0.35	30.4 ± 0.33	23.6 ± 0.34	27.3 ± 0.30
56	31.1 ± 0.32	25.1 ± 0.37	32.1 ± 0.34	24.1 ± 0.35	28.3 ± 0.31
Males					
0[1]	1.8 ± 0.03	1.3 ± 0.03	1.5 ± 0.03	1.6 ± 0.03	1.6 ± 0.03
10	9.4 ± 0.13	6.3 ± 0.15	8.0 ± 0.14	8.5 ± 0.14	8.4 ± 0.13
21	18.0 ± 0.26	11.6 ± 0.29	14.6 ± 0.27	16.4 ± 0.28	16.3 ± 0.25
28	29.0 ± 0.28	19.2 ± 0.32	24.4 ± 0.30	25.6 ± 0.31	25.7 ± 0.27
35	32.8 ± 0.29	24.3 ± 0.33	30.9 ± 0.31	27.3 ± 0.32	29.0 ± 0.28
42	34.9 ± 0.29	26.8 ± 0.34	33.8 ± 0.31	26.9 ± 0.33	30.9 ± 0.29
49	36.5 ± 0.31	29.2 ± 0.36	36.5 ± 0.33	27.5 ± 0.35	32.4 ± 0.31
56	38.1 ± 0.32	30.8 ± 0.37	38.6 ± 0.35	28.0 ± 0.36	34.0 ± 0.32

[1]Least squares means for birth weight were estimated from average birth weights by litter and are therefore identical for both sexes.

TABLE 4. Least squares means for tail lengths (mm) from day 10 to 8 weeks of age by sex and line

	Line				
Age (days)	E^+	E^-	L^+	L^-	C
Females					
0[1]	37.9 ± 0.48	32.0 ± 0.48	34.7 ± 0.43	36.1 ± 0.44	34.8 ± 0.39
28	76.2 ± 0.67	67.3 ± 0.75	71.2 ± 0.71	72.7 ± 0.73	72.9 ± 0.66
35	77.9 ± 0.62	69.6 ± 0.70	73.3 ± 0.65	75.2 ± 0.66	76.2 ± 0.59
42	82.8 ± 0.65	76.5 ± 0.22	79.9 ± 0.68	79.3 ± 0.69	82.6 ± 0.62
49	86.1 ± 0.55	79.9 ± 0.62	83.2 ± 0.58	81.3 ± 0.59	84.6 ± 0.52
56	87.1 ± 0.52	82.0 ± 0.59	85.2 ± 0.55	82.6 ± 0.56	86.4 ± 0.50
Males					
10	38.1 ± 0.47	32.2 ± 0.48	34.8 ± 0.43	36.3 ± 0.44	35.2 ± 0.39
28	76.4 ± 0.67	68.1 ± 0.76	70.7 ± 0.71	72.5 ± 0.74	72.9 ± 0.66
35	79.5 ± 0.62	71.2 ± 0.70	73.7 ± 0.66	76.8 ± 0.67	77.4 ± 0.59
42	86.6 ± 0.65	79.3 ± 0.73	81.4 ± 0.68	81.7 ± 0.70	84.7 ± 0.63
49	89.6 ± 0.55	82.9 ± 0.62	85.2 ± 0.58	83.8 ± 0.60	87.2 ± 0.53
56	90.7 ± 0.52	85.1 ± 0.59	87.2 ± 0.56	84.9 ± 0.57	89.0 ± 0.50

growth curve traits were observed. In contrast, within-line variation in body weights did contribute to significant replicate variation in growth curve traits for late selection lines. Lines L^+, L^-, and C lines each showed significant replicate differentiation for estimated mature weight (A). Additionally, the control line replicates differed significantly for shape parameter b, and L^- replicates differed significantly for intrinsic growth rate (k).

Compensatory growth

Similarity in body weight growth curve traits (Table 5) results from similarities in underlying growth rates. Lines E^+ vs. C showed relative growth rate increasing from birth to a mean age of 17.6 days (± 0.5) in E^+ males and 14.5 days (± 0.5) in females and decreasing thereafter (Figs. 3a–d show distributions for males as differences from the randomly selected control). E^- mice similarly showed early divergence in growth rate, peaking at age 15.6 days (± 0.2) in males and 14.6 days (± 0.2) in females.

TABLE 5. Body weight growth curve traits means and least significant differences[1]

	Least squares means			Age of maximum	
Line	A	b	k	growth rate (t')	mgr
E^+	34.43^a	2.432^a	0.1260^a	19.4^a	1.08^c
E^-	28.50^b	2.609^b	0.1114^b	23.5^b	0.79^d
L^+	35.84^c	2.613^b	0.1133^b	23.2^b	1.01^a
L^-	26.10^d	2.397^a	0.1510^c	16.1^c	$0.98^{a,b}$
C	30.99^e	2.425^a	0.1244^a	19.5^a	0.96^b

[1]Means with no superscripts in common differ at $P < 0.05$. Units for values are as follows: A (estimated mature weight) in grams, b (shape parameter) is unitless, k (intrinsic growth rate) is natural log of growth rate per day, t' (age of maximum growth rate) in days, and mgr in grams/day.

However, E^- mice also experienced a compensatory growth period (i.e., directionally opposite in relation to response in the selected character) in the constrained interval. This compensatory phase began near day 30, reaching maximum divergence near day 40 (38.7d ± 0.4 for males, 39.5d ± 0.5 in females), after which growth rates between E^- and C appear to have converged throughout the late selection period. Differences observed between L^+ and C males reflected growth rate diverging near day 20 and peaking in the late selection period (at age 34.0d ± 0.3 in males and 31.6d ± 0.5 in females). A small compensatory response was apparent during the early selection period (maximum divergence at age 12.3d ± 0.3 in males and 10.6d ± 0.4 in females). The difference equation between L^- and C also shows two responses in growth rate, one during the early selection period (reaching maximum divergence at age 12.7d ± 0.3 in males and 9.7d ± 0.4 in females), and the sec-

TABLE 6. Analysis of variance for tail length, and tail length gain from age 10 days to 8 weeks of age in mouse selection lines (adjusting for body weight covariance)[1]

		Age (days)					
Source	d.f.	10	28	35	42	49	56
Line	4		***	***	***	***	***
Rep(line)	10	***	***	***	***	***	***
Sex	1		***	***			
Line × Sex	4		***	*	***	**	**
Rep × Sex	10			*		***	***
Litter size[2]	1						
Body weight[3]	1	***	***	***	***	***	***

[1]Empty cells represent values not significant at $P < 0.05$ level.
[2]Litter size at birth covariate.
[3]Age-specific body weight covariate.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

TABLE 7. Tail length growth curve traits means and least significant differences[1]

Line	Least squares means			Age of maximum growth rate (t')	*mgr*
	A	*b*	*k*		
E^+	89.74[a]	1.381[a]	0.1087[a]	12.8[a]	2.43[a]
E^-	84.89[b]	1.418[a]	0.0941[b]	15.2[c]	2.00[b]
L^+	87.13[b,c]	1.350[a]	0.0960[b,c]	14.2[b]	2.09[b]
L^-	83.93[b]	1.402[a]	0.1133[a]	12.5[a]	2.37[a]
C	88.62[a,c]	1.450[a]	0.1035[a,c]	14.1[b]	2.29[a]

[1]Means with no superscripts in common differ at $P < 0.05$. Units for values are as follows: *A* (estimated mature tail length) in mm, *b* (shape parameter) is unitless, *k* (intrinsic growth rate) is natural log of growth rate per day, t' (age of maximum growth rate) in days, and *mgr* in mm/day.

ond, with a more pronounced response peaking near day 30 (30.0d ± 0.3 in males and 27.8 ± 0.4 females). Similarities in growth curve parameters between E^+ and L^- lines and E^- and L^+ can be seen in cases where compensatory response was observed either during intervals of restricted selection on growth rate or during the day 10 to day 28 interval of unconstrained growth. However, compensatory response observed in late selected lines does not appear to peak at the same age as primary response in their early counterpart. In both cases, response reaches maximum divergence for late selection lines a few days prior to early selection lines (+4.9 days for E^+ vs. L^-, and +3.3 days for E^- vs. L^+).

Growth curves response across generations

The appearance of patterns of compensatory growth, especially during constrained selection intervals (e.g., Fig. 1), prompts consideration of when and how such response originated. While extensive longitudinal data was not collected prior to generation 19, we can still use the same logistic model and estimate population growth curves by generation and line. Such results are not anticipated to be identical to analyses using either individual growth curves or a larger array of measurements. However, within-line comparison of generation 19 population and average individual growth curves revealed a close similarity in results (data not shown). By contrasting growth rates for selection lines growth with those from the control, we adjust for effects of inbreeding depression and average seasonal effects on growth rates. This permits us to observe evolutionary trends (Fig. 4a–d).

TABLE 8. Means and least significant differences for significant within line effects[1]

Line	Rep	Least square means:			Age of maximum growth rate (t')
		A	*b*	*k*	
E^+	1	34.00[a]	2.440[a]	0.1260[a]	19.4[a]
	2	34.35[a]	2.448[a]	0.1268[a]	19.4[a]
	3	35.26[a]	2.413[a]	0.1256[a]	19.3[a]
E^-	1	28.59[a]	2.541[a]	0.1128[a]	22.8[a]
	2	29.38[a]	2.706[a]	0.1126[a]	24.2[a]
	3	27.25[a]	2.590[a]	0.1100[a]	23.6[a]
L^+	1	36.10[a]	2.576[a]	0.1122[a]	23.0[a]
	2	33.14[b]	2.684[a]	0.1177[a]	23.2[a]
	3	38.09[c]	2.594[a]	0.1108[a]	23.4[a]
L^-	1	26.47[a]	2.455[a]	0.1604	15.6[a]
	2	26.88[a]	2.329[a]	0.1447[a]	16.0[a]
	3	24.58	2.425[a]	0.1489[a]	16.5[a]
C	1	30.25[a]	2.348[a]	0.1250[a]	18.7[a]
	2	30.12[a]	2.518[b]	0.1247[a]	20.1[a]
	3	32.69	2.434[a,b]	0.1233[a]	19.8[a]

[1]Replicate means within each line with no superscripts in common differ significantly ($P < 0.05$).

Estimates of genetic parameters used in this experiment were initially derived from a separate population of ICR mice (Riska et al., '85). The general lack of response to selection in all selection lines through generation four indicates that genetic parameter estimates from one ICR population did not accurately predict the genetic parameters of the same strain of outbred mice obtained from another source. Selection indices were recalculated at generation four and maintained through generation 19, during which time divergence in growth trajectories is shown to fluctuate in each of the lines. Response in selected characters, estimated by regressing response by line on generation, has been previously shown to be significant in all selection lines, while response in constrained characters through generation 14 has been shown to be either not statistically significant or trivial (Atchley et al., '97).

In comparing selection lines with the control, divergence in E^+ growth rate was first observed during generation 5, disappearing in generation 7 and recurring in generations 9–14 (Fig. 4a). In the following five generations, the E^+ line was relatively free of response in the constrained late interval while remaining lines exhibit various degrees of response during constrained intervals. The E^+ line is also unique in that deviation from the control during the selection interval is between a half to a third what is observed in other lines. The E^- line showed similar patterns of lack of response through generation four, with divergence occurring after genetic parameters were re-estimated in generation 5 (Fig. 4b). In generation 5 and 6, growth rates in the 28 to 56 day interval exceeded those of the control, disappearing until generation 10, then reappearing and persisting until generation 19.

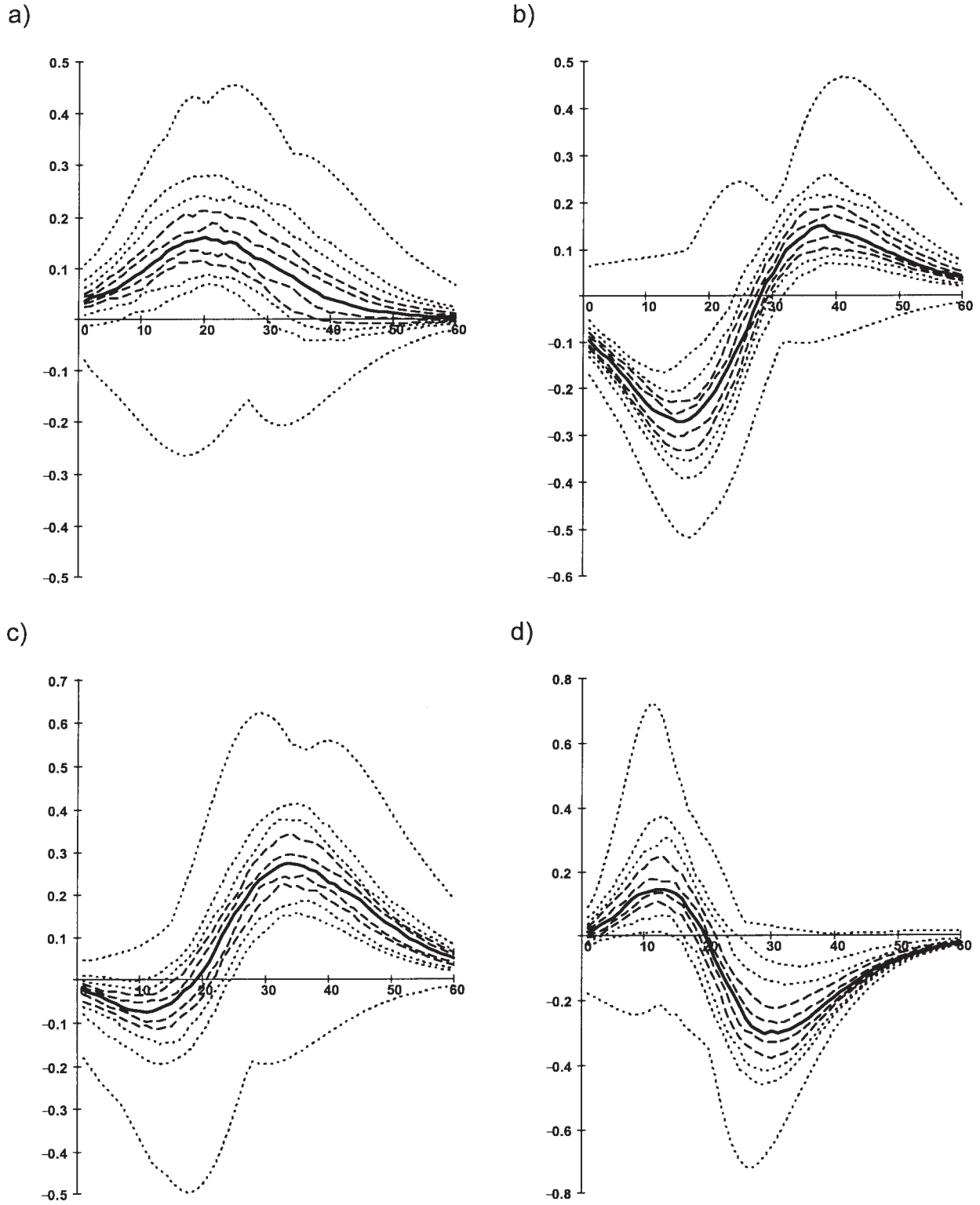

Fig. 3. Distribution of growth curves for selection lines relative to the control (males only) from birth to 56 days: (**a**) E^+ vs. C, (**b**) E^- vs. C, (**c**) L^+ vs. C, (**d**) L^- vs. C. In each figure, bands represent 10% increments in the distribution of individual growth curves (i.e., all growth curves fall between the two outermost lines, 80% of growth curves fall between the next two interior bands, etc.) with the heavy center band indicating the median.

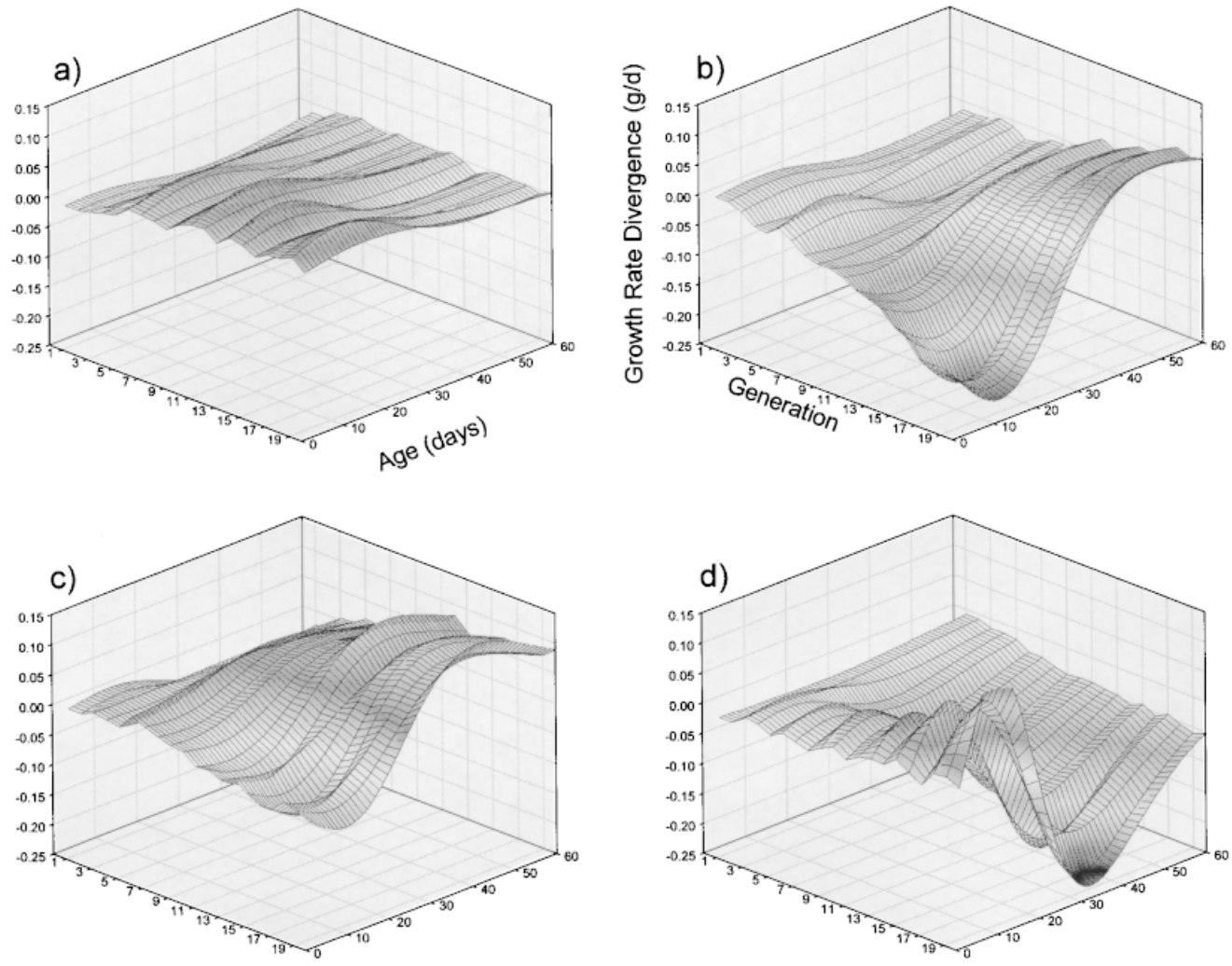

Fig. 4. Effects of long-term restricted index selection on growth rate across generations. Surface plot represent estimated growth rates for selection lines against control line growth rates from birth to 56 days and from generation 1 to 19. Scaling is fixed across lines: (**a**) E^+ vs. C, (**b**) E^- vs. C, (**c**) L^+ vs. C, (**d**) L^- vs. C

The late selection lines, L^+ and L^- have markedly different profiles (Fig. 4c,d). Departure in growth rates during the late interval occurred as early as generation 5 while slight departures from the control during the constrained early interval appeared near generation 8 in both lines, growing through generation 19. In the L^- line, response during the early-constrained interval was particularly extreme, being more than half as large in magnitude as the departure during the late selection interval, though it nearly disappears in generations 13 and 16.

DISCUSSION

Our purpose in fitting individual growth curves was to characterize growth trajectories of mice selected for differential rate of development. Therefore, we have used analyses of age-specific traits to demonstrate periods of statistically divergent growth, and growth curves to reveal patterns in the shape of selection response. Summarized briefly: (1) the selection lines have unique overall growth trajectories while retaining similarities in shape; (2) response during selected growth intervals was accompanied by a "compensatory" response during restricted intervals in E^-, L^+, and L^- lines; (3) correlated response was also seen in tail lengths, even after adjusting for age-specific effects of body weight; (4) selection resulted in divergence in age and rate of maximum growth among selection lines; and (5) within-line variation for age-specific traits

was significant, but its effect on growth trajectories was either small or non-significant.

One interesting aspect of this experiment was the convergence of body weight between up and down selected lines during the late selection interval—i.e., E^+ vs. L^+ and E^- vs. L^-. In other words, selection lines achieve identical phenotypes by different underlying growth mechanisms. This phenomena has been termed "developmental homoplasy" (Atchley et al., '97). Resolving which causal factors are responsible for these divergent trajectories will ultimately require a partitioning of response at the genetic and cellular levels. For instance, a portion of this divergence may be attributed to indirect genetic differences such as maternal effects (which can arise through genetic correlation between selected and maternal traits in offspring). The developmental quantitative model predicts that response may also be partitioned into differences in cell size and number.

Evolution of growth trajectories

This restricted index selection protocol has resulted in substantial change in the shape of individual growth curves. Response in shape parameters differs both in degree and direction from that observed in single trait selection experiments (Timon and Eisen, '69). While single-trait selection may dramatically alter rate of gain, it generally does not alter the basic shape of the growth curve (Malik, '84). In addition we found significant change in both shape parameters, which has not been observed in previous restricted index experiments in mice (McCarthy and Baker, '79).

Selection response in growth trajectories of E^-, L^+, and L^- lines was seen during intervals where selection response is constrained by the restricted index selection model. In the E^- line, divergence from the control during the 28 to 56 day restricted interval appears large, though not of the same magnitude as the response viewed in the early unconstrained selection interval. Accumulation of such response appears to have occurred gradually over the entire selection experiment. Since the genetic parameter structure was not re-estimated after generation 5, observed differences between selection lines and the control during constrained intervals may represent biased estimates of genetic covariance from generation 5 or gradual change in the covariance structure between early and late gain in subsequent generations. Because accumulated response in constrained characters does not appear until after generations 12–14, any bias in the original estimate of trait covariance should be slight.

Restricted index selection has been shown to be effective in reducing response in restricted (or constrained) characters, but there are few examples where predicted responses for either restricted or selected characters are achieved (e.g., Garwood and Lowe, '78; Matzinger et al., '89). Response in restricted characters is usually significant while response in unconstrained characters is often below expectation (Okada and Hardin, '67; Scheinberg et al., '67; Campo and Villanueva, '87). Such results are often attributed to initially biased parameter estimates or accumulated change in genetic parameters (i.e., additive genetic covariance) due to selection and genetic drift (e.g., Abplanalp et al., '63; McCarthy and Doolittle, '77; and Eisen, '92). Relatively few restricted index selection experiments have been performed in rodents but in these studies response in constrained characters is common.

However, estimate bias does not account for the consistently compensatory direction of response during intervals of restricted growth observed in the current study. It is likewise interesting that E^-, L^+, and L^- selection lines did not exhibit compensatory response in earlier generations where response in the selected interval was of the same magnitude as is currently observed in the E^+ line.

It is worthwhile to consider the potential consequences of architectural constraints on the evolution of growth trajectories. Using ICR mouse growth data from Riska et al. ('84), Kirkpatrick and Lofsvold ('89) decomposed the genetic covariance function of the population. Results showed two eigenfunctions that accounted for over 90% of additive genetic variation. The first eigenfunction was associated with genes which simultaneously increased or decreased size at all ages (e.g., growth hormones that exhibit systemic and positively correlated effects). The second eigenfunction showed a negative correlation between early and late growth that increased in magnitude proportionally with age difference. These observations indicate that the majority of additive genetic variation in random-bred mice is potentially constrained to response patterns undesirable in our restricted index selection program. Such findings are in agreement with other studies that have examined the underlying structure of genetic covariation between age-specific growth traits (e.g., Leamy and Cheverud, '84).

The explicit purpose of the restricted index selection program was to alter cell number by selecting

on early growth rate (0–10 days) and cell size by selecting on late growth rate (28–56 days). Indeed, response in growth curves indicates that in E^+ mice increased body size is predominately due to increased growth rate prior to day 28 (Fig. 3a). Therefore, E^+ mice are anticipated to have an average increase in cell number without change in cell size. However, E^-, L^+, and L^- lines all experienced some compensatory response both during constrained growth intervals and the unselected interval (10–28 days) (Fig. 3b–d). We might therefore anticipate that a decrease in cell number in E^- mice would also be accompanied by some increase in cell size. Similarly, increased cell size in L^+ mice may be accompanied by some decrease in cell number. Response in cell size and cell number may well be more complex for these lines.

Indeed, brain cell counts showed that E^+ mice have increased numbers of brain and liver cells relative to E^- mice at day 28, without any significant difference in cell size (Atchley et al., 2000). Moreover, these differences persist until 70 days of age. In similar fashion, L^+ mice were observed to have fewer brain cells at 28 days of age than all other lines except E^-. While E^- mice were not found to have increased cell size, cell size differences were generally not as pronounced as cell number differences. In conclusion, the compensatory growth phases observed in this study appear to explain the relative order of cell numbers at 28 and 70 days.

Correlation between t′ and reproduction onset

We may also consider whether or not change in age of maximum growth rate is correlated with change in onset of reproductive maturity. The inflection point in a growth curve represents the age (t') at which absolute growth is maximum, and has been associated with sexual maturation (Monteiro and Falconer, '66; Eisen et al., '69; and Kachman et al., '88). If the association between t' and reproductive onset is valid, change in timing of the inflection point between lines should be reflected in altered onset of puberty.

Differences in reproductive onset traits have been observed in these selection lines. Mean ages of vaginal opening for selection lines obtained for generation 23 show that vaginal opening is delayed in E^- lines, relative to L^- (Ernst et al., '99). First vaginal cornification, which is an accurate estimate of the onset of puberty, was also shown to be accelerated in the E^+ line relative to remaining lines (with L^- reaching vaginal cornification last). However, these differences are not characteristic of differences in t' from generation 19. Maximum growth rate was reached first by L^- females and last by E^- and L^+. Reproductive onset in female mice, therefore, does not appear to be correlated with age of maximum growth rate in these particular selection lines.

Response in maximum growth rate

Divergence in age of maximum weight gain (t') was accompanied by change in maximum growth rate (*mgr*). Of the three selection lines, only L^- failed to alter *mgr* relative to the control. Selection on early growth rate produced the largest changes in *mgr*, with E^+ selection resulting in a 12% increase and E^- selection resulting in an 18% decrease. L^+ selection similarly resulted in an increase *mgr*, though this estimate was not significantly greater than *mgr* for L^- mice. Therefore, alteration of maximum growth rate appears to be most closely correlated to response in early growth traits. Kachman et al. ('88) report that for ICR mice, estimated mature weight and maximum growth rate are moderately correlated (0.43 for *mgr* estimated assuming homogeneous variance). Therefore, selecting for either mature weight or maximum growth rate may result in correlated response in body weight at all ages. Results from the present study indicate that it is possible to obtain response in mature weight without significantly altering maximum growth rate.

Convergence and divergence in mature weight and tail length

The substantial changes in adult weight in these mice are reflected in differences in asymptotic or estimated mature weight (A). However, the logistic model fixes asymptotic weight at twice the body weight obtained at t', while terminal adult weight in mice approaches three times mature body at t' (Timon and Eisen, '69). Terminal weight is achieved, however, only after 25 weeks of growth (Malik, '84), or well after the age of reproductive onset and maturity. Therefore, while estimated mature weight (A) is likely to be a downwardly biased estimate for the terminal adult phenotype, it represents the weight of sexually mature mice. Restricted index selection has produced lines with divergent mature weights. However, we note that during the late selection interval, both the body weight and tail length phenotypes of early selection lines are converged upon by their late selection counterparts—i.e., E^+ vs. L^+ and E^- vs. L^- obtain identical weights during late selection.

The absolute magnitude of body weight gained

or lost by these selection lines was smaller than that seen in single trait selection experiments for body weight gain. For example, in one experiment Timon and Eisen ('69) show selection for 3 to 6 week post weaning gain over only 9 generations results in somewhat larger difference in asymptotic weights (5 g increase, averaged across sexes for 3–6 week gain, vs. 3.5 g and 4.8 g for E^+ and L^+).

Within-line differentiation

Divergence between replicates was seen primarily in age-specific traits and to a lesser extent in growth curve traits. This indicates that underlying differences in growth patterns between replicates were either transient or that their overall effects on the shape of the growth trajectory were small. However, trait divergence between replicates results from random gene fixation (genetic drift) operating on genetic variation from all underlying developmental pathways. Therefore, response in populations subjected to the same selection index may result from fixation of genetic variation in different developmental systems. Homogeneous growth curves between within-line replicates does not, therefore, indicate that replicates have achieved response via the same developmental mechanisms (e.g., Rutledge et al., '74).

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