

Effect of selection for development rate on reproductive onset in female mice

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Summary

This research reports analyses of correlated response in reproductive onset in ICR mice after 23 generations of restricted index selection for divergent body weight gain, early (birth–10 days) or later (28–56 days) in life. Long-term selection altered growth trajectories and 56 day body weight of individuals under different selection regimes in this study. Mice in lines under early selection have the same percentage mature weight at vaginal opening as controls (63%). Vaginal opening is delayed in mice selected for slow early growth, which take longer to reach what appears to be a weight threshold. In contrast, individuals in lines selected for later slow growth undergo vaginal opening at the same age as controls, but at a lower weight and increased percentage mature weight. Pre-compensation or ‘counter-balance growth’ is observed in these lines, with mice selected for late enhanced growth reaching 52% of mature weight at vaginal opening while mice with late slow growth attain 71% of mature weight prior to vaginal opening. Only 42% of mice with late slow growth attain first oestrus by 56 days. We speculate this is a function of growth rate and fat/lean ratio. Mice with early slow growth show compensatory growth, reaching first oestrus at a similar time to controls. We conclude that selection for growth rate has asymmetrically affected reproductive onset, with lines selected for suppressed gains experiencing delays in the reproductive onset traits measured.

1. Introduction

Long-term selection for growth rate at different developmental periods during ontogeny influences many aspects of later development. Development is regulated by a hierarchical cascade of genetic and biochemical processes. Therefore, a complex trait such as growth rate may be controlled by different genes and gene products at different stages of development (Atchley & Hall, 1991; Atchley *et al.*, 1991, 1994; Cowley & Atchley, 1992; Cheverud *et al.*, 1996; Atchley & Zhu, 1997). Consequently, selection on the components of such a complex trait at different intervals during ontogeny is expected to have manifold effects. Genetic selection may be applied at different stages of development using a restricted index selection strategy (Walsh & Lynch, 1999). Under a developmental quantitative genetic model, genetic selection

could have differential effects on different sets of genes regulating the trait being selected, as well as correlated traits through pleiotropic gene effects.

One important set of traits potentially correlated with rate of development in body weight are those involving reproductive maturity in females. Previous work suggests that body weight significantly influences onset of puberty in female mice (Monteiro & Falconer, 1966). There is extensive evidence that management factors such as feed intake (Kennedy & Mitra, 1963; Vandenberg *et al.*, 1972; Hansen *et al.*, 1983), population density (Champlin *et al.*, 1973) and exposure to pheromones (Vandenberg *et al.*, 1972; Vandenberg, 1973; Drickamer, 1988; Jemiolo & Novotny, 1993) also influence onset of puberty in mice. Effects of age-specific selection on age and weight at reproductive maturity are well documented (Crane *et al.*, 1972; Bakker *et al.*, 1977; Barria & Bradford, 1981; Falconer, 1984). However, there is no consensus of opinion on effects of age and weight on reproductive maturity in animals undergoing selection

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for growth, probably due to differences in the developmental stage when selection was applied. Individuals undergoing selection early in postnatal ontogeny may develop differently from those undergoing selection later in life. Research into correlated reproductive responses in individuals under restricted index selection regimes is lacking.

The definition of onset of reproductive maturity in mice has changed over time. Initially, vaginal opening was believed to be a sufficient estimate of reproductive maturity in female mice (Crane *et al.*, 1972 and references therein; Falconer, 1984). However, the female mouse is not reproductively capable until first oestrus, and there may be substantial delay between vaginal opening and first oestrus (Cooper *et al.*, 1993). It may well be that various stages of reproductive onset are regulated by different genes (Nelson *et al.*, 1990). Mouse vaginal opening was recently identified as an apoptotic event under hormonal mediation restricted to suprabasal layers of vaginal epithelium over an extremely short period of time (Rodriguez *et al.*, 1997). First vaginal cornification, in contrast, results from increased sex hormone activity and development of negative feedback mechanisms along the hypothalamic–pituitary–gonadal axis (Rossmanith *et al.*, 1994). Therefore, vaginal opening and first oestrus measure different reproductive maturity landmarks (Vandenbergh *et al.*, 1972; Bakker *et al.*, 1977; Hansen *et al.*, 1983; Nelson *et al.*, 1990).

Herein, we report on the correlated response of these particular reproductive traits in mouse selection lines that have undergone 23 generations of restricted index selection for increased or decreased body weight gain. These populations were selected in a standardized environment for early (0–10 days) or late (28–56 days) rate of gain to test the null hypothesis that there is no correlated response in onset of puberty.

2. Materials and methods

(i) Husbandry

Female mice, from generation 23 of a continuing long-term restricted index selection experiment were studied for puberty onset. Five selection regimes were involved in this restricted index selection experiment: (1) E^+L^0 [E^+] was selected to increase early body weight gain (rate of change from birth to 10 days of age) holding late weight gain (28–56 days) constant; (2) E^-L^0 [E^-] was selected to decrease early gain holding late gain constant; (3) E^0L^+ [L^+] was selected to increase late gain holding early gain constant; (4) E^0L^- [L^-] was selected to decrease late gain holding early gain constant; and (5) E^0L^0 [C] was a randomly selected control line. Each line was replicated three

times resulting in 15 genetically independent lines. Selection for subsequent generations occurs within family, with a planned selection intensity of 25% for both sexes. A detailed description of these lines and the direct response to selection up to generation 15 can be found in Atchley *et al.* (1997).

Females from two randomly chosen litters from each line/replicate combination were averaged in the present study. One hundred and one females from generation 23 were studied in January and February 1996, for age and body weight at vaginal opening (VO) and first vaginal cornification (FVC).

Females were reared in litters standardized at birth to eight pups and an even sex ratio. Body weights were recorded at birth and 10 days of age. At 21 days of age, mice were weaned, separated by sex, and body weights recorded. Females were caged by litter and a density of four mice per cage was maintained. Cages containing males were interspersed between cages of females throughout the duration of the experiment to remove male pheromonal bias. Each female was examined for reproductive maturity, as measured by (1) VO and (2) FVC.

Beginning at weaning (21 days), females were inspected daily between 0800 hours and 1000 hours for VO. Age and body weight were recorded at VO. After the appearance of an open vaginal orifice, a wet-mount vaginal lavage was taken daily between 0800 hours and 1000 hours until first oestrus was achieved. Vaginal lavage (Vandenbergh, 1967; Cooper *et al.*, 1993) was used to determine FVC.

Cell types were recorded from daily vaginal smears as relative numbers of leucocytes, polynucleated epithelial cells and cornified epithelial cells. The first day an entire cornified epithelial cell smear was observed was recorded as FVC. Age and body weight were recorded at this time. Females were examined for FVC from VO until 56 days of age, at which time body weight was recorded. Fifty-six days was considered to be mature age and weight for purposes of transformation. Individuals that had not reached FVC were lavaged and inspected for an additional week.

(ii) Statistical analysis

Traits investigated were weaning weight (WNWT), age (VOA) and body weight (VOBW) at vaginal opening, as well as age (FCA) and body weight (FCBW) at first vaginal cornification. Time between VO and FVC (VOFCT), body weight gain between FVC and VO (VOFCWG), as well as average daily gains VOFCADG, were recorded.

Body weight was not recorded past 56 days of age in this study (the terminal weight in the continuing selection experiment). Mice from the various selection lines have reached asymptotic growth by 56 days of

age; consequently 56 days is a valid estimate of mature weight (Rhees & Atchley, unpublished data). Thus, for simplicity, 56 day weight is considered as the 'mature weight' for the sake of discussion in this study. To analyse growth across divergent lines, weight and age at each reproductive event were transformed to percentage of maturity values as follows:

Percentage mature weight = weight at event/56 day weight,

Percentage mature age = age at event/56 days.

Therefore, all animals at the same percentage 'mature age' are also the same age. However, animals at the same percentage mature weight do not have the same body weight.

Data were analysed by analysis of variance and regression using a fixed effects linear model as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \epsilon_{ijk},$$

where Y_{ijk} is the trait value of the k th individual in the j th replicate nested in the i th selection line, μ is the trait mean, α_i is the fixed effect of the i th selection line, $\beta_{j(i)}$ is the j th replicate nested within the i th selection line, and ϵ_{ijk} is the residual.

Statistical analysis was carried out using the SAS GLM procedure (SAS Institute, 1989). Preliminary statistical analysis showed that litter effects were not significant; therefore, litter component was not included in this model. Pairwise product-moment correlations among reproductive and growth traits were computed to describe the correlated response of reproduction to selection for growth rate.

The matrices of pairwise correlations were analysed using principal factor analysis that permits description of simultaneous covariability among several traits. Principal factor analysis was carried out using Varimax (orthogonal) rotation and the SAS FACTOR procedure (SAS Institute, 1989) on the six reproductive traits (VOA, VOBW, FCA, FCBW, VOFCT and VOFCADG).

Three eigenvectors were extracted from the correlation matrix for each selection line and the congruence between pairs of eigenvectors \mathbf{e}_i and \mathbf{e}_j was calculated as:

$$\cos \theta = \mathbf{e}_i^T \mathbf{e}_j / (\mathbf{e}_i^T \mathbf{e}_i \mathbf{e}_j^T \mathbf{e}_j)^{1/2},$$

where T denotes a matrix transpose, and \mathbf{e}_i and \mathbf{e}_j are the i th and j th eigenvectors (Rummel, 1970).

3. Results

(i) Direct response

Means for body weight, pooled by line, reproductive measurements and mature equivalents are presented

in Table 1. This information describes the ontogenetic changes in these measurements and summarizes the consequences of selection on rate of development in body weight between birth and 56 days of age.

At 56 days of age E^+ and L^+ line mice have converged to an average body weight of 31.5 g. E^- individuals have undergone compensatory growth between 10 and 56 days, and have converged with C mice at 25.5 g. L^- responded to late selection by nearly ceasing growth during the late selection period (28–56 days).

(ii) Correlated response in sexual maturity

Several different reproductive trends are apparent from these data. For example, only 42% of individuals measured from L^- attain oestrus (FVC). Body weight of L^- individuals at 6.5–8 weeks of age is similar to that of other lines at 3–4 weeks of age. Percentage of mature weight at reproductive landmarks is presented to assist in comparing these divergent lines. Detailed interpretation of growth patterns in L^- mice will be provided later.

While litter effects were not significant, some litters were observed to have outliers. The large standard deviations observed at FVC for E^+ and C are due to some mice maturing later than others in their cohort do. In line E^+ , 16 of 20 individuals reached FVC by 40 days. Three E^+ individuals (two were full-sibs) reached FVC after 60 days. Similar results occurred in the C line, where five individuals (four were full-sibs) attained FVC after 60 days. The large standard deviation in line L^- may be attributed to few observed FVC in this line. Individuals in L^- that failed to attain FVC had an average 56 day body weight of 18.6 g, which is not significantly different from the average FVC weight of 17.5 g for L^- individuals that attained FVC.

Analysis of variance

Least squares means for reproductive maturity intervals are presented in Table 2. Note that only eight individuals from L^- successfully attained FVC. Vaginal opening occurs at or near weaning, independent of selection line. E^+ individuals were significantly heavier than all other lines at VO (mean 19.2 g). The mean for L^+ mice that had not undergone selection before VO was 16.3 g, and not significantly different from the mean for C (16.2 g). Lines E^- and L^- were lightest at VO with means of 15.2 and 13.7 g, respectively. These lines, selected for decreased growth rate, were significantly lighter than lines selected for enhanced growth and controls. Furthermore, L^- was significantly lighter than E^- at VO in spite of selection being applied only to E^- before VO.

Table 1. Means (standard deviations) pooled within line, for age in days, weight in grams for longitudinal measurements at vaginal opening, first vaginal cornification and unsuccessful cornification; percentages of mature values are included for reproductive traits

Line	E ⁺	E ⁻	C	L ⁺	L ⁻
<i>Longitudinal growth</i>					
<i>n</i>	20	20	22	20	19
Birth weight (g)	1.88 (0.10)	1.50 (0.17)	1.50 (0.11)	1.51 (0.08)	1.66 (0.14)
10 day weight (g)	9.05 (0.78)	6.52 (1.12)	7.89 (0.67)	7.81 (0.55)	6.81 (1.25)
21 day weight (g)	17.10 (1.25)	12.27 (2.24)	15.36 (0.99)	14.50 (1.62)	13.45 (2.45)
28 day weight (g)	21.18 (1.57)	16.68 (2.81)	18.91 (1.10)	19.96 (2.07)	16.28 (2.30)
56 day weight (g)	31.37 (2.83)	25.52 (3.92)	25.79 (2.23)	31.72 (3.24)	19.27 (2.05)
<i>Vaginal opening</i>					
<i>n</i>	20	20	22	20	19
Age (days)	23.75 (1.80)	27.35 (4.50)	23.36 (2.48)	23.95 (2.58)	22.42 (1.22)
Weight (g)	19.17 (1.48)	15.39 (1.66)	16.16 (1.42)	16.42 (2.13)	13.68 (2.10)
Mature weight (%)	61.40 (5.46)	61.06 (8.07)	62.90 (6.03)	51.92 (6.27)	71.57 (12.85)
<i>First vaginal cornification</i>					
<i>n</i>	19	19	21	20	8
Age (days)	35.32 (12.95)	40.37 (8.58)	45.81 (12.50)	38.75 (10.25)	46.00 (17.35)
Weight (g)	23.43 (3.02)	21.08 (1.63)	22.80 (3.19)	24.28 (3.08)	17.55 (2.35)
Mature weight (%)	74.66 (13.34)	83.10 (9.71)	87.90 (11.22)	77.37 (13.03)	87.85 (14.80)
<i>Unsuccessful vaginal cornification</i>					
<i>n</i>	1	1	1	0	11
Weight (g)	28.04	21.57	21.70	N/A	18.62 (2.04)

Table 2. Least squares means for all measured, calculated and transformed traits, pooled across lines

Trait	E ⁺ (n = 20)	E ⁻ (n = 20)	C (n = 22)	L ⁺ (n = 20)	L ⁻ (n = 19*) ^b	LSD ^a
<i>Vaginal opening</i>						
Age (days)	23.79	26.59	23.44	23.91	22.38	<u>L⁻</u> <u>C</u> <u>E⁺</u> <u>L⁺</u> <u>E⁻</u>
Body weight (g)	19.18	15.16	16.20	16.31	13.69	<u>L⁻</u> <u>E⁻</u> <u>C</u> <u>L⁺</u> <u>E⁺</u>
<i>First vaginal cornification</i>						
Age (days)	35.65	41.47	45.43	39.18	48.14	<u>E⁺</u> <u>L⁺</u> <u>E⁻</u> <u>C</u> <u>L⁻</u>
Body weight (g)	23.52	21.36	22.65	24.34	17.95	<u>L⁻</u> <u>E⁻</u> <u>C</u> <u>E⁺</u> <u>L⁺</u>
Weaning weight (g)	17.07	12.42	15.37	14.43	13.47	<u>E⁻</u> <u>L⁻</u> <u>L⁺</u> <u>C</u> <u>E⁺</u>
<i>Vaginal opening to first vaginal cornification</i>						
Time (days)	11.84	14.74	21.97	15.28	25.79	<u>E⁺</u> <u>E⁻</u> <u>L⁺</u> <u>C</u> <u>L⁻</u>
Body weight gain (g)	4.25	6.11	6.39	8.03	4.06	<u>L⁻</u> <u>E⁺</u> <u>E⁻</u> <u>C</u> <u>L⁺</u>
Average daily gain (g/day)	0.41	0.52	0.31	0.61	0.18	<u>L⁻</u> <u>C</u> <u>E⁺</u> <u>E⁻</u> <u>L⁺</u>
<i>Percentage mature weight</i>						
Vaginal opening (%)	61.30	60.35	63.07	51.73	71.41	<u>L⁺</u> <u>E⁻</u> <u>E⁺</u> <u>C</u> <u>L⁻</u>
First vaginal cornification (%)	75.35	84.53	87.21	77.81	91.01	<u>E⁺</u> <u>L⁺</u> <u>E⁻</u> <u>C</u> <u>L⁻</u>

^a Least significant difference: for each trait, underscored values identify means not significantly different ($p > 0.05$).

^b Only 8 individuals attained first vaginal cornification for L⁻.

FVC occurred well after the early selection interval had ended (10 days) and during the late selection phase (28–56 days). E⁺ and L⁺, selected for increased growth, were heaviest at FVC (23.5 g and 24.3 g, respectively), and did not differ significantly from each other; however, L⁺ was significantly heavier than C (22.7 g). E⁻ and L⁻ weighed the least at FVC and differed significantly from each other (21.4 g and

17.9 g, respectively). L⁻ was also significantly lighter than C. Furthermore, individuals that experienced divergent selection for growth from 0 to 10 days experienced FVC at weights similar to controls, but significantly different from each other. Individuals in lines undergoing selection at FVC (28–56 days) differed significantly from each other and from the controls with respect to weight.

Table 3. Comparison of means (standard deviations) of body weight (g) and average daily gain (g) across late selection interval (28–56 days) in line L^- individuals that reach first vaginal cornification (FVC) versus those that do not

FVC achieved	n	Weight (g) at day					Average daily gain (g) from 28 to 56 days
		0	10	21	28	56	
Yes	8	1.62 (0.10)	6.02 (1.23)	12.57 (2.44)	15.24 (2.06)	20.17 (1.82)	0.18 (0.03)
No	11	1.69 (0.16)	7.38 (0.95)	14.08 (2.35)	17.04 (2.25)	18.62 (2.04)	0.06 (0.10)

In E^+ , E^- and L^+ mice the VO to FVC time interval was significantly shorter than in C. Lack of statistical significance between L^+ and L^- was probably an artifact due to lack of successful FVC in L^- . Calculating average daily gain across the interval revealed that E^- and L^+ were growing at similar rates (0.52 and 0.61 g/day, respectively). E^+ grew at a slightly slower rate, but experienced an early growth spurt probably due to selection. E^- experienced compensatory growth resulting in increased growth rate over the interval from VO to FVC. L^- individuals that successfully reached FVC grew slowest (0.18 g/day) across the same interval.

A more detailed evaluation of FVC in L^- mice is presented in Table 3. There is an interesting trend in individuals that reached FVC compared with those that did not. The two groups had similar birth weights, but at 10 days of age, individuals that failed to reach FVC were slightly larger than their successful counterparts. This gap widened to a standard deviation difference by 28 days, and at 56 days the successful FVC group outweighed the unsuccessful group by a standard deviation. Indeed, five of 11 individuals that failed to attain FVC lost weight from 28 to 56 days of age. Only three individuals that were unsuccessful at attaining FVC had average daily gains within a standard deviation of the successful group (0.01–0.20).

Data transformed to percentage mature age and weight are also presented in Table 2. Transformation of age data did not change the level of statistical significance with respect to untransformed data. Evaluation of VO as percentage mature age and mature weight reveals several trends. VO in E^+ , L^+ and L^- mice occurred at the same percentage mature age as controls (C), i.e. at approximately 42% of mature age. Individuals selected for divergent late growth (L^+ and L^-) differ significantly in percentage mature age at VO with respect to each other (43% and 40%, respectively). E^- individuals attain VO at a significantly later percentage mature age than all counterparts. However, percentage mature weight between E^- and E^+ or C does not differ significantly. Percentage mature weight at VO in late selected lines varies significantly with respect to each other, and the

other three lines. L^+ individuals attain 52% of mature weight, and L^- individuals have reached 71% of mature weight, at VO; while individuals in C, E^+ and E^- lines reach 63%, 61% and 60% of mature weight by VO, respectively.

When calculating FVC as a percentage of mature age, transformation of age data did not change statistical significance with respect to untransformed data. As expected, E^+ and L^+ are not significantly different in percentage mature age. Both are significantly younger and heavier than controls at FVC.

When calculating FVC with respect to percentage of mature weight, L^- individuals require a significantly larger percentage mature weight (91%) to attain FVC compared with their counterparts. Lack of significance between L^- and E^- or C line is most probably attributable to sampling artifact, as only those individuals that attained FVC (42% of L^- sample) contribute to this trait. This results in a two-fold increase in standard error for L^- compared with the other four lines. E^+ individuals reach FVC requiring the least percentage of mature weight (75%), while L^- individuals must reach 91% of mature weight before FVC. This observation suggests that body weight plays an integral role in FVC.

Regression analysis

Linear and quadratic regression analysis of VOBW on VOA as well as FCBW on FCA is presented in Fig. 1. Unlike the results of Falconer (1984), quadratic regressions did not significantly improve fit when compared with linear regression of reproductive trait weights on reproductive trait ages.

There are no significant differences between intercepts for selected lines versus controls, for any of the regression analyses presented. There are significant differences in slope between controls and L^- ($P < 0.05$) for VOBW regressed on VOA. There is also a significant difference in slope for VOBW regressed on VOA between late selected lines (L^+ and L^- ; $P < 0.001$). When calculating differences in slopes for both linear ($P < 0.02$) and quadratic ($P < 0.01$) regression of FCBW on FCA, significant differences occur in slopes between E^- and E^+ as well as C.

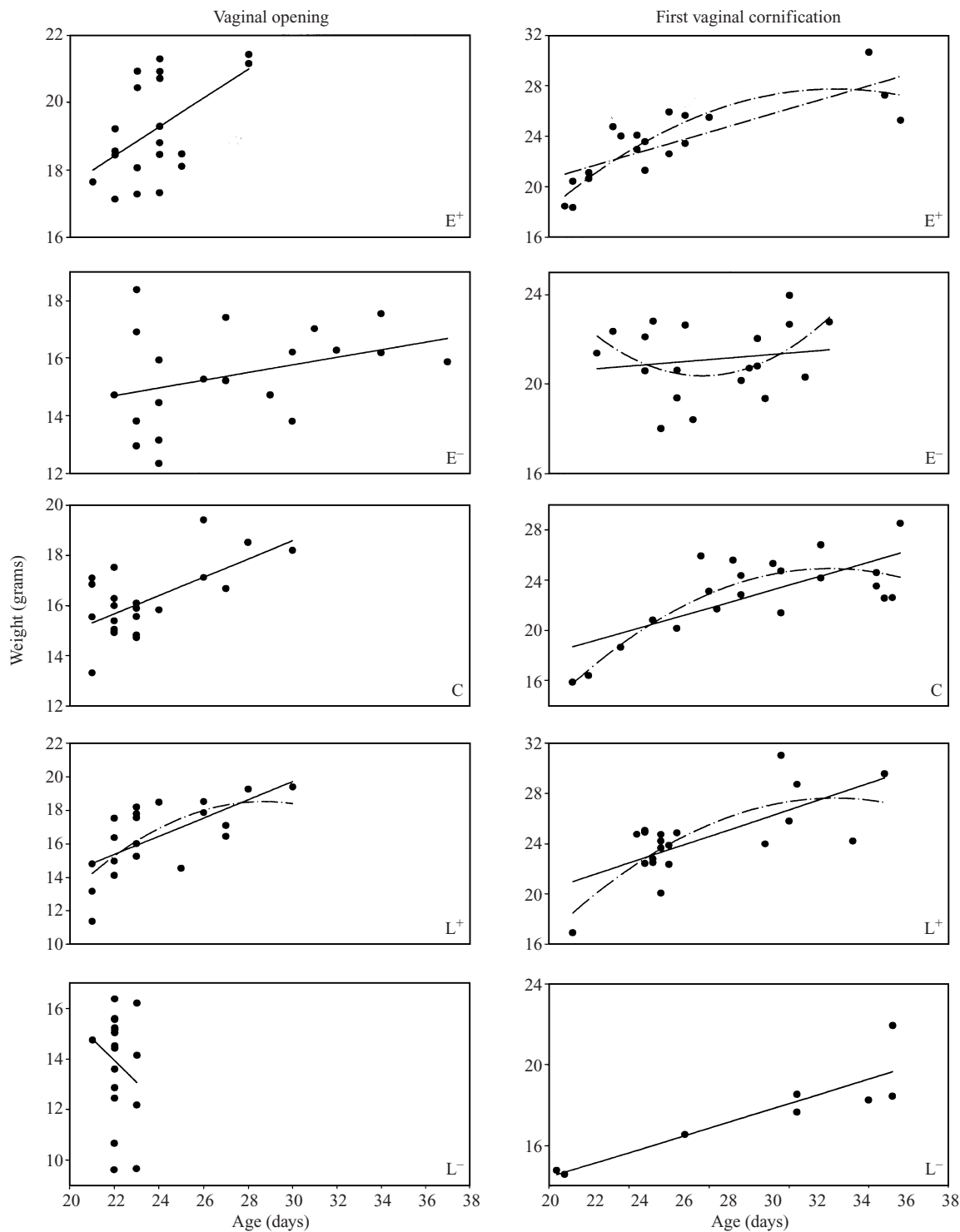


Fig. 1. Individual values of weight and age at vaginal opening and first vaginal cornification, by line.

Multivariate analysis

Principal factor analysis results permit a description of the simultaneous or multivariate patterns of covariation among the various reproductive traits within each selection line. These eigenvectors were orthogonally rotated to simple structure using the Varimax criterion (Rummel, 1970) to facilitate more

interpretable results, i.e. statistically independent vectors exhibiting either high or low eigenvector coefficients and few intermediate values. Eigenvector coefficients in factor analysis describe the multi-dimensional correlated response in these traits to selection for rate of development at different stages of postnatal ontogeny. The three patterns of covariation (eigenvectors) summarize the common information

Table 4. Varimax rotated principal factor components of phenotypic correlation matrices, by line

Trait	E ⁺			E ⁻			C			L ⁺			L ⁻		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
VOA*	0.418	0.811	0.114	0.482	-0.738	0.437	0.207	0.814	-0.293	0.033	0.909	-0.135	0.125	0.028	0.983
VOBW	-0.248	0.927	-0.017	-0.153	0.035	0.978	0.201	0.913	-0.004	-0.111	0.906	0.192	0.035	0.993	-0.017
FCA	0.985	-0.002	-0.128	0.993	0.022	-0.069	0.912	0.282	-0.247	-0.817	-0.064	-0.548	0.960	0.075	0.267
FCBW	0.838	0.284	0.305	0.147	0.959	0.163	0.856	0.230	0.357	0.967	0.082	0.090	0.744	0.447	-0.069
VOFCT	0.973	-0.128	-0.152	0.819	0.453	-0.330	0.962	0.130	-0.208	0.802	-0.291	-0.510	0.991	0.074	0.075
VOFCADG	-0.056	0.038	0.992	-0.959	0.214	-0.004	-0.081	-0.187	0.934	-0.131	-0.012	0.955	-0.546	-0.663	-0.152
Eigenvalue	2.859	1.616	1.130	2.854	1.717	1.288	2.580	1.680	1.190	2.276	1.743	1.536	2.772	1.637	1.071

Values in **bold** are indicative of high coefficient values.

* VOA, vaginal opening age; VOBW, vaginal opening body weight; FCA, first vaginal cornification age; FCBW, first vaginal cornification body weight; VOFCT, time from vaginal opening to first vaginal cornification; VOFCADG, average daily gain from vaginal opening to first vaginal cornification.

(= covariation) among these six reproductive traits. Traits with high factor coefficients represent high correlations between the traits and the particular statistically independent multivariate patterns of covariation. These high values are given in bold type in Table 4.

Lines selected for increased growth rate and the controls show very similar patterns of eigenvector coefficients for these three vectors, suggesting concordance in patterns of covariation in reproductive traits in each of these three lines. The first eigenvector has large and positive coefficients on age and weight at FVC together with the length of time between VO and FVC. Coefficients for the remaining traits are very small and contribute little to this axis of covariation. The second eigenvector has large and positive coefficients for age and weight at VO. Because these eigenvectors are orthogonal (statistically independent), these results suggest that variation in the age and weight at VO is independent of the age and weight of the mice at FVC.

The third orthogonal vector has a large coefficient for the rate of average daily weight gain between VO and FVC but no other traits. This indicates that this trait varies more or less independently of all the others for these three lines.

Coefficients of congruence between the eigenvectors in these three analyses indicate a high level of similarity (cosines between pairs of vectors) among E⁺, L⁺ and C. The first eigenvector has congruence coefficients greater than 0.95 among the three lines. Similar patterns are observed for the second eigenvector (coefficients > 0.90) and the third eigenvector (coefficients > 0.84). Thus, the patterns of covariation among the six reproductive traits are very similar between E⁺, L⁺ and C.

The correlations among reproductive traits in the lines selected for decreased growth rate (E⁻ and L⁻) differ from E⁺, L⁺ and C and from each other as well. For E⁻ mice, the first eigenvector has large positive coefficients for age at FVC, the time between VO and FVC, and a large negative coefficient rate of average daily gain between VO and FVC. Selection for decreased rate of development early in postnatal ontogeny has genetically decoupled age and weight at FVC in E⁻ mice. The product-moment correlation between age and weight at FVC in this experiment is 0.71 in C mice, 0.78 in E⁺ and 0.89 in L⁻. This correlation is only 0.15 in the E⁻ mice.

Eigenvector II represents an inverse relationship between age at VO and weight at FVC. Eigenvector III reflects positive covariation between age and weight at VO.

Thus, the E⁻ mice differ from the E⁺, L⁺ and C mice in two major patterns of covariation among reproductive traits, and there is a lower vector congruence between E⁻ mice and either E⁺ or C for eigenvectors

I and II. Eigenvector III in E⁻ mice shows high congruence (cosine > 0.92) with E⁺ and L⁺ and 0.83 with the controls.

For L⁻ mice, eigenvector I describes an inverse relationship between large and positive coefficients on age and weight at FVC together with the time between VO and FVC on the one hand, and a negative coefficient for average daily gain between VO and FVC. Eigenvector II describes an inverse relationship between average daily gain between VO and FVC and weight at VO. Eigenvector III has a high coefficient on VO age but no other variables suggesting that, in L⁻ mice, this trait is uncorrelated with the others. Examination of the individual pair-wise correlation coefficients of age at VO with all the other traits shows only a single significant correlation coefficient (VO and the time between VO and weaning weight).

4. Discussion

Mice in this study are the products of lines that have undergone 23 generations of restricted index selection. The results of this selection are lines very divergent for body weight. The early selected (E⁺ and E⁻) and late selected lines (L⁺ and L⁻) have diverged from each other as the result of selection. In contrast, lines selected for increased growth (E⁺ and L⁺) have achieved the same average body weight at 56 days. These results demonstrate that the same phenotype can be achieved by quite different developmental processes. This phenomenon has been termed 'developmental homoplasy' (Atchley & Zhu, 1997). Given the nature of the selection index restriction, it is anticipated that different genes are mediating development in early and late selected lines (Atchley & Zhu, 1997; Cheverud *et al.*, 1996).

Reproductive onset (puberty) is caused by a cascade of hormonally regulated events occurring in the hypothalamic–pituitary–gonadal axis (Rossmannith *et al.*, 1994). The present experiment tests the null hypothesis that 23 generations of restricted index selection for early or late postnatal divergent growth rate has had no significant effect on reproductive onset (defined as first vaginal cornification or complete cornification of vaginal epithelial cells). We reject this null hypothesis and present evidence that selection on growth rate has asymmetrically affected reproductive onset, with lines selected for decreased weight gain experiencing delays in puberty traits measured.

There are several theories regarding control of VO. There is a large body of evidence that VO is weight- rather than age-dependent in rodents (Kennedy & Mitra, 1963; Frisch *et al.*, 1975, 1977; Bakker *et al.*, 1977; Eisen, 1978; Drickamer, 1981; Falconer, 1984; Nelson *et al.*, 1990). Work by Drickamer (1981) demonstrated that age at VO was under genetic

control, was easily changed by selection and was not correlated with body weight at VO. Recognition that weight plays an integral role in VO led to the hypothesis that food intake and/or body composition, and not weight, mediates VO (Hansen *et al.*, 1983) and oestrus onset (Kennedy, 1969; Frisch *et al.*, 1975, 1977).

In this study, with the exception of E⁻, there was no difference in age at VO between selected and control lines, although weight did vary significantly, and in expected directions, for E⁺ and L⁻ lines. Furthermore, E⁻ mice, that experienced selection for decreased growth rate for their first 10 days, also experienced a significant delay in VO with respect to all other lines. Hansen *et al.* (1983) identified a similar VO delay in animals with restricted dietary intake, with VO occurring after dietary restrictions were removed. Similarly, E⁻ mice experience compensatory growth after early growth suppression.

These VO results may be due, in part, to the selection experiment protocol and postponing VO evaluation until weaning in these females. The selection experiment protocol provides selection on growth rate early or late in ontogeny. After 23 generations there are real differences in growth trajectories and mature body weight of individuals in lines exposed to the different selection regimes set out in this study. When expressing body weight as a percentage of mature weight, mice in lines under selection prior to VO have the same percentage mature weight as controls. E⁻ mice require longer to reach that threshold, resulting in delayed VO. Hence, mice selected for growth rate prior to or during VO demonstrate that VO is weight-dependent. These results are consistent with previous reports (Bakker *et al.*, 1977; Eisen, 1978; Drickamer, 1981; Falconer, 1984; Nelson *et al.*, 1990).

In contrast, individuals in lines that undergo selection later in ontogeny (i.e. L⁻ and L⁺) express VO at the same age as controls, but differ in body weight and percentage mature weight. Their early growth is inversely proportional to their later growth under selection. L⁻ individuals reach 71% of their mature weight by VO, while L⁺ individuals are at 52% of mature weight. Monteiro & Falconer (1966) define compensatory growth as changes in growth rate as a result of, or in compensation for, prior growth trends. In lines selected for late growth rate we observe a pre-compensation or 'counter-balance growth'. While age at VO is not significantly different between late selected lines and controls, there is a significant difference in age at VO between late selected lines. In terms of percentage mature weight, individuals with a lower percentage mature weight experience VO later. This result parallels results for E⁺ and E⁻ individuals.

First cornification of vaginal epithelial cells signals the onset of hormonal expression necessary to support

reproduction (Rossmann *et al.*, 1994). Our results show trends similar to those seen for VO. Lines L^+ and L^- , which are under selection during FVC onset, exhibit an onset pattern similar to that observed by Falconer (1984) for VO in animals under selection for divergent 6 week weight. Individuals selected for increased growth are heavier and younger at FVC, while individuals selected for decreased growth are lighter and older at FVC. It is interesting to note that the percentage mature weight required to reach FVC is inversely proportional to 56 day body weight. Animals with the greatest 56 day weight (E^+ and L^+) reach less than 80% of their mature body weight to undergo FVC, while L^- individuals must reach 91% of mature body weight to achieve the same outcome. This suggests weight and not age is essential for FVC, which is consistent with earlier findings in mice, rats and humans (Kennedy & Mitra, 1963; Frisch *et al.*, 1975, 1977; Drickamer, 1981; Frisch, 1980, 1990).

Body weight is similar in L^- mice that reach FVC compared with those that do not. This suggests that body composition (ratio of fat to lean mass) may play an integral role in puberty. Kennedy & Mitra (1963) and Frisch *et al.* (1975, 1977) demonstrated that a minimum ratio of fat to lean mass is required for puberty in rat and human. Normal human females of reproductive age maintain 26–28% body fat (Frisch, 1990 and references therein). Body fat has long been known as a source of extra oestrogen via aromatization of androgens to oestrogen (Nimrod & Ryan, 1975) and may affect oestrogen metabolism (Frisch, 1990). It will be interesting to compare body composition in L^- individuals that fail to attain FVC with those that are successful.

Generally, in long-term selection studies attention is paid only to changes in means of individual traits. However, long-term selection response is a multi-dimensional phenomenon in which a host of correlated traits respond differentially depending upon the time during ontogeny at which selection is focused. Multivariate analyses can facilitate understanding of simultaneous and differential response within and between selection lines. As a consequence, a better understanding of the genetic architecture of quantitative traits can be achieved by considering the effect of selection of the covariances among traits.

The principal factor analyses of intertrait correlations indicated that the early and late up-selected lines (E^+ , L^+) show patterns of covariances that are quite similar to the control (C). These patterns suggest that selection has not disrupted the covariances among these reproductive traits in these three selection treatments. The largest component of variability in E^+ , L^+ and C reflects covariation in age and body weight at FVC together with the time interval between VO and FVC. Similarly, the second orthogonal axis reflects covariation in age and body weight at VO

while the third independent axis reflects variation in average daily gain from VO to FVC.

On the other hand, multivariate analyses of the E^- and L^- lines indicate significant changes in the covariances over that seen in the control line. First, in E^- there is a significant positive relationship between age at FVC and the amount of time between VO and FVC. However, unlike the patterns seen in the control, up-selected (E^+ , L^+) and the late down-selected (L^-) lines, the E^- line shows no significant correlation between these two traits and body weight at FVC. Secondly, in both down-selected lines (E^- , L^-) there is a significant inverse relationship between these two traits and the average daily weight gain from VO to FVC. Thus, selection in the E^- mice has genetically decoupled age and weight at vaginal cornification. Further, there is a significant change between time interval and the amount of weight gain between these two reproductive traits in both down-selected lines.

These multivariate analyses indicate that the time during ontogeny at which selection occurred and its direction have significantly altered the covariances among these various reproductive traits. In some cases, selection has decoupled two traits (VOA, VOBW) in E^- and L^- , FCBW from FCA and VOFCT in E^- , and generated a significant association between VOFCT and VOFCADG in L^- . These results, which are important in understanding the developmental aspects of selection on quantitative traits, can be obscured by analyses of single traits.

Impact of long-term selection for decreased growth early or late in ontogeny (E^- or L^-) is detrimental to puberty onset, and potentially to reproductive performance. Individuals selected for decreased rate of early growth achieve VO later, but then undergo compensatory growth permitting them to achieve FVC at an age and weight similar to controls. Individuals selected for later decreased growth rates may not attain FVC and are therefore not reproductively viable. These results may be potentially applicable to miniature breeds of swine, horses and the like.

Results from L^- may have far reaching implications. This line may prove useful for investigating the effect of body composition on reproductive onset, for elucidating quantitative trait loci specific to reproduction, or as a model for studying primary amenorrhoea in humans.

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