# Uterine and Postnatal Maternal Effects in Mice Selected for Differential Rate of Early Development

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ABSTRACT

A series of mouse lines was produced by long-term restricted index selection for divergent rate of growth during early and late postnatal development. The selection program was based on the following treatments: $E^+$ and $E^-$ lines were selected to alter birth to 10-day weight gain while holding late gain for both lines constant and a control line was established via random selection. Using embryo transfer and crossfostering methodology, we partitioned postnatal growth for $E^+$, $E^-$, and C lines into progeny genetic, uterine maternal, and nurse maternal components. Selection for differential early growth resulted in correlated response in uterine and nurse maternal effects on body weights, with significant genetic-by-environment interactions. Significant uterine effects were also observed in tail length measurements. Direct uterine effects on body weight were relatively small and resulted in growth rate differences early in development. Nurse effects were large, resulting in modification of progeny growth trajectory especially during early postnatal development. Genetic-by-uterine interactions were large and demonstrate progeny-specific effects of the prenatal uterine environment.

EARLY development in all organisms is influenced by numerous forces extrinsic to their genome. While many such factors are directly due to environmental conditions, an important subset are controlled by the maternal genome. Though organism specific, these maternal/offspring interactions may include maternal mRNA transcripts, hormone secretions, antibodies, placental permeability, growth factors in milk, and maternal nesting behavior (reviewed by Rossiter 1996; Mousseau and Fox 1998). Of these, we define all heritable maternal contributions, beyond those of the nuclear genome, as maternal effects (Willham 1963; Falconer 1965).

Any additive genetic correlation that exists between maternal effect traits and offspring fitness will shape the expression and evolution of developmental processes. Genetic pleiotropy, linkage disequilibrium, and epigenetic interaction between selected traits in progeny and its own maternal abilities may result in direct, indirect, and even cyclic selection responses. Consequently, the appropriateness of genetic and evolutionary models is determined by the nature and magnitude of maternal effects (Cheverud 1984; Atchley and Newman 1989; Kirkpatrick and Lande 1989; Lande and Kirkpatrick 1990; Cheverud and Moore 1994; Wolf *et al.* 1998).

While maternal effects have long been recognized as potentially important factors in artificial selection and evolution, their precise role remains unclear. Studies have been performed to characterize maternal effects in various organisms including plants (*e.g.*, Schaal 1984; Roach and Wulff 1987; Thiede 1998), insects (Mousseau and Dingle 1991; Rossiter 1991; Beeman *et al.* 1992), and fish (Reznick 1981; Heath and Blouw 1988; Beacham 1989). In mammals the role of maternal effects is especially complicated by the fact that progeny experience two distinct maternal environments—prenatal uterine and postnatal nursing. Of the two, the nursing environment is better documented and is generally thought to account for most of the maternal effect (*e.g.*, Rutledge *et al.* 1972; Riska *et al.* 1985; Kurnianto *et al.* 1998). This does not, however, preclude the uterine environment from an evolutionarily important role. In addition, the nature of uterine and nurse maternal interaction on postnatal development is not well characterized (Wolf *et al.* 1998). Even in systems where maternal effects are simpler to model or the magnitude of their effects is well described, the underlying mechanism behind the response is generally unknown.

Some insight into the roles of the uterine environment has been provided by embryo manipulation studies using inbred mice. Reciprocal embryo exchange between different uterine environments has shown that the effects of different uterine environments on progeny may last well into sexual maturity (Cowley *et al.* 1989; Atchley *et al.* 1991). However, such research does not clarify whether the difference in impact of uterine environments is a consequence of random drift or a response to selection. In addition, the role of progeny genotype by maternal environment interactions has been largely ignored. While embryo transfer studies of

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inbred organisms are important, it is also crucial to understand the role maternal contributions play in the evolution of natural populations. Specifically, do pre- and postnatal maternal effects respond to selection on progeny traits? If so, what is the magnitude and direction of their response, and to what degree do they interact with the progeny genotype and each other?

Herein we describe a series of embryo transfer/crossfostering experiments on mice carried out to resolve these questions. Mice were taken from a restricted index selection experiment designed to alter early growth rate (0–10 days) while constraining response during a late interval (28–56 days). Correlated response of long-term selection on maternal effects was estimated for several traits including body weight, weight gain, tail length and gain, and growth curve traits. We examine how maternal effects evolve in the absence of direct selection on maternal environments and address the following questions: (i) Is observed divergence in early growth rate a result of a correlated response in maternal environments? (ii) Has response in developmental rates been enhanced or diminished by pre- and postnatal maternal effects? (iii) Do maternal effects interact in conditioning progeny growth?

## MATERIALS AND METHODS

**Selection lines:** Mouse lines used in these analyses were taken from an ongoing restricted index selection experiment (Atchley *et al.* 1997). The goal of this experiment is to produce lines of mice that differ in hyperplasia (cell number) and hypertrophy (cell size). Lines were originally derived from the random-bred ICR mouse strain obtained from Harlan Sprague-Dawley (Indianapolis). Three selection treatments were 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; and (3) a control line, $E^0L^0$ [C], randomly selected. Each line was replicated three times, for a total of nine independent lines. Selection was performed within families to prevent direct response in maternal effects (Falconer and Mackay 1996).

**Husbandry:** Mice were housed in opaque cages on hardwood chips and supplied with food and water *ad libitum.* Room temperature was maintained between 23° and 26° with a controlled 14-hr light:10-hr 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 caged separately by sex. Measurements on substituted pups are not included in analyses. (A more detailed description of these lines and their direct response to selection can be found in Atchley *et al.* 1997.)

**Partitioned effects by embryo transfer:** Reciprocal embryo transfers were performed between embryos and infundibuli of $E^+$, $E^-$, and C line mice within 12 hr of conception. At birth, litters were standardized to eight pups and as even a sex ratio as possible and were crossfostered to $E^+$, $E^-$, and C dams who had birthed within the same 24-hr interval [additional details provided in Ernst *et al.* (1999b)].

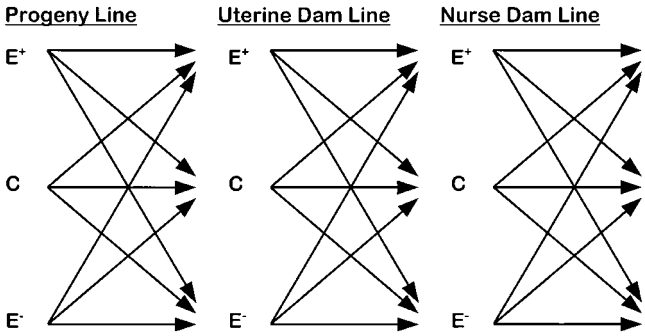


Figure 1.—Embryo transfer and crossfostering design. Embryos from $E^+$, $E^-$, and C lines were transferred within 12 hr of conception to $E^+$, $E^-$, and C uteri. At birth, litters were crossfostered among dams that had birthed within the same 24-hr interval.

To obtain sufficient sample sizes, embryo transfers were performed across generations 27, 28, and 29. Thus, we assume relative homogeneity within lines over short selection intervals. In addition, random sampling of embryos and maternal environment was performed across replicates. This has the consequence of restricting inference to the level of selection line, thereby reducing the effects of drift. The $3 \times 3 \times 3$ factorial arrangement (Figure 1) permits partitioning of direct genetic, uterine maternal, and nurse maternal effects on postnatal growth. In addition, a factorial experiment provides various intrinsic controls. Postnatal performance of pups transferred or crossfostered to dams from a different line may be directly compared to those transferred or crossfostered within line. In this way, effects of foreign maternal environments on pups may be measured. Also, a subset of untransferred and unfostered pups from the main selection experiment were measured contemporaneously to control for the specific impacts of surgery and crossfostering.

Measurements of individual mouse body weight and tail length were taken at birth, 5, 10, 15, 21, 28, 35, 42, 49, and 56 days of age. Litter weight at birth was also recorded as the total weight of both live and dead pups.

**Growth curve trait estimation:** Age-specific selection invariably produces correlated response in traits at other stages in ontogeny. Therefore, we used growth curves to summarize 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 1969; Eisen 1976; Kasser *et al.* 1983; Bailey *et al.* 1988), Gompertz (Laird and Howard 1967; Kidwell *et al.* 1969), and Bertalanffy (Di Masso *et al.* 1990). These models provide a reasonable fit of growth data (which generally follow a pattern of exponential decay), relate model parameters to growth curve traits (*e.g.*, asymptotic weight, age of maximum gain, and maximum growth rate), and permit estimation of instantaneous weight.

We used a logistic growth model to summarize growth trends and to estimate growth curve traits, such as age of maximum growth rates and mature body weight. For our data a three-parameter logistic model produced the least biased residuals as well as the smallest variance for individual growth curves. The equation was of the form

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

where $Y(t)$ is the response (body weight) at age $t$ days, $A$ is

**TABLE 1**

**Number of transferred progeny**

| Genetic line | Nurse line | Uterine line $E^+$ | Uterine line $E^-$ | Uterine line C |
|---|---|---|---|---|
| $E^+$ | $E^+$ | 16 (2)[a] | 15 (2) | 16 (2) |
| | $E^-$ | 8 (1) | 31 (5) | 12 (2) |
| | C | 18 (3) | 16 (2) | 6 (1) |
| $E^-$ | $E^+$ | 22 (3) | 18 (3) | 24 (3) |
| | $E^-$ | 24 (3) | 20 (3) | 24 (4) |
| | C | 15 (2) | 27 (4) | 21 (4) |
| C | $E^+$ | 28 (4) | 48 (6) | 35 (5) |
| | $E^-$ | 36 (5) | 33 (5) | 24 (5) |
| | C | 37 (5) | 32 (5) | 47 (6) |

[a] Number of litters per catergory are given in parentheses.

the asymptotic or mature body weight, and $k$ is the intrinsic growth rate parameter (rate of change in logarithmic weight per unit of time) for individual $i$. Shape parameter $b$, by itself, lacks an explicit biological interpretation. However, individuals obtain at postnatal age $t' = b/k$ a maximum growth rate (mgr) $= Ak/4$. Growth curve parameters were estimated using weighted nonlinear regression (Rawlings *et al.* 1998) with SAS procedure NLIN (SAS Institute 1988). In total, regressions on 653 pups from 95 embryo-transferred litters were analyzed.

**Statistical methods:** To test for effects of progeny genetic background and maternal environments on offspring growth traits, measurements on embryo-transferred and postnatally crossfostered pups were analyzed by analysis of variance (ANOVA). Embryo transfer data were fit to a model that included progeny genetic background and uterine and nurse environments as fixed effects. Traits were tested for all possible combinations ($3 \times 3 \times 3 \times 2$ factorial) of genetic, uterine dam, nurse dam, and sex effects. Litter was also included as a random effect to account for common environmental and genetic effects between observations within full-sib families. A covariate for litter size was added to explicitly account for litter size effects on postnatal development (Falconer 1965). The model was fit using SAS procedure MIXED (SAS Institute 1992). Sample sizes for each $G \times U \times N$ cell are given in Table 1 for number of progeny and litters represented.

We also wished to resolve which uterine and nurse environments differed in their effects and to determine if these effects were specific to the genetic background of their progeny. To this end we used linear contrasts to investigate specific differences in maternal and genetic factors. Contrasts were only examined when justified by significant values for factors in the ANOVA. Because genetic, uterine, and nurse main effects had only three levels ($E^+$, $E^-$, and C), pairwise comparisons were performed without multiple comparison adjustment (Steel *et al.* 1997). Treatment combinations and means for interaction terms were also restricted to orthogonal comparisons, eliminating the need for multiple comparison adjustment there as well.

## RESULTS

**Effect of embryo transfer and crossfostering:** In an embryo transfer and crossfostering experiment it is critical to determine if either procedure has a phenotypic impact in offspring and to identify if any such effect is line specific. That is, is there an effect of surgery or crossfostering over and above that due to genetic strain and other biological variables? Because of the complexity required to individually partition embryo transfer and crossfostering effects, we limited our experiment to consider their joint effect. Thus, progeny produced without embryo transfer and crossfostering were compared with those that were transferred and crossfostered. Comparisons were restricted to those transfers and crossfosterings that occurred within line. Therefore, pups produced naturally from the $E^+$ line were compared with $E^+$ progeny transferred to $E^+$ uterine dams and crossfostered to $E^+$ nurse dams. Equivalent comparisons were made for $E^-$ and C progeny. In general, results indicate that embryo transfer and crossfostering, considered together, had a small but significant effect on postnatal weights. On average, birth weights for untransferred pups were not significantly greater ($P < 0.05$) than weights from their transferred counterparts ($+0.13$ g $\pm$ 0.14). At day 5, however, untransferred and uncrossfostered controls achieved significantly greater weights ($+0.52$ g $\pm$ 0.12). This difference was observed across all genetic lines, with no significant line-by-treatment interaction, indicating that the effects of crossfostering and embryo transfer on pups led to a uniform decrease in postnatal body weight. Similarly, at days 10, 28, and 42 weights in untransferred/uncrossfostered mice were also found to be significantly greater ($+0.43$ g $\pm$ 0.16, $+1.25$ g $\pm$ 0.42, and $+1.11$ g $\pm$ 0.51, respectively). At all other ages, no significant differences due to transfer or crossfostering were found.

**Uterine litter size effects in the embryo transfer experiment:** Uterine litter size, measured as the number of live and dead pups at birth, had a highly significant ($P < 0.01$) linear effect on early postnatal body weight, estimated mature weight $A$, as well as shape parameter $b$ (Table 2). Increased litter size had a negative effect on birth ($-0.021$ g/pup), 5-day weight ($-0.044$ g/pup), and mature weight ($-0.123$ g/pup) and a slightly positive effect ($+0.77\%$/pup) on shape parameter $b$. However, nonsignificant effect of litter size on $t'$ and mgr indicates that litter size did not significantly affect either maximum growth rate or age of maximum growth rate. Similarly, litter size had a highly significant effect on early postnatal tail length ($-0.064$ mm/pup at birth and $-0.11$ mm/pup at 5 days). Because of these significant effects, litter size was included in the model as a covariate for all analyses. Additional uterine effects, such as viability of embryo transfers, are described in Ernst *et al.* (1999b).

**Sex effects in embryo transfer experiment:** Males and females were highly significantly different ($P < 0.01$) for body weight and weight gain means at all observed ages, except gain for the 49- to 56-day interval. Age and rate of maximum growth also showed significant sexual dimorphism (Table 2). Males invariably had higher mean body weights and achieved a heavier mature weight than

**TABLE 2**

**Analysis of variance for body weight, weight gain, and growth curve traits from birth to 8 wk of age in embryo transferred (ET) mice**

| | | Age (days) | | | | | | | | | | Gains | | | | | | | | | Growth curve traits | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | d.f. | 0 | 5 | 10 | 15 | 21 | 28 | 35 | 42 | 49 | 56 | 0–5 | 5–10 | 10–15 | 15–21 | 21–28 | 28–35 | 35–42 | 42–49 | 49–56 | *A* | *b* | *k* | *t′* | mgr |
| Genetic (*G*) | 2 | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | | *** | *** | *** | *** | *** | * | *** | *** | *** | *** | *** |
| Uterine env (*U*) | 2 | | | | | | | | | | | | ** | | | | | * | | | | | | | |
| Nurse env (*N*) | 2 | | | * | *** | ** | * | ** | ** | * | * | | *** | ** | | | | | * | | * | | ** | * | *** |
| Sex (*S*) | 1 | *** | *** | *** | ** | *** | *** | *** | *** | *** | *** | *** | *** | ** | *** | *** | *** | *** | *** | | *** | *** | *** | *** | *** |
| *G* × *U* | 4 | * | * | ** | ** | ** | ** | * | ** | ** | * | | | | | | | | | | | | ** | ** | ** |
| *G* × *N* | 4 | | | | | | | | | | * | | | | | | ** | | | * | * | | | | |
| *N* × *U* | 4 | | | | | | | | | | | | | | | | | | | | | | | | |
| *G* × *U* × *N* | 8 | | | | | | | | | | | | | | | | | | | | * | | | | |
| *G* × *S* | 2 | | * | * | | | *** | *** | * | ** | ** | * | | | | *** | *** | *** | | | | ** | | | * | *** |
| *U* × *S* | 2 | | | | | | | | | | | | | | | | | | * | *** | | | | | |
| *N* × *S* | 2 | | | | | | | | | | | | | | | * | | | *** | ** | | | | | |
| *G* × *U* × *S* | 4 | | | | | | | | | | | | | | | | | | ** | * | | | | | |
| *G* × *U* × *S* | 4 | | | | | | | | | | | | | | | * | ** | | *** | * | | | | | |
| *U* × *N* × *S* | 4 | * | ** | * | | | | * | | | | ** | | | | ** | | *** | | | | | | | |
| *G* × *U* × *N* × *S* | 8 | | | * | | | | * | | | | * | ** | | | | *** | | ** | ** | | | | | |
| Litter size | 1 | *** | *** | * | | | * | * | | * | * | * | | ** | * | | | | * | | ** | *** | | | |

*($P < 0.05$); **($P < 0.01$); ***($P < 0.001$); all empty cells represent values not significant at $P < 0.05$ level.

**TABLE 3**

**Analysis of variance for tail length gain and growth curve traits from birth to 8 wk of age in ET mice**

| | | Age (days) | | | | | | | | | | Gains | | | | | | | | | Growth curve traits | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | d.f. | 0 | 5 | 10 | 15 | 21 | 28 | 35 | 42 | 49 | 56 | 0–5 | 5–10 | 10–15 | 15–21 | 21–28 | 28–35 | 35–42 | 42–49 | 49–56 | *A* | *b* | *k* | *t′* | mgr |
| Genetic (*G*) | 2 | | ** | * | | | | | | | | *** | | | | | * | | | | | * | | | |
| Uterine env (*U*) | 2 | | * | | | | | | | | | | | | | | | | | | | * | | | |
| Nurse env (*N*) | 2 | | | | | | * | * | | | | * | | | | | | | | | | | | | * |
| Sex (*S*) | 1 | *** | ** | * | | | | *** | *** | *** | *** | | | * | | * | *** | *** | | | | | *** | | |
| *G* × *U* | 4 | | * | * | ** | * | * | * | * | * | | ** | | | | | | | | | | | * | | * |
| *G* × *N* | 4 | | | | | | | | | | | | | | | | | | | | | | | | |
| *U* × *N* | 4 | | | | | * | * | | | | | | | | | | | | | | | | | | |
| *G* × *U* × *N* | 8 | | | | | | | | | | | | | | | | | | | * | | | | | |
| *G* × *S* | 2 | | | | | | | | | | | | | | | | | | | | | | | | |
| *U* × *S* | 2 | | | * | | | | | | | | | * | | | | | | | | | | | | |
| *N* × *S* | 2 | | | | | | | | | | | | | | | | * | | | | | | | | |
| *G* × *U* × *S* | 4 | | | | | | | | | | | | | | | | | | * | | | | | | |
| *G* × *N* × *S* | 4 | | * | | | | * | | | | * | | | | | | | * | | | | | | | |
| *U* × *N* × *S* | 4 | | | * | | | | | | | | | | | | | | | | | | | | | |
| *G* × *U* × *N* × *S* | 8 | | | | | | | | | | | | ** | | | * | * | | | | | | * | | |
| Litter size | 1 | *** | *** | * | | | | | | | | | | | * | | | | | * | | *** | | * | |

*($P < 0.05$); **($P < 0.01$); ***($P < 0.001$); all empty cells represent values not significant at $P < 0.05$ level.

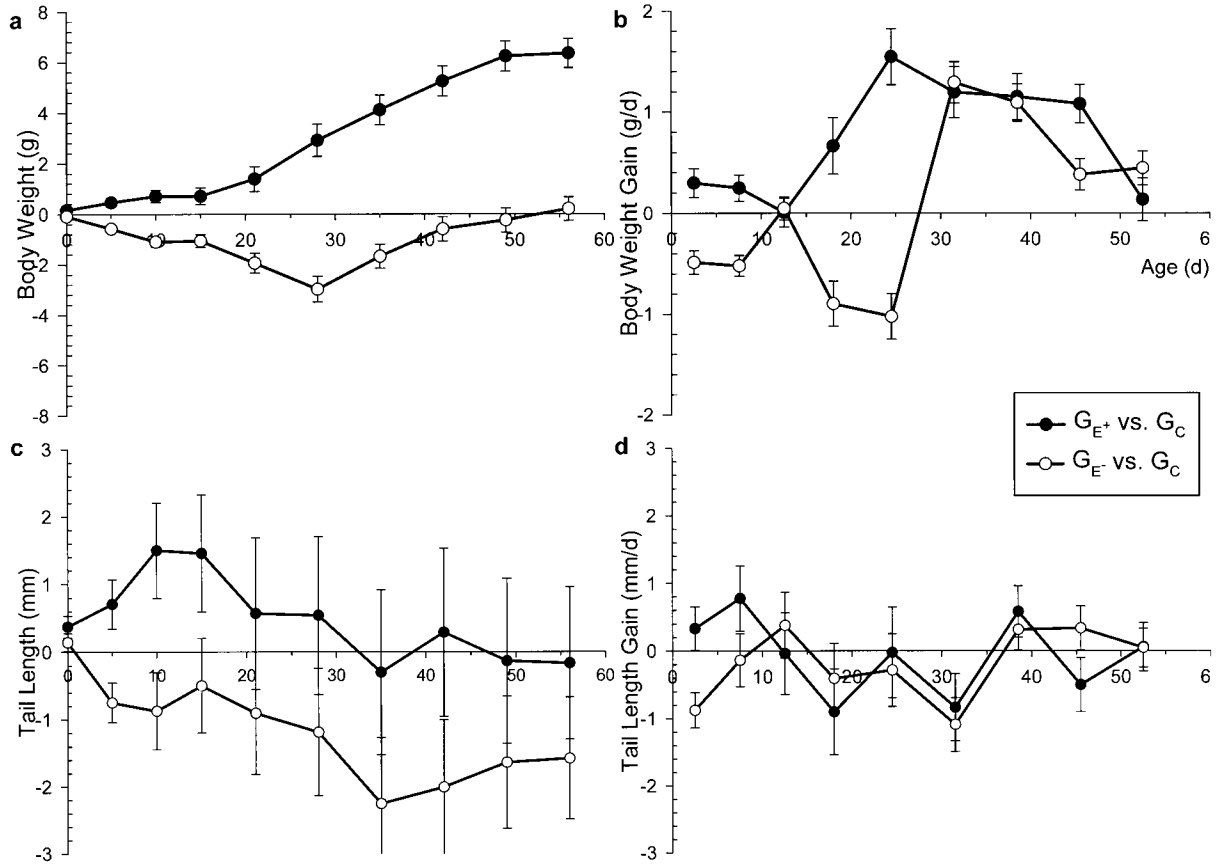


Figure 2.—(a–d) Direct genetic effect on postnatal growth in embryo-transferred mice. Lines represent deviations for $E^+$ and $E^-$ progeny from control mean growth trajectory. Genetic effects are estimated by averaging across all combinations of uterine and nurse maternal environments: (a) body weight; (b) body weight gain; (c) tail length; (d) tail length gain.

females (+6.9 g ± 0.19) independent of uterine and nurse environments. Sex effects on tail length were similar (Table 3). Mean tail lengths for males were larger than females, irrespective of age or maternal effects, and these differences were generally significant except between days 15 and 28. Significant sex × line interactions in selection lines are partially accounted for by sex × progeny and/or sex × maternal environment interactions. A trend of significant interactions between sex and progeny line for body weights was observed. However, sexual dimorphism did not have a significant effect on response to either uterine or nurse environments. Additional significant interactions of higher order involving uterine and nurse maternal environments were observed. However, the multicomparison aspects of the experiment suggest caution in interpreting such results.

**Genetic line effects:** Statistical analysis of the embryo transfer/crossfostering experiment permits us to partition variation in postnatal growth into genetic and maternal components. Genetic effect of progeny line was highly significant for all body weights and most weight gain intervals as well as age and rate of maximum growth (Table 2). $E^+$ progeny have an increased postnatal weight gain during the early selection interval relative to both $E^-$ or C progeny, resulting in significantly different body weights (Figure 2, a and b). Weight gains again diverge during the unselected interval (10–28 days) after which they generally converge. $E^+$ progeny reach a significantly higher maximum growth rate than control progeny (+0.25 g/day ± 0.03) at an earlier age (−1.2 day ± 0.5) and a higher estimated mature weight (+6.7 g ± 0.6; Figure 7, a–c). $E^-$ mice have a lower (−0.08 g/day ± 0.02) maximum growth rate at a later age (3.0 day ± 0.4) relative to control progeny. While genetic effects in $E^-$ mice eventually converge with those of control mice, means for $E^-$ mice reach a maximum difference at 28 days (−3.0 g ± 0.5).

Genetic effects on tail lengths are not as pronounced (Figure 2, c and d). Although tail length means are consistently higher for $E^+$ *vs.* $E^-$ progeny, genetic differences in tail length are significant only between day 5 ($P < 0.001$) and day 15 ($P < 0.04$). $E^+$ progeny do experience a significantly increased growth rate from birth to day 5 ($P < 0.001$). No significant effects were seen on age or rate of maximum growth rates for tail length.

**Effects of uterine environment:** What are the conse-

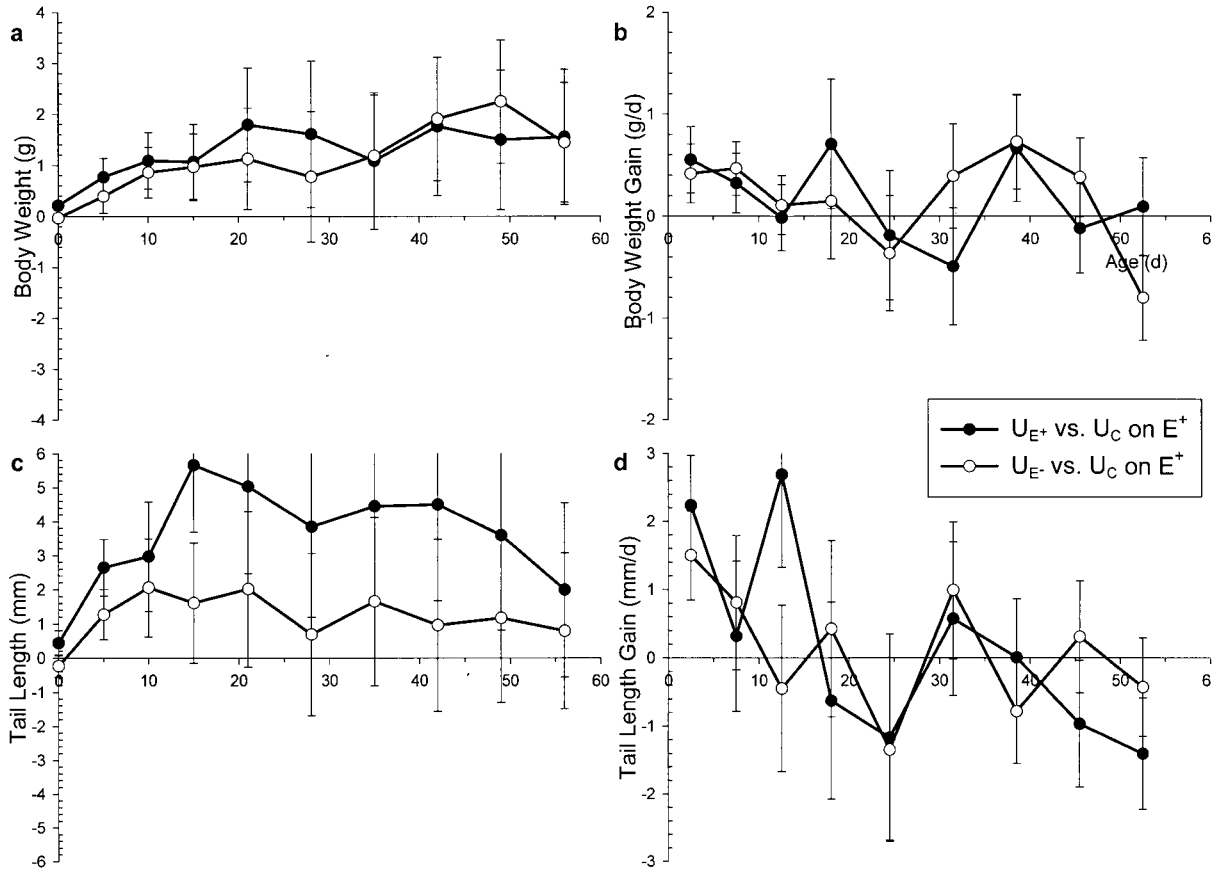


Figure 3.—(a–d) Uterine maternal effect on postnatal growth in $E^+$ progeny. Lines represent effect of uterine environments on $E^+$ progeny averaged across all nurse maternal environments: (a) body weight; (b) body weight gain; (c) tail length; (d) tail length gain.

quences of being gestated in the uterus of a dam from a different selection line? Uterine environment was found to have a significant main effect ($P < 0.05$) only on the 5- to 10-day gain interval for body weight, 5-day tail length measurement, and one weight growth curve variable (*b*; Tables 2 and 3). The effect, however, was not strong enough to significantly alter either age or rate of maximum growth. Significance for the 5- to 10-day weight gain interval was due to a small increased postnatal gain in progeny gestated in an $E^-$ uterus relative to both $E^+$ (0.27 g ± 0.12) and C (0.36 g ± 0.12) uteri.

While uterine main effects were generally nonsignificant, genetic-by-uterine interactions ($G \times U$) had significant effects on all body weight measurements. Similarly, $G \times U$ was significant for tail lengths from 5–49 days. Significant $G \times U$ values result from variable direction and magnitude of response to uterine environments for mouse lines. Mean body weights and tail lengths for $E^+$ progeny gestated in selection line uteri were consistently higher than those for $E^+$ progeny gestated in a control uterus (Figure 3, a and c). In general, however, these differences were not individually significant. Body weight means were significant ($P < 0.05$) only at birth and 5 days for $E^+$ progeny gestated in an $E^+$ uterus. Similarly, tail lengths were significantly greater at 5 and 15 days for $E^+$ progeny gestated in an $E^+$ uterus. Tail length gains (Figure 3d) were significantly greater from birth to 5 days for $E^+$ progeny gestated in either $E^+$ or $E^-$ lines. Detectable postnatal effects of uterine environment on $E^+$ progeny are therefore primarily slight and occur shortly after birth.

Direction and magnitude of uterine effect on $E^-$ progeny followed a different profile (Figure 4, a–d). $E^-$ progeny transferred to either an $E^-$ or control uterus were not significantly different for any body weights, tail lengths, weight gains, or tail length gains. However, $E^-$ progeny transferred to $E^+$ uteri experienced a significant and persistent decrease in postnatal growth. Body weights were significantly less from days 10–56, with largest effect at day 28 (−3.22 g ± 0.99). Weight gains were significantly decreased from 5- to 10- through 21- to 28-day intervals. Tail lengths were also significantly shorter at 10, 28, 35, 42, and 49 days with decreased gains from 5–10 and 10–15 days of age. The largest decrease in mean tail length was observed at 35 days (−4.53 mm ± 1.91). $E^-$ progeny transferred to $E^+$ uteri experienced a significant reduction in postnatal growth

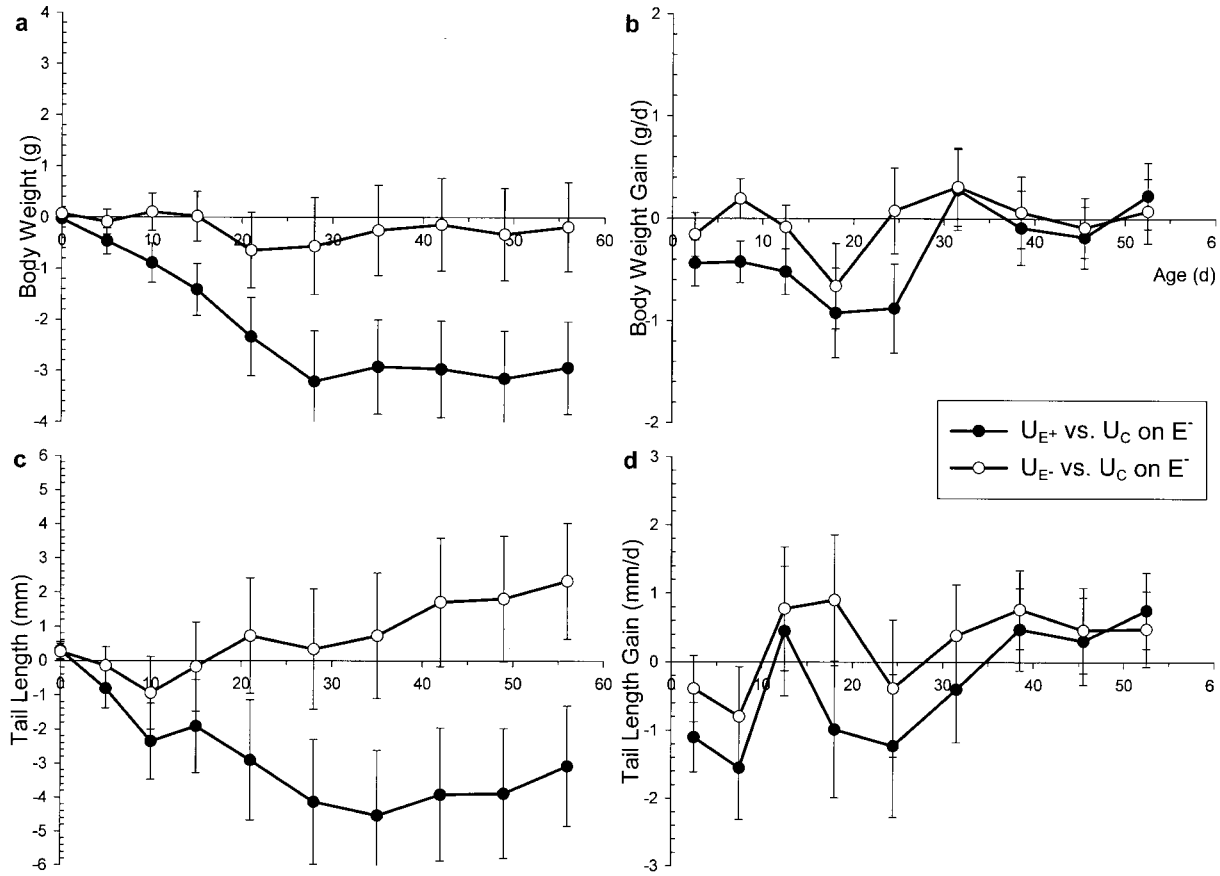


Figure 4.—(a–d) Uterine maternal effect on postnatal growth in $E^-$ progeny: (a) body weight; (b) body weight gain; (c) tail length; (d) tail length gain.

relative to those transferred to either a control or their native $E^-$ uterine environments. Such mice also achieve a smaller mgr ($-0.16$ g/day $\pm$ 0.04) at a later time ($+1.93$ day $\pm$ 0.76) relative to those gestated by control dams (Figure 7, b and c).

Effects of uterine environments on control progeny were similar in direction to those on $E^+$ progeny but less variable (Figure 5, a–d). Consequentially, means for postnatal body weights were significantly greater in mice transferred to $E^+$ uterine dams at 10, 15, and 21 days and for mice transferred to $E^-$ dams at day 15. In both cases mice transferred to selection line dams experienced a period of increased postnatal gain prior to weaning and decreased gain thereafter. The largest estimated response was observed at day 28 in $E^+$ gestated mice ($+1.43$ g $\pm$ 0.77) and day 21 in $E^-$ gestated mice ($+1.10$ g $\pm$ 0.58). Likewise, gestation in an $E^+$ uterus resulted in a moderate postnatal increase in tail lengths. Tail lengths were significantly greater at 5, 15, 21, and 28 days with the largest response seen at day 28 ($+3.33$ mm $\pm$ 1.43). Tail length means for $E^+$-gestated control mice decreased thereafter though they remained greater until day 56. Tail lengths were also significantly greater at 15, 21, and 28 days in control mice transferred to $E^-$ uteri with maximum effect seen at 28 days ($+3.92$ mm $\pm$ 1.39). In both cases, selection line uterine effects did not alter magnitude of maximum growth rate for body weight in control mice but did affect its timing. Control mice transferred to $E^+$ uterine dams reached mgr on average 1.67 $\pm$ 0.58 days early (Figure 7b). Those transferred to $E^-$ uterine dams reached mgr 1.85 $\pm$ 0.57 days early.

$G \times U$ interaction was marginally significant ($P = 0.06$) for estimated mature weight, suggesting that uterine environment may play a more long-term effect on mouse growth, at least for certain combinations of genetic progeny-by-uterine environment. However, significant $G \times U$ interactions were seen for shape parameter $k$ ($P < 0.006$), maximum growth rate ($P < 0.016$), and age of maximum growth ($P < 0.004$). Three orthogonal contrasts proved to be significant for $k$—$E^- \times E^+$ *vs.* $E^- \times C$ ($P < 0.004$), $C \times E^+$ *vs.* $C \times C$ ($P < 0.037$), and $C \times E^-$ *vs.* $C \times C$ ($P < 0.002$)—with a similar trend for $t'$. Results indicate that for $E^-$ and C progeny, the postnatal response growth trajectory is not merely accounted for by additive effects of either progeny or uterine dam lines. Uterine environment was also seen to have a nonsignificant direct effect on parameter $k$ or

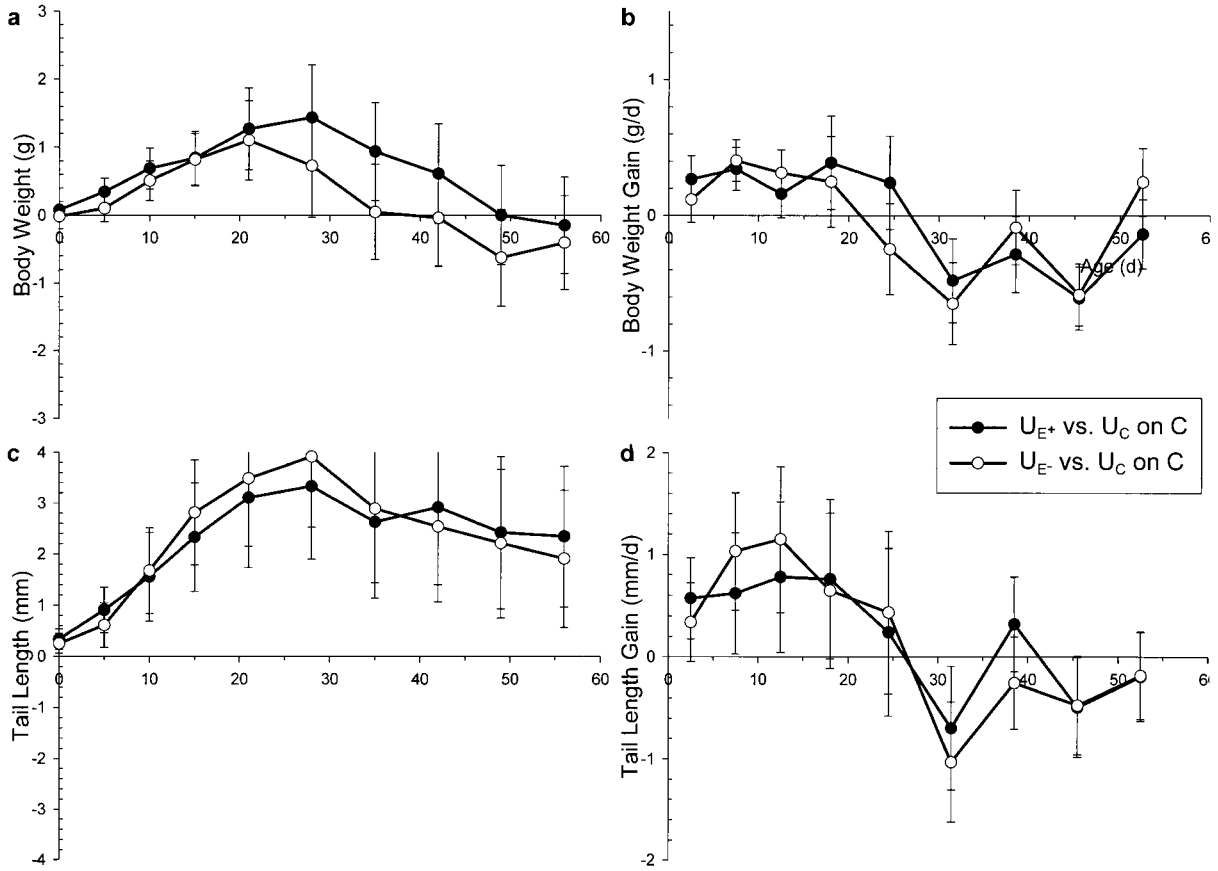


Figure 5.—(a–d) Uterine maternal effect on postnatal growth in control progeny: (a) body weight; (b) body weight gain; (c) tail length; (d) tail length gain.

$t'$ in general. These observations suggest a particular susceptibility for $E^-$ and C progeny to specific uterine environments as compared with $E^+$ progeny.

**Effects of postnatal nurse environment:** Similarly, we have examined the extent to which the postnatal nursing environment has contributed to selection response. In general, nurse environment was found to have a greater influence on postnatal body weight growth trajectories compared with uterine environment. Nurse environment significantly altered pre- and postweaning body weights as well as age and size of mgr for body weight. Mice nursed by $E^+$ dams experienced a period of increased growth rate up until approximately weaning, resulting in increase relative body weights until 21 days ($+0.9$ g $\pm$ 0.5; Figure 6, a and b). However, irregular postweaning growth rates resulted in a mean estimated mature weight not significantly greater than mature weight for control mice (0.1 g $\pm$ 0.5). While maximum growth rate is not significantly different between $E^+$ and C nursed mice, those nursed by $E^+$ dams reach their maximum growth rate at an earlier age ($-0.98$ days $\pm$ 0.46; Figure 7b).

Relative growth rates for pups reared in an $E^-$ nursing environment were consistently smaller even beyond weaning and relative body weight decreased in $E^-$-nursed mice up to day 42 ($-1.2$ g $\pm$ 0.6). Estimated mature weight indicates that an $E^-$ nurse environment caused a persistent decrease in body weight ($-1.2$ g $\pm$ 0.6). Maximum growth rate for body weight in $E^-$-nursed mice was also significantly decreased ($-0.059$ g/day $\pm$ 0.026; Figure 7c).

Nurse maternal effect on tail length was markedly different (Figure 6, c and d). $E^+$ nurse dam environment resulted in a pattern of increased tail growth rate until shortly after weaning. Consequentially, relative tail length in $E^+$-nursed mice increased until day 28 ($+2.4$ mm $\pm$ 1.1). $E^+$ and C nursing environments were found to have a nonsignificant difference in their effects on estimated mature tail length due to high variability associated with those estimates. However, their effects on tail length were significantly different at both 49 and 56 days, suggesting some persistence of $E^+$ nursing effects. Mice reared in an $E^-$ nursing environment, however, did not appear to experience relative change in tail length. Postnatal gains and length differences were uniformly nonsignificant relative to those of mice nursed by control dams.

**Effects involving environmental interactions:** While progeny genotype and uterine and nurse maternal environments all contribute to postnatal response, it is necessary to determine the degree to which these factors interact. Significant interactions between progeny ge-

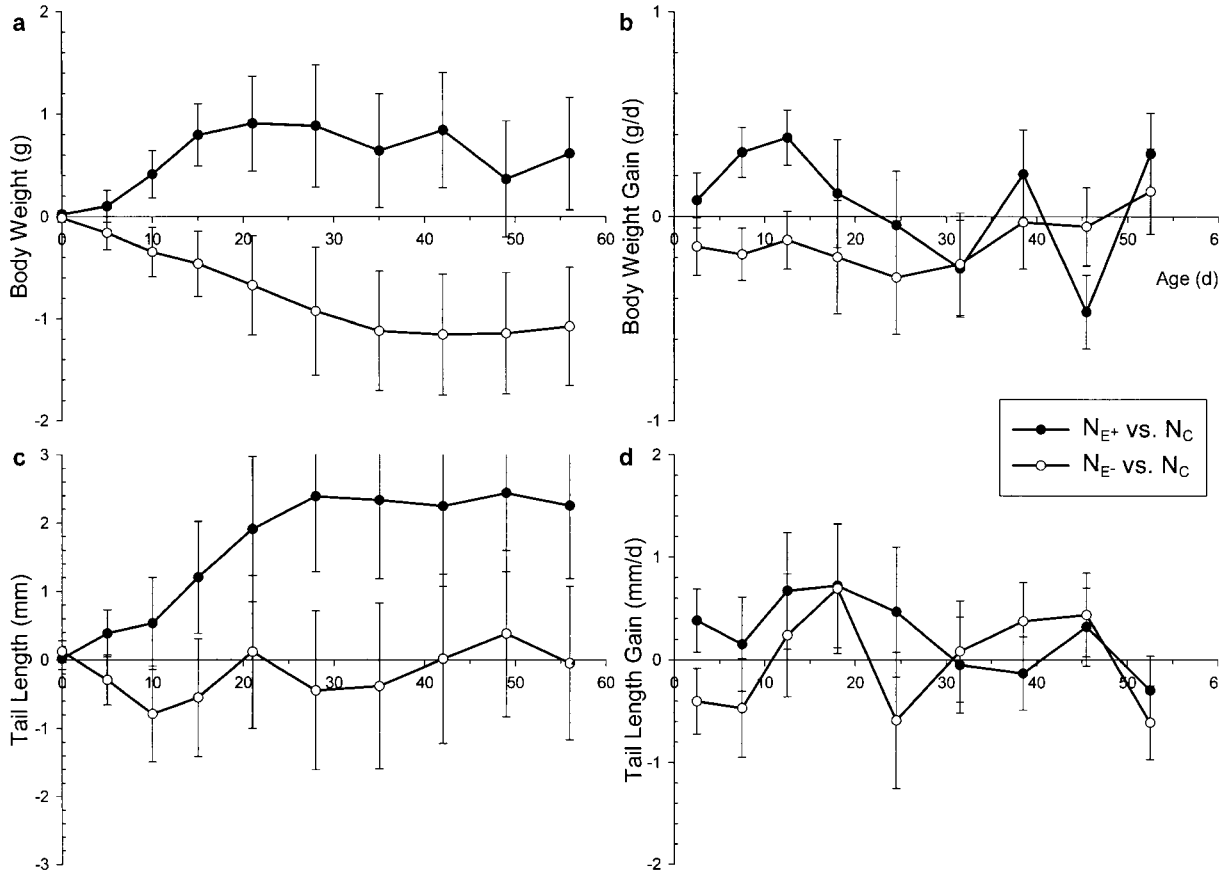


Figure 6.—(a–d) Nurse maternal effect on postnatal growth in ET mice. Lines indicate effect of $E^+$ and $E^-$ maternal environments relative to control: (a) body weight; (b) body weight gain; (c) tail length; (d) tail length gain.

netic and uterine or nurse environments indicate genetic differences found only under specific environments. Interactions detectable in this experiment include genetic-by-nurse ($G \times N$), uterine-by-nurse ($U \times N$), genetic-by-uterine-by-nurse ($G \times U \times N$), the previously discussed genetic-by-uterine ($G \times U$), and those involving sex. To control for false positives that might arise from performing multiple analyses, we limited our attention to either highly significant effects or those that show trends of significant values. In general, effects due to maternal environmental interactions were nonsignificant for body weights, tail lengths, tail length gains, and growth curve traits. Uterine-by-nurse-by-sex ($U \times N \times S$) interaction was statistically significant for body weights from 0, 5, and 10 days. However, nursing environment had no impact on birth weights (because birth weights were measured prior to crossfostering). Therefore, significant effects result both from sampling error and subsequent within-individual measurement correlation. Because maternal interactions were otherwise nonsignificant for body weight, tail lengths, tail length gains, and growth curve traits, these environments contributed primarily in an additive fashion to these traits.

However, body weight gains had several highly significant environmental interaction effects, especially after weaning. $G \times U \times S$ was significant between days 42–49 and 49–56. $G \times N \times S$ had significant effects between days 21–28, 28–35, 42–49, and 49–56. $U \times N \times S$ was significant between days 0–5, 21–28, 35–42, and $G \times U \times N \times S$ was significant between days 0–5, 5–10, 28–35, 42–49, and 49–56. Significant interactions indicate that genetic, uterine, nurse, and sex factors are not independent in their effects on weight gain. However, subsequent partitioning of significant interactions into orthogonal contrasts of simple effects proved to be uninformative. Simple effects for highly significant interactions were uniformly significant. Further partitioning with appropriate multicomparison adjustment resulted in a loss of significance for virtually all contrasts. Environmental interactions therefore appear to contribute to weight gains, especially after weaning, in a nonadditive fashion. However, it should be noted that significant gains did not result in significant differences in body weights. Interactions, therefore, do not appear to contribute to a systematic departure of body weights for any particular combination of uterine and dam nurse environment despite the role they play in heterogeneous postweaning weight gain.

**Magnitude of maternal effects:** Selection for increased or decreased early growth rate resulted in statistically significant changes in pre- and postnatal maternal environ-

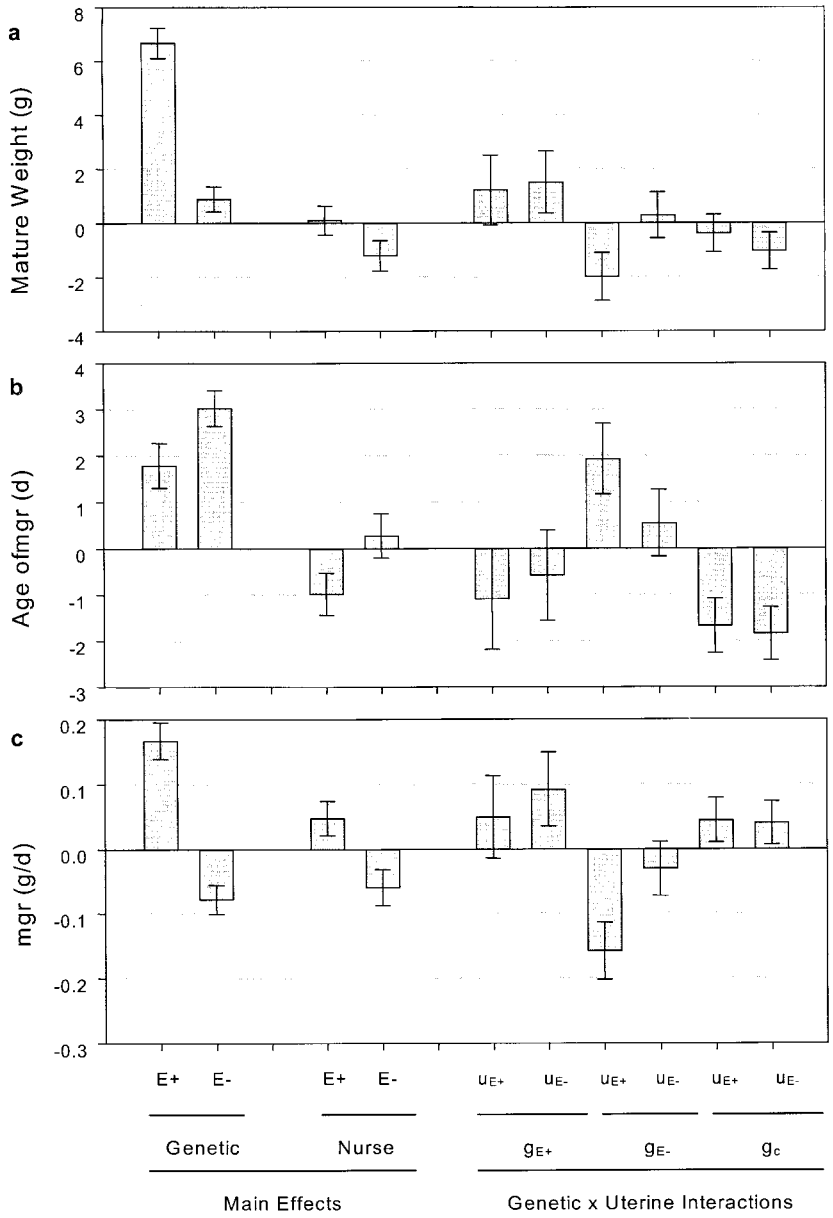


Figure 7.—(a–c) Genetic and maternal effects on growth curve traits: (a) mature weight; (b) age of maximum growth rate (mgr); (c) mgr. Least squares means for main effects are expressed as differences from the control line. Means for genetic × uterine interactions are expressed as differences from the appropriate uterine control for each specific level of genetic effect [*e.g.*, the least squares mean for the interaction between $E^+$ progeny and $E^+$ uterine environment ($gu_{E^+,E^+}$) is expressed as the difference $gu_{E^+,E^+} - gu_{E^+,C}$].

ments. However, are these changes of equal biological relevance? Uterine response contributed less to postnatal development compared with direct genetic and nurse effects. Figure 8, a and b, provides a description of the relative contributions of main effects by line. For the $E^+$ line, progeny genotype accounted for ~60% of response at birth, the effect decreased until shortly before weaning, and then increased to >80%. Uterine effects were greatest at birth (>30%) and steadily decreased until shortly after weaning, where they contributed <10% between days 28 and 56 to response in body weight. Nurse effects were initially small, but increased until day 15 at which time their estimated contribution to response was greater than that due to direct genetic effect. Because genetic contribution to response in the 0- to 10-day interval remained below 60%, nurse and uterine postnatal effects contributed to body weight gain observed during the early selection interval.

Response in $E^-$ effects is primarily explained by direct genetic effects during the early selection interval. However, genetic effects on relative body weight diminished after day 28 while nursing effects remained constant. Response in body weight during the late selection interval increasingly became a product of nurse maternal effects.

## DISCUSSION

Results of this experiment demonstrate that both uterine and nurse maternal components respond indirectly to selection for rate of development in evolving populations. Furthermore, we show that the uterine component has a large genotype-specific impact on early postnatal develop-

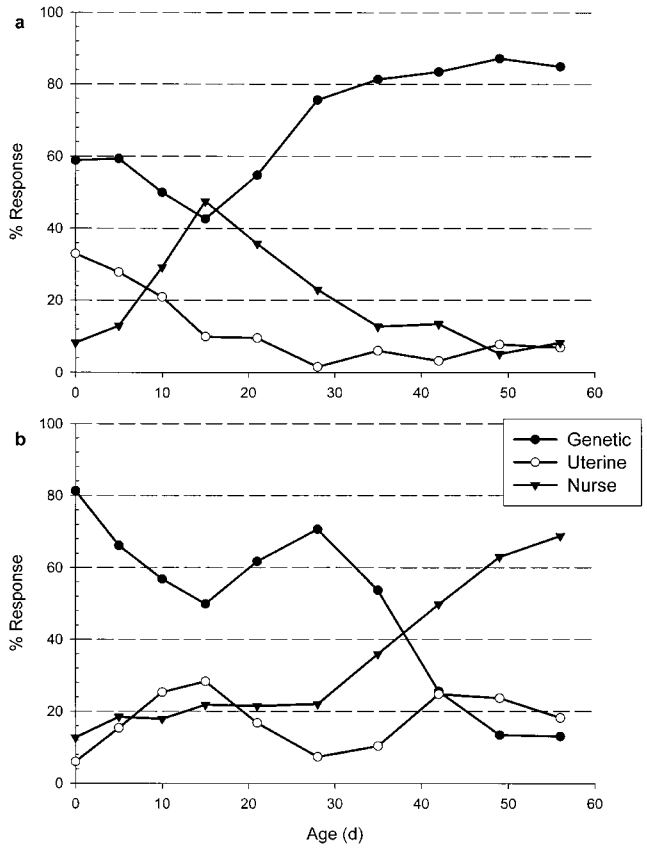


Figure 8.—Relative contribution of genetic, uterine, and nurse maternal effects to selection response in lines (a) $E^+$ and (b) $E^-$. Response to selection in each effect was computed as the difference between either $E^+$ or $E^-$ least-squares means and the control. Magnitudes of these differences were summed by age to express total departure from controls accounted for by simple effects. Relative contribution of each simple effect was calculated as the ratio of the magnitude of the difference function for that particular effect over the total.

ment. Nurse maternal effects on weight and tail length traits were also large and independent of uterine maternal effects. Therefore, uterine and nursing effects on these traits may be considered as independent factors in artificial selection programs and studies of natural populations.

**Embryo transfer and postnatal crossfostering effect:** Comparison of progeny produced by embryo transfer and crossfostering with those from natural contemporaneous litters shows that the observed maternal effects were not artifacts of either the embryo transfer or crossfostering procedures. The joint effect of both of these procedures was a consistent decrease in early body weights across all progeny types. Therefore, while embryo transfer and crossfostering procedures resulted in initially lower weights, they did not contribute to observed differences between progeny.

**Uterine maternal effects:** In this study, the uterine environment had a significant impact on weight and tail length traits at nearly all ages, demonstrating both its importance and persistence. This is in contrast to previous studies that failed to find evidence of prenatal uterine effects on body size in selection lines (Brumby 1960; Al-Murranni and Roberts 1978). Moore *et al.* (1970) reported evidence of significant uterine effects in mice selected for 6-wk body weight. However, their findings showed uterine effects to be both small and transient in comparison to direct genetic and nurse effects.

Uterine effects in this study were observed principally in the interaction between uterine environments and progeny genotype. Previous work on inbred mouse lines showed that magnitude and direction of uterine maternal effects were dependent on progeny genotype (Cowley *et al.* 1989). Specifically, progeny developed more rapidly when gestated in their natural uterine environment. While significant uterine × progeny effects were observed in our experiment, they followed no clear pattern. Specifically, the $E^+$ uterine environment had a pronounced negative impact on body weights from $E^-$ progeny and a positive impact on control weights. Similarly, the $E^-$ uterine environment had a positive impact on control progeny but a nonsignificant effect on $E^+$ mice. Results suggest that uterine components have responded to selection and their effects are conditioned by requirements specific to progeny genotype. However, progeny gestated in their natural uterine environment did not experience an increased postnatal gain.

The effect of the uterine maternal environment on growth curve traits was significant and, for specific combinations of progeny and uterine environment, larger than the nurse effect. However, as for body weights, the effects of the uterine environment on weight growth curve traits are seen primarily in the uterine × progeny interactions. For instance, $E^-$ progeny gestated in $E^+$ uteri experience a decrease in maximum growth rate, which occurs ~2 days later than for those $E^-$ progeny gestated in a control environment. This decreased rate is followed by a marginally significant decrease in estimated mature weight for this combination of dam and offspring. These results indicate persistence of uterine effects and their potential impact on reproductive traits.

Uterine effects on tail lengths were pronounced. In general, those genetic-by-uterine interactions observed in postnatal body weight were also seen in tail lengths. However, while $E^+$ uteri had a positive but nonsignificant effect on $E^+$ body weights in mice, corresponding tail lengths were significantly greater at 5 and 15 days. Such effects were evident even in the absence of significant direct genetic or nurse maternal effects on tail size. Therefore, the importance of the uterine environment varies both with age and trait.

$E^+$ uterine main effects accounted for >30% of response in body weight at birth and >20% at day 10 (Figure 8, a and b). In contrast, effects of $E^-$ uteri were initially negligible though they increased to >20% by day 10 and maximized shortly thereafter. Cowley *et al.* (1989) observed that inbred uterine genotypes ac-

counted for 8% of total weight gain from 3–12 days and 26% from 42–63 days postpartum. Such a pattern is most compatible with response seen in the $E^-$ uterine environment. However, $E^+$ uterine effects were initially greater and followed a reversed trend.

**Nurse maternal effects:** Age-specific total maternal variance usually accounts for a maximum of 50–70% of phenotypic variance with peaks coinciding with maximum growth rate in progeny (*e.g.*, El Oksh *et al.* 1967; Herbert *et al.* 1979). The majority of this variation arises from postnatal nursing effects. Rutledge *et al.* (1972) partitioned phenotypic variance for growth curve traits and demonstrated (except for age of maximum growth rate) small or negligible amounts of variation arising from the postnatal nursing environment. The maternal × direct genetic variance component was found to be as large as the direct genetic variance component by itself for mature weight. To some extent the findings of Rutledge *et al.* are consistent with this study. In our results, nurse maternal effects are generally much greater on body weight than uterine effects in $E^+$ mice. Nurse maternal effects in $E^+$ mice resulted in an earlier inflection point ($t'$) with no significant change in maximum growth rate. In $E^-$ mice, $t'$ did not occur at a different age than control mice, although maximum growth rate was lower than for the control. Compensatory postweaning growth was observed in mice reared by $E^+$ and, to a lesser extent, $E^-$ dams (Figure 6, b and d).

In contrast to uterine effects, nurse maternal effects were positively correlated with selection on early growth. In addition, nurse maternal effects were largely independent of progeny or uterine genotype, especially for early postnatal weight. Mice nursed by $E^+$ dams uniformly experienced a period of increased growth. Similarly, mice nursed by $E^-$ dams experienced decreased postnatal growth, though as a consequence of less abrupt but more persistent maternal effects. In contrast, Nagai *et al.* (1976) found either negative or nonsignificant correlation in selection response in ICR mice selected for increased weight and postweaning gain. This may be due, in part, to fundamental differences between selection indices in the Nagai *et al.* experiment compared to ours.

Response in $t'$ and maximum growth rate due to maternal effects prompts consideration as to whether or not maternal effects are also responsible for line-specific differentiation in sexual traits. Differential reproductive onset as a function of growth rate in females has been observed in a previous generation (Ernst *et al.* 1999a) and we are currently exploring the role of pre- and postnatal maternal effects on reproduction.

**Maternal interactions:** Estimates of additive direct × additive maternal covariance in mouse body weight have been shown to vary between populations. Positive (Cheverud 1984; Riska *et al.* 1985) as well as negative covariance structures (Eisen 1970) have been reported for mouse lines with standardized litter size. Therefore, we did not anticipate any particular form of selection response in maternal effects. Significant maternal interactions were primarily found in weight gains and included all higher order interactions involving sex. However, these effects did not result in significant differences in overall body weights. Interactions, therefore, may be responsible for differences in gain, especially postweaning, but do not appear to contribute to a systematic departure of body weights for any particular combination of uterine and dam nurse environment.

**Conclusions:** $E^-$ and control progeny are more sensitive to change in uterine environment than $E^+$ mice. While the $E^+$ uterine environment had a positive influence on postnatal body weights in control mice, $E^-$ mice gestated in that environment experienced a decrease in postnatal growth. Such differences emphasize the role that genetic-by-uterine interactions play in conditioning postnatal growth. While genetic-by-uterine effects were significant for body weight, their role was considerably more pronounced in tail lengths. Both nurse environments had a positively correlated response to selection. Nurse effects were also primarily additive with respect to uterine and direct genetic factors early in postnatal development. In $E^+$ mice, nurse environment main effect accounted for a relatively large portion of preweaning response in body weight. The $E^-$ nursing environment, conversely, had a larger effect on postweaning response. Significant maternal effects and their interactions in postweaning body weight gains indicate persistency of maternal effects in growth trajectories. Because selection was performed within family, maternal traits were altered in the absence of direct selection pressure.

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