The Impact of Maternal Uterine Genotype on Postnatal Growth and Adult Body Size in Mice

David E. Cowley,* Daniel Pomp,† William R. Atchley,* Eugene J. Eisen† and Diane Hawkins-Brown*

**Department of Genetics and †Department of Animal Science, North Carolina State University, Raleigh, North Carolina 27695*

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ABSTRACT

Embryo transfers were used to demonstrate that the genotype of the mother providing the uterine developmental environment significantly influences postnatal growth and adult body size of her progeny. Irrespective of their own genotype, mouse embryos transferred into the uterus of an inbred strain with large body size (C3H) had greater body weights, longer tails and higher growth rates than those transferred into the uterus of a strain with small body size (SWR). Uterine heterosis on body size was smaller than progeny heterosis, and both progeny and uterine heterosis persisted in adult mice. Uterine litter size was significantly negatively associated with body weight, tail length, growth rate and the timing of developmental events. The inbred SWR strain was more sensitive to the embryo transfer procedure than the C3H strain, but effects due to embryo transfer were moderate. Prenatal uterine effects have ramifications for biotechnologies utilizing embryo transfers as well as predictions about evolutionary change by selection.

IN mammals, female parents make more complex contributions to the phenotype of their progeny than do male parents. In addition to nuclear genes, females contribute mitochondria, egg cytoplasm, and the developmental environment from conception to weaning. Complex interactions, known as "maternal effects" (WILLHAM 1963; FALCONER 1965; ROBISON 1981), may arise from interplay between progeny and their uterine and postnatal nursing environments. Through these maternal effects, the mother has the potential to modify the expression of genes in her progeny.

Progeny phenotype at any age can be influenced by uterine effects, postnatal nursing effects, and other environmental effects in addition to the progeny genotype (Figure 1). Relative contributions of the progeny genotype and postnatal factors have been shown to vary as organisms develop (EL OKSH, SUTHERLAND and WILLIAMS 1967; RUTLEDGE *et al.* 1972; ATCHLEY and RUTLEDGE 1980; RISKA, ATCHLEY and RUTLEDGE 1984). However, little is known about effects of uterine genotype on complex and continuously varying traits, such as body weight or skeletal dimensions. "Uterine genotype" in this sense refers to all components of prenatal maternal effects, and it is defined as the genotype of the female in which the embryo develops. Prenatal uterine effects are mediated through such factors as the age and condition of the mother as well as prenatal fraternity size, body size of the female and quality of the environment which the mother experiences during gestation. Histocompatibility genotype of fetus and mother are also potentially important components of prenatal maternal effects (HEDRICK 1988; HEDRICK and THOMSON 1988).

The impact of the uterine environment on the developing fetus has been amply demonstrated by the teratogenic effects of various chemicals (SCHARDEIN 1985; BRIGGS, FREEMAN and YAFFE 1986). However, the effects of uterine genotype on normal patterns of development are poorly understood. Knowledge of these latter prenatal effects has special significance for biotechnologies such as embryo manipulation and cryopreservation which rely on embryo transfer for their ultimate success. Further, maternal effects are of potential significance in predicting evolutionary change by selection in natural populations as well as in livestock (VAN VLECK, ST. LOUIS and MILLER 1977; FALCONER 1981; ATCHLEY and NEWMAN 1989; KIRKPATRICK and LANDE 1989). Herein, an embryo transfer experiment is described which quantifies the magnitude and postnatal dynamics of the effects of uterine genotype on the expression of quantitative polygenic traits in laboratory mice. The impact of prenatal maternal effects on reproductive traits is described elsewhere (POMP *et al.* 1989).

MATERIALS AND METHODS

Four strains of mice were utilized in this experiment. All mice were obtained from The Jackson Laboratory, Bar Harbor, Maine, including the inbred strains C3HeB/FeJ and SWR/J, an F_1 hybrid between them (C3SWF1/J), and an unrelated F_1 hybrid between the inbred strains BALB/cJ and C57BL/6J (CB6F1/J). Inbred strains were chosen over random-bred strains because the variation within an isogenic inbred strain is completely environmental, whereas

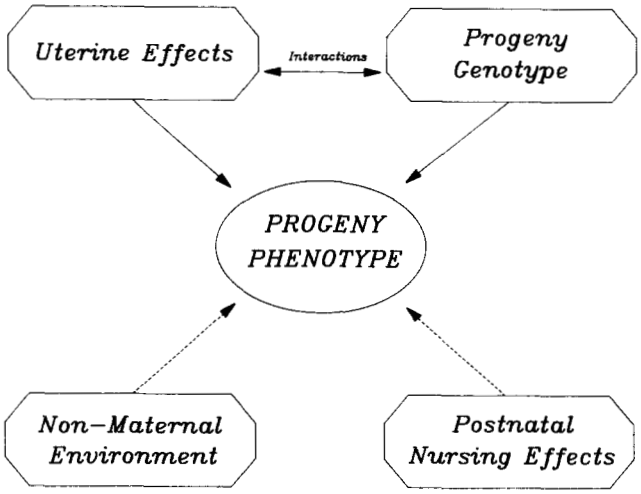

FIGURE 1.—Contribution of maternal, environmental and direct genotype effects on progeny phenotype. Dashed arrows indicate effects held constant in the experimental design. All other possible interactions between major components which are not shown were assumed to be zero.

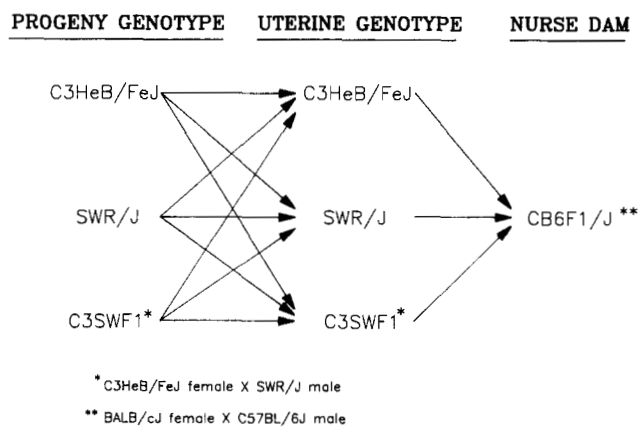

FIGURE 2.—Experimental design for reciprocal embryo transfers. At parturition, pups were fostered to CB6F1/J females with postnatal litter size standardized to six pups. A total of 148 progeny was produced.

the variation between strains is genetic. C3H and SWR were chosen because they differ significantly in adult body size and coat color pigment and they are not closely related through genealogy. ATCHLEY, NEWMAN and COWLEY (1988) have shown that C3H and SWR differ at many major gene loci described by GREENE (1981) and that these two strains also differ widely in polygenic craniomandibular traits. Furthermore, C3H and SWR differ in genotype at the major *H2* histocompatibility locus as well as at the minor *H1*, *H4*, *H7* and *H12* histocompatibility loci (GREENE 1981).

A replicated embryo transfer experiment (Figure 2) was carried out where embryos were reciprocally transferred between C3H, SWR and C3SWF1. Details of embryo recovery and transfer were given by POMP *et al.* (1989). Contemporaneous control litters were also produced without embryo transfer. All pups, both transfer and control, were postnatally nursed by mothers from the unrelated isogenic CB6F1 strain that had given birth the same day. These nurse mothers provided a uniform postnatal maternal environment for all experimental progeny.

This experimental design enabled measurement of genetic uterine effects without extraneous postnatal environmental biases. This design separated uterine effects from direct progeny genotype effects by standardizing postnatal nursing effects and postnursing environmental effects across all genotypes. There were two control comparisons for these embryo transfers. First, embryos were transferred into mice of the same inbred genotype. Second, contemporaneous natural litters were produced without embryo transfer. The sample sizes for each cell of this experiment are given in Table 1.

TABLE 1

Number of pups born by donor (progeny genotype) and recipient (uterine genotype) categories

	Recipients			
Donors	C3H	C3SWF1	SWR	Controls[a]
C3H	18	38	10	104
C3SWF1	15	21	16	132
SWR	8	16	6	95

[a] Contemporaneous control litters produced without embryo transfer.

Beginning at 12 days post-transfer, recipient females were checked twice daily for parturition. On the day of parturition (day 0), litter size (including both live and dead pups), pup weights and tail lengths were recorded, and pups were fostered to CB6F1/J females. All litters were standardized to 6 pups on day 0. If necessary, a foster mother's own pups were used to reach a postnatal litter size of 6 when fewer than 6 pups were born in an embryo-transfer litter. Mice were housed in cages on hardwood shavings with feed and water supplied *ad libitum*. Room temperature was maintained between 23° and 26° with a controlled light:dark cycle (12 hr:12 hr).

Progeny weights and tail lengths were recorded at birth and at 3, 6, 9, 12, 15, 18, 21, 28, 35, 42, 49, 56, 63 and 70 days of age. Body weight at each age was recorded as the arithmetic mean of 15 measurements on an electronic programmable balance. Growth rates were expressed as gains between 3 and 12 days, 12 and 21 days, 21 and 42 days, and 42 and 63 days. These intervals mark important events during ontogeny, *i.e.*, eye opening occurred at about 12 days, weaning occurred at 21 days and most postweaning growth was between 21 and 42 days. Age and body weights were also recorded for traits representing developmental landmarks, *i.e.*, ear, eye and vaginal opening.

The fixed-effects linear model for trait Y in the analysis of variance is

$$Y = \mu + \text{REP} + \text{SEX} + p + u + (\text{SEX} \times p) + (\text{SEX} \times u) + (p \times u) + b(LS) + \text{residual}$$

where μ is the overall mean, REP is replicate, p is progeny genotype, u is uterine genotype, LS is the covariate uterine litter size measured as the sum of live and dead pups on the day of parturition and b represents the regression coefficient on the covariate litter size. The two degrees of freedom associated with uterine genotype and with progeny genotype were partitioned into single degree of freedom linear contrasts for inbreds and for heterosis.

Differences between inbred strains were computed as the difference in their least squares means, *i.e.*, C3H minus SWR, and plotted as a percent of the mean of the inbred lines (Figures 3c and 4c). Negative values indicate that C3H had a smaller least squares mean than did SWR. Heterosis was calculated as the least squares mean of the hybrid strain, C3SWF1, minus the least squares mean of the inbred parental strains C3H and SWR and expressed as a percent of the mean of the inbred strains.

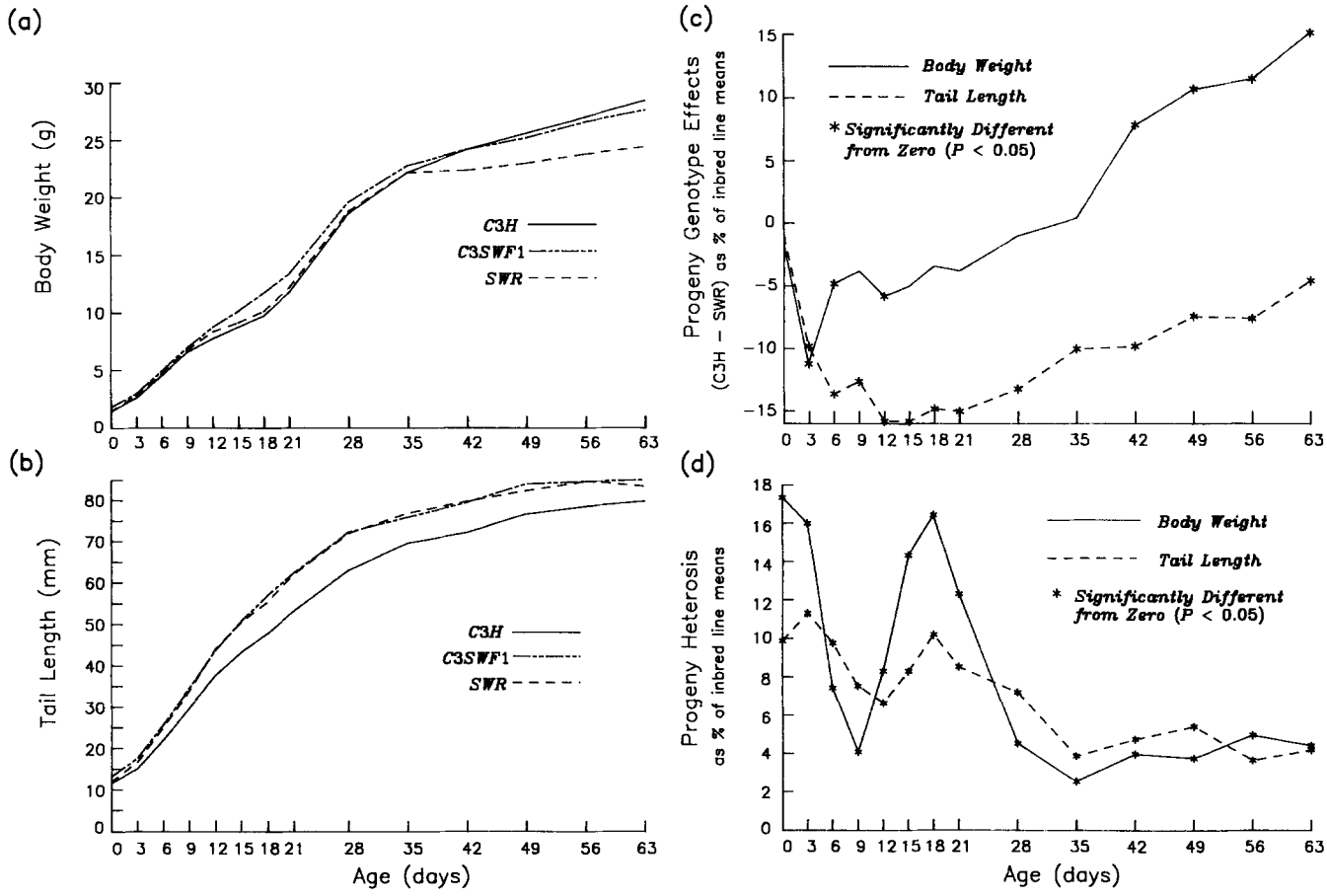

FIGURE 3.—Progeny genotype effects independent of uterine and postnatal maternal effects. (a) Growth curves for body weight. (b) Growth curves for tail length. (c) Inbred line difference computed as C3H minus SWR and expressed as a percent of the mean of C3HeB/FeJ and SWR/J strains. (d) Percent progeny heterosis expressed as a percent of the inbred strain means. Note that (a) through (d) are based on the least squares marginal means for progeny genotype.

All analyses used partial sums of squares to obtain estimates of each fixed effect independent of all other model components. Statistical analyses were carried out using the PC-1 LSMLMW program (HARVEY 1987).

RESULTS

Effect of embryo transfer: In an embryo transfer experiment to assess the role of maternal genotype, one of the critical issues is whether the act of transferring embryos from one female to another produces a phenotypic effect in the progeny. To test this hypothesis, we compared the performance of progeny produced by transferring isogenic embryos between females of the same genotype with contemporaneous control matings produced without embryo transfer. Thus, C3H progeny produced by embryo transfer to C3H mothers were compared to C3H progeny produced by their natural mother. An equivalent comparison was made for SWR mice. To test the effect of embryo transfer on growth of C3SWF1 mice, the C3SWF1 embryos transferred into C3H uteri were compared to the C3SWF1 control litters. This is because the control C3SWF1 litters were made by mating an SWR male with a C3H female and the resulting C3SWF1 progeny were gestated in a C3H uterus. These comparisons are free of postnatal maternal effects since both embryo-transfer and control litters were nursed by the isogenic C6BF1 females.

In general, the results indicate that the embryo transfer procedure had modest but variable effects on the expression of traits in the progeny mice (Table 2). For body weight, embryo transfer had no effect on C3H mice. SWR embryos that were transferred resulted in progeny that were 0.75 g *heavier* than SWR control animals between 3 and 9 days. At all other ages, there were no significant differences between transferred and control SWR mice. The hybrid strain, C3SWF1, displayed a slightly negative effect on body weight at 12, 18 and 21 days ($P < 0.05$) but not thereafter.

For tail length, C3H progeny from embryo transfers had significantly shorter tails (about 2 mm, $P < 0.05$) than controls from 21 through 49 days, but did not differ from controls at other ages. SWR transfer progeny differed significantly from controls ($P < 0.05$) only at 49 and 63 days and had tails about 2.5 mm shorter. The C3SWF1 mice showed no significant embryo transfer effect on tail length.

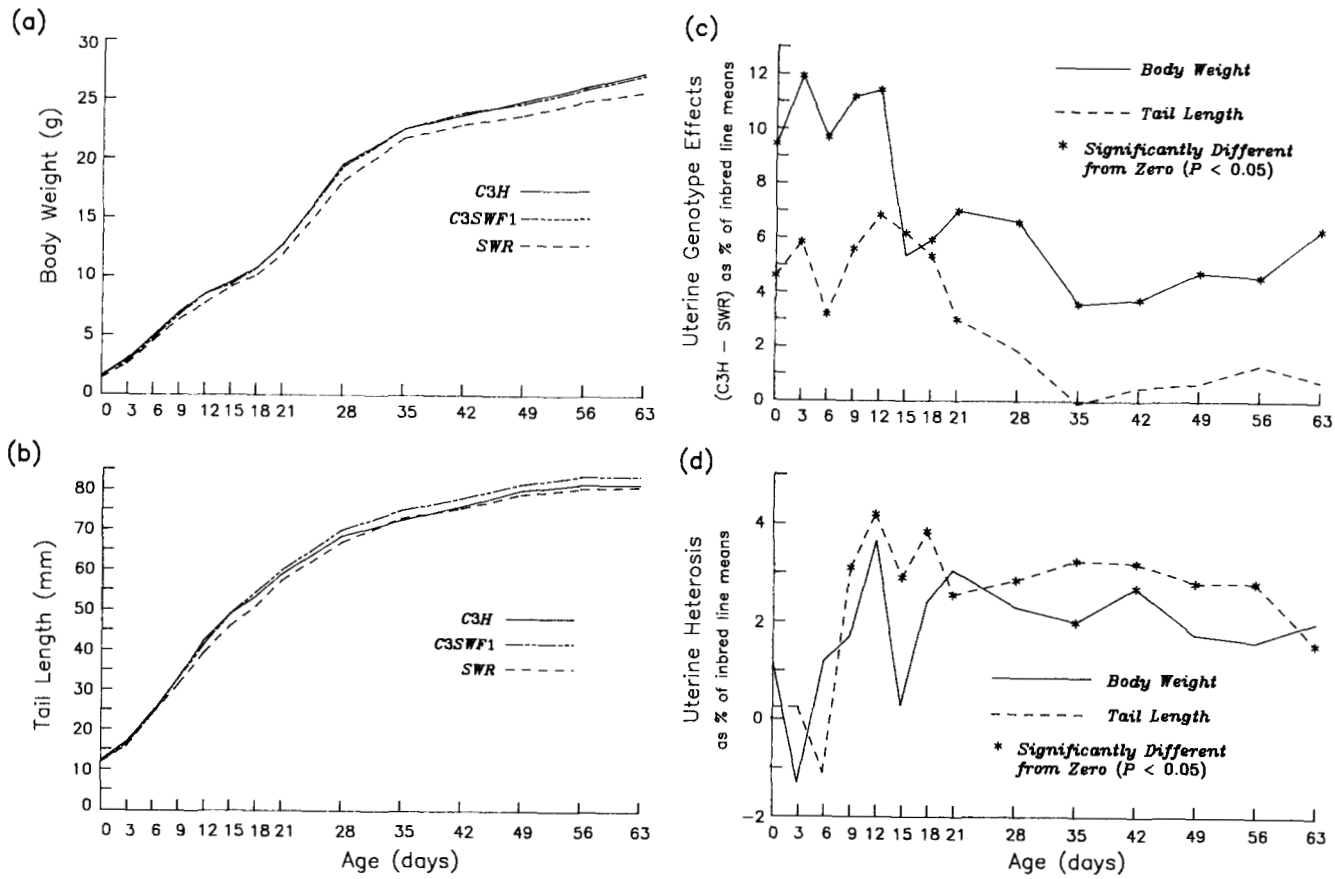

FIGURE 4.—Impact of uterine genotype effects independent of progeny genotype and postnatal maternal effects. (a) Growth curve for body weight. (b) Growth curve for tail length. (c) Difference in inbred line uterine effects (C3H − SWR) as a percent of the mean of the inbred strains. (d) Percent uterine heterosis. Note that (a) through (d) are based on the least square marginal means for uterine genotype.

For body weight gain, there were no significant differences between transfer and control C3H mice in any interval. However, SWR mice exhibited a significant effect with regard to body weight gain from 3 days to 6 weeks, but not thereafter. The results for SWR were interesting in that SWR mice produced by embryo transfer had smaller body weight gains in the 3–12-day and 12–21-day intervals but had greater weight gains in the 21–42-day intervals.

For tail length gains, the effect of embryo transfer was significant for C3H only between 42 and 63 days ($P < 0.01$), and transfer mice had a greater gain for tail length than did the control mice. With SWR, the transfer mice were significantly different from the controls ($P < 0.05$) for the 12–21-day and 42–63-day intervals. In both instances, the transfer mice had smaller tail length gains. Tail length gains of C3SWF1 mice were not influenced by embryo transfer.

Only two developmental landmark traits were influenced by the embryo transfer procedure. SWR embryo transfer progeny had earlier ear opening (about ½ day, $P < 0.01$) and decreased body weight at eye opening (about 6% or 0.5 g, $P < 0.05$). Expression of developmental landmark traits in C3H and C3SWF1 progeny were not influenced by the embryo transfers.

Effects of uterine litter size: Uterine litter size for the embryo transfer litters, measured as the sum of both live and dead pups at birth, had an average of 4.4 pups with a standard deviation of 1.8. This was about half the size of naturally produced contemporaneous control litters which had a mean size of 8.4 pups and a standard deviation of 1.8. Uterine litter size had a highly significant linear and negative effect on many traits which was uniform across inbred strains (slopes homogeneous). Differences in uterine litter size affected body weight ($P < 0.01$) from birth through 63 days, age and weight at ear and eye opening, and weight at vaginal opening. Increased uterine litter size reduced ($P < 0.01$) tail length through 42 days, and growth rates for weight and tail length over all intervals except 21–42 days. Because of these significant effects, uterine litter size was included as a covariate in all statistical analyses.

Effect of sex: There was sexual dimorphism in body weight ($P < 0.05$, Table 3) at all ages except at 3, 12 and 15 days. Sex effect was also highly significant ($P < 0.001$) for tail length after 28 days (Table 4). Rates of growth of body weight and tail length, measured as incremental gains, were also influenced by sex in all intervals for body weight (Table 3) and over the

TABLE 2

Effect of embryo transfer on body weight, body weight gain, tail length and tail length gain

Age (day) or interval	Body weight in strain: C3H	C3SWF1	SWR	Tail length in strain: C3H	C3SWF1	SWR
0						
3			*** (+)			
6			*** (+)			
9			** (+)			
12		* (−)				
15						
18		* (−)				
21		* (−)		* (−)		
28				*** (−)		
35				* (−)		
42				*** (−)		
49				** (−)		* (−)
56						
63						** (−)
—*GAINS*—						
3–12			** (−)			
12–21			* (−)			* (−)
21–42			*** (+)			
42–63				* (+)		* (−)

A "+" indicates that the trait value is larger for the transfer animals as opposed to the control. A "−" indicates that the trait value is smaller for the transfers than for the controls. Table entries symbolically represent significance levels in the ANOVA, *i.e.*, * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

21–42-day interval for tail length. Sex was also an important effect ($P < 0.01$) for body weight at ear and eye opening, but did not influence the age at which these developmental landmarks occurred (Table 5). In general, males of all three progeny genotypes (C3H, C3SWF1 and SWR) were heavier than females of the same strain, and males also had longer tails. There were also significant interactions between sex and progeny genotype and between sex and uterine genotype for several traits. The nature of these interactions is described in more detail below.

Effects of progeny genotype: Progeny genotype was a highly significant effect in the analysis of variance for body weight, tail length and their respective gains (Tables 3 and 4) as well as the expression of developmental landmark traits (Table 5). Figure 3, a and b, shows the growth curves for body weight and tail length, based on least squares marginal means for progeny genotype. The percent difference in the two inbred progeny genotypes as well as the percent heterosis during postnatal ontogeny for body weight and tail length are also described in Figure 3, c and d.

Independent of uterine genotype and after adjustment for differences in litter size at birth, mice of the two inbred genotypes exhibited different growth patterns for body weight and tail length (Figure 3, a and b). SWR mice were heavier than C3H mice between 3 and 12 days ($P < 0.05$). Between 12 and 42 days, these strains did not differ in weight; however, after 42 days C3H mice were larger ($P < 0.05$) and were about 4 g heavier at 63 days. C3H and SWR mice did not differ in tail length at birth. After 3 days, SWR had longer tails ($P < 0.05$) and had tails about 4 mm longer than C3H mice at 63 days.

C3SWF1 were heavier than either C3H or SWR from birth to 42 days. Thereafter, body weight of C3SWF1 mice did not differ from C3H. C3SWF1 mice also had longer tails from birth to 9 days, but after 12 days, tail length for the C3SWF1 strain was generally not different from that for the SWR mice. Thus, significant ($P < 0.05$) positive progeny heterosis occurred for both body weight and tail length throughout postnatal ontogeny.

Over all genotypes, ear opening occurred at 12 days on average and eye opening was observed nearly ½ days later on average (Table 6). The average age at vaginal opening was 16 days. There were significant inbred line differences in progeny genotype effects on age at ear, eye and vaginal opening. C3H mice had earlier eye and vaginal opening but later ear opening than did SWR mice (Table 6). The inbred genotypes also differed in body weight at eye and vaginal opening. Progeny genotype heterosis was observed for all developmental landmark traits ($P < 0.01$) except vaginal opening, and the level of heterosis was marginally significant ($P < 0.08$) for that trait.

Significant interactions between sex and progeny genotype occurred for body weight at 56 and 63 days and for body weight gain from 42 to 63 days (Table 3). At 56 days, the interaction was due to males being heaviest when they were from strain C3SWF1, whereas the heaviest females were C3H mice. At 63 days, C3H and C3SWF1 males were equivalent in body weight and larger than SWR males, while there was a clear ranking in body weight for females (C3H > C3SWF1 > SWR). An analogous situation was responsible for the sex × progeny interaction for body weight gain at 42–63 days. However, the rankings of body weight gains for females differed from the former (C3H > SWR > C3SWF1).

For tail length, significant interaction between sex and progeny genotype occurred only at 35 and 56 days (Table 4). At 35 days, SWR males had longer tails than males from C3H or C3SWF1. In contrast, C3SWF1 and SWR females had similar tail lengths but both had longer tails than C3H females. At 56 days, C3SWF1 males had greatest tail length, whereas SWR females had longest tails for that sex.

Effects of uterine genotype: Does being reared in the uterus of a different genotype affect the magnitude of differences in weight and tail length or their rates of growth? Uterine genotype was a significant effect in the analysis of variance ($P < 0.05$) for body weight at all ages except 15 and 18 days (Table 3) and for tail length at all ages except 63 days (Table 4).

TABLE 3

Analysis of variance for body weight and body weight gain from birth to 9 weeks of age

Source	d.f.	Age (days)														Gains (intervals)			
		0	3	6	9	12	15	18	21	28	35	42	49	56	63	3–12	12–21	21–42	42–63
Replicate	1		*				**				*	*	***	***					
Sex	1	*		*	*			**	***	***	***	***	***	***	***	*	***	***	***
Progency genotype	2	***	***	***	*	***	***	***	***	**	*	***	***	***	***	*	***	***	***
Uterine genotype	2	***	***	***	***	***			*	**	*	**	*	*	**	*			*
Inbreds	1	***	***	***	***	***		*	**	***	***	**	**	**	***	*			**
Heterosis	1										*	*				*			
$p^a \times$ sex	2													**	**				***
$u^b \times$ sex	2										***	**	**	**	**			**	
$p \times u$	4			**	**		***	***	***	**						***	***	**	
Litter size[c]	1	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***		*
Residual (d.f.)		131	124	121	121	120	119	120	120	120	120	119	120	120	118	120	120	119	117
Total (d.f.)		147	140	137	137	136	135	136	136	136	136	135	136	136	134	136	136	135	133

[a] p is abbreviation for progeny genotype.
[b] u represents uterine genotype.
[c] Linear regression on the covariate litter size at birth.
* ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

TABLE 4

Analysis of variance for tail length and tail length gains from birth to 9 weeks of age

Source	d.f.	Age (days)														Gains (intervals)			
		0	3	6	9	12	15	18	21	28	35	42	49	56	63	3–12	12–21	21–42	42–63
Replicate	1						***	**	***	**				***	*	***	***	***	**
Sex	1									*	***	***	***	***	***			***	
Progency genotype	2	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	*	***
Uterine genotype	2	*	*	*	**	***	***	***	**	**	***	***	**	***		**		*	
Inbreds	1	**	**	*	***	***	***	***	**							*			**
Heterosis	1				*	**	**	***	**	**	***	***	***	***	*	***			
$p \times$ sex	2										*			*					
$u \times$ sex	2						*							*					
$p \times u$	4								**	**		*							*
Litter size	1	***	***	***	***	***	***	***	***	*		*				***	***		**
Residual (d.f.)		127	125	121	121	121	121	120	120	120	118	119	120	120	119	120	120	119	118
Total		143	141	137	137	137	137	136	136	136	134	135	136	136	135	136	136	135	134

* ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

Body weight gain was impacted by the uterine genotype between 3 and 12 days and between 42 and 63 days ($P < 0.05$). Uterine effects on tail length gains were important between 3 and 12 days and 21 to 42 days ($P < 0.05$).

Growth curves of progeny gestated by mothers of the three different uterine genotypes are shown in Figure 4, a and b. These curves are based on the least squares marginal means for uterine genotype. In general, differences in growth of progeny imparted by the three uterine genotypes are less than that observed for progeny genotype (Figure 3, a and b). This is especially true for tail length.

Irrespective of genotype, pups that developed in uteri of the strain with larger body size (C3H) were always heavier on average than pups that developed in uteri of the strain with smaller body size (SWR). For body weight, the proportion of inbred line differences due to uterine genotype effects varied from 9 to 12% of the mean from birth to 12 days (Figure 4c) and decreased to 4–7% from 15 to 63 days. These inbred line differences for uterine effects were significant at all ages except 15 days.

Uterine genotype effects on tail length (Figure 4c) ranged from 3 to 7% of the mean from birth until weaning (21 days, $P < 0.05$) and were not different from zero thereafter. Mice reared in the uterus of C3H females had longer tails from birth to 21 days. Thereafter, mice which developed in SWR females achieved equivalent tail lengths to those reared in C3H uteri.

Differences between inbred uterine genotypes accounted for about 8% of the average weight gain of 5.5 g from 3 to 12 days ($P < 0.05$) and about 26% of the average gain of 3.3 g from 42 to 63 days ($P < 0.01$). The postnatal pattern of uterine genotype ef-

TABLE 5

Analysis of variance for developmental landmarks

Source	d.f.	Ear opening		Eye opening		Vaginal opening	
		Age	Weight	Age	Weight	Age	Weight
Replicate	1			*	*		*
Sex	1		**		***		
Progeny genotype	2	***	***	***	***	***	***
Uterine genotype	2	***	**	***	**		
Inbreds	1		**	***	**		
Heterosis	1	**	**		**		
$p \times$ sex	2						
$u \times$ sex	2						
$p \times u$	4	***	**	**	*		**
Litter size	1	*	***	**	***		
Residual (d.f.)		119	119	119	119	56	56
Total (d.f.)		135	135	135	135	67	67

* ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

fects on tail length gains was similar to that observed for weight gains. From 3 to 12 days, uterine genotype effects were about 7% of the average gain of 24.6 mm in tail length ($P < 0.05$), but were not significant thereafter. Weight and tail length gains were generally greater for embryos that developed in uteri of the large-body strain (C3H).

Uterine heterosis for body weight was 2 to 3% of the mean after eye opening (about 12 days) and for tail length was about 3% after 6 days (Figure 4d). Percent uterine heterosis was different from zero for body weight only at 35 and 42 days ($P < 0.05$). For tail length, uterine heterosis was significant ($P < 0.05$) after 6 days. This contrasts with the nonsignificant inbred line differences for tail length after 21 days, and the magnitude of heterosis is sufficient to yield significance in the overall uterine genotype effect in the analysis of variance (Table 4). In general, uterine heterosis was positive, indicating superiority of the hybrid uterus over the mean performance of uteri of either inbred strain. Uterine heterosis for both weight and tail length gains was positive and significant ($P < 0.05$) only in the 3–12-day interval.

Differences between inbred lines were observed for age and body weight at ear and eye opening, but inbred line differences were not observed for age or weight at vaginal opening. Uterine heterosis was also observed for age and weight at ear and eye opening. In general, mice reared in a C3H uterus had earlier age and greater body size at ear and eye opening (Table 6).

Interactions between uterine and progeny genotypes were significantly different from zero ($P < 0.01$) from 6 to 28 days for body weight and from 21 to 42 days for tail length. For body weight, the significant interaction at 6 days arose from SWR mice being heavier when developed in SWR uteri, whereas C3H and C3SWF1 mice were heavier when developed in C3H uteri. At 9 through 28 days, uterus × progeny interaction resulted from superior performance of hybrid mice reared in hybrid or SWR uteri. Both C3H and SWR mice were heavier at these ages when they developed in C3H uteri. Significant uterine × progeny interactions for tail length at 28 and 42 days involved superior performance of the hybrid mice when reared in SWR uteri. In contrast, C3H and SWR mice had longer tails at these ages when developed in a hybrid uterus.

Highly significant interactions between uterine genotype and sex of the progeny occurred for body weight at 35, 42, 49, 56 and 63 days ($P < 0.001$) and for weight gain between 21 and 42 days ($P < 0.01$). Male mice, irrespective of progeny genotype, were consistently larger when they had been gestated in hybrid uteri. In contrast, female mice were consistently heavier when they developed in C3H females. Both male and female mice were smallest when they developed in the uteri of SWR females.

Effects of histoincompatibility: The C3H and SWR strains differ at the MHC *H2* locus and at the minor *H1*, *H4*, *H7* and *H12* loci (GREENE 1981). Therefore, these strains offer the possibility of investigating the influence of histoincompatibility between fetus and mother on postnatal growth. A linear contrast was used to test, across all categories of embryo transfer, whether histoincompatibility between fetus and mother resulted in enhanced postnatal growth. This linear contrast compares syngenic (histocompatible) embryo transfer categories with allogenic (histoincompatible) categories. For body weight there was no effect of histoincompatibility at birth but syngenic transfer progeny had significantly heavier weights at 3, 6 and 9 days. At 15 through 28 days, allogenic transfer progeny were heavier than syngenic transfer progeny. For tail length, allogenic transfer progeny had significantly longer tails than syngenic transfer progeny ($P < 0.05$) at 15, 18, 21, 28, 42, 49 and 63 days.

To test whether these results could simply be a result of heterozygosity of the mother, a second linear contrast was used to compare inbred embryos transferred into the same genotype against inbred embryos transferred into hybrid C3SWF1 uteri. This latter contrast should test for the effect of heterozygosity of the uterine mother for syngenic embryo transfers. The results generally parallel those described above for the contrast comparing syngenic and allogenic embryo transfers.

DISCUSSION

This experiment demonstrates that the genotype of the mother providing the uterine developmental environment significantly influences postnatal growth and adult body size of her progeny. Biotechnologies

TABLE 6

Least squares means for age and body weight at occurrence of developmental landmark traits

Trait	Progeny genotype			Uterine genotype			Overall mean
	C3H	C3SWF1	SWR	C3H	C3SWF1	SWR	
Age (days) at:							
Ear opening	12.42	11.89	11.97	11.91	12.26	12.11	12.09
	±0.07	±0.06	±0.08	±0.06	±0.06	±0.09	±0.04
Eye opening	12.35	12.04	12.87	12.12	12.51	12.62	12.42
	±0.07	±0.06	±0.08	±0.06	±0.06	±0.10	±0.04
Vaginal opening	15.54	13.52	18.98	15.45	16.44	16.14	16.01
	±0.45	±0.38	±0.55	±0.47	±0.30	±0.59	±0.27
Body weight (g) at:							
Ear opening	8.11	8.83	8.38	8.62	8.71	7.99	8.44
	±0.13	±0.11	±0.14	±0.11	±0.11	±0.17	±0.08
Eye opening	8.08	8.96	8.71	8.74	8.85	8.16	8.58
	±0.13	±0.11	±0.14	±0.11	±0.10	±0.16	±0.08
Vaginal opening	9.00	9.27	10.69	9.66	10.03	9.27	9.65
	±0.28	±0.23	±0.34	±0.29	±0.19	±0.36	±0.17

relying on embryo transfer (*e.g.*, *in vitro* fertilization, gene transfer, cloning and cryopreservation) should therefore consider the potential consequences of prenatal uterine effects. Further, since the efficiency of natural selection depends upon a high correlation between genotype and phenotype, conditioning of the progeny's phenotype by the maternal uterine genotype can reduce the accuracy of predictions about evolutionary change by selection in mammals (FALCONER 1965, 1981; WILLHAM 1972; ATCHLEY and NEWMAN 1989; KIRKPATRICK and LANDE 1989).

Embryo transfer effect: The comparison of progeny produced by embryo transfer with those produced from natural contemporaneous litters demonstrates that the observed genetic uterine effects are not an artifact of the experimental procedure. The comparison of transfer against control litters within inbred strains reveals that strain differences exist in the sensitivity to embryo transfer. In this experiment, the SWR strain appears to be more sensitive to embryo transfer than does the C3H strain. Data on embryo survival and pregnancy rate from this experiment (POMP *et al.* 1989) indicates that SWR also has a generally lower reproductive performance than C3H. However, in spite of these strain differences, embryo transfer is a useful tool for partitioning the components of maternal effects on mammalian development.

Uterine effects: In this study, uterine effects were a significant influence at nearly all ages on both body weight and tail length. The surprising result here was the persistence of uterine effects on progeny into adult ages. These results are in contrast to previous reports suggesting that uterine effects on body weight disappear after about 2 weeks of age (MOORE, EISEN and ULBERG 1970). Other authors have failed to find evidence of prenatal uterine effects on body size of mice (BRUMBY 1960; AL-MURANNI and ROBERTS 1978). The results on selection line mice suggest that uterine effects have probably not contributed to direct or correlated selection response. In contrast, the results of this study may indicate that inbreeding has produced lines with different randomly fixed sets of genes affecting maternal uterine performance.

The results described here reflect differences between the inbred C3H and SWR strains in their uterine effects as well as superiority of the hybrid C3SWF1 uterus over the mean of the two inbred strains. The inbred line differences demonstrate genetic determination of the uterine effects, whereas the performance of hybrid uteri suggests at least some nonadditive gene action involved in prenatal maternal performance.

The importance of uterine genotype effects relative to progeny genotype effects varies not only with age but also with the trait under consideration and whether inbred or hybrid individuals are considered. For body weight in an inbred line comparison, uterine effects are more important than the progeny's own genotype from birth to about 35 days, but thereafter progeny genotype effects dominate. In contrast, from 3 days through all later ages progeny genotype is a more important determinant of inbred line differences in tail length. When comparing heterosis of hybrid C3SWF1 mice, progeny heterosis exceeds uterine heterosis at all ages for body weight and tail length. In situations where postnatal maternal effects are not controlled as they are in the present experiment, one might expect uterine effects to be a smaller overall determinant of progeny phenotype, especially when postnatal fraternity size is not standardized.

This is the first study to demonstrate heterosis in uterine effects on continuously varying morphological traits. IIDA, MIZUMA and NAGAI (1987) failed to find significant uterine heterosis for survival of transferred embryos. Significant uterine heterosis for body weight

was limited to a brief period from 35 to 42 days of age. In contrast, uterine heterosis for tail length was significant at all ages after 6 days. It is interesting that uterine heterosis was exhibited by a skeletal trait, tail length, but generally not for body weight. Studies are underway to determine if uterine effects have a general impact on skeletal traits.

In general, irrespective of their own genotype, pups which developed in the uteri of the larger body size C3H mice were heavier on average than pups which were gestated by the smaller size SWR females. Further, mice produced by embryo transfer into C3H mothers had longer tails until weaning, earlier ear and eye opening and greater body weights at ear and eye opening. ROTH and KLEIN (1986) reported similar uterine effects on body size for embryo transfers between the smaller body size *Peromyscus maniculatus gambelii* and the larger body size *P. m. santacruzae*. However, the latter study made no adjustment for uterine litter size or sex of progeny, and further comparison of results are not possible. In comparing lines selected for high (H) and low (L) 6-week body weight, MOORE, EISEN and ULBERG (1970) reported that the uterine environment provided by the H mothers was superior to that of the L females.

An important but yet unresolved question involves which maternal factors operating *in utero* are responsible for the observed differences in fetal growth. These factors can include maternal hormones, uterine space and uteroplacental blood flow of substrates and nutrients (MCLAREN and MICHIE 1960). Uterine litter size can be visualized as a linear combination of several factors. Litter size at birth is determined by the number of eggs ovulated minus the number of failed fertilizations minus the number of embryos failing to implant minus postimplantation embryonic death. In naturally produced litters in mice, several authors have demonstrated a line of male effect on litter size (FINN 1964; NAGAI, MCALLISTER and MASAKI 1985) which appears to be mediated primarily through male fertility. However, the effect on litter size contributed by the male is generally much smaller than the contribution of the female (FALCONER 1960). MCLAREN and MICHIE (1960) demonstrated that uterine space was more important than embryonic competition for humoral factors circulating in the maternal blood. In contrast to these studies, embryo transfers in this experiment produced litters that were smaller than natural litters. Thus, uterine litter size in this experiment is a reflection of the environmental factors inherent in embryo transfer rather than of genotypic differences in sires.

Our estimates of uterine effects are corrected for one aspect of uterine space, i.e. uterine litter size. The results reported here indicate a strong negative relationship between prenatal fraternity size and body weight, tail length, their respective gains and the expression of developmental landmark traits. KIRKPATRICK and RUTLEDGE (1987) and KIRKPATRICK, ARIAS and RUTLEDGE (1988) found that uterine litter size significantly affected age at vaginal opening and litter size at second parity. All estimates of maternal uterine effects reported here reflect adjustment for differences in uterine litter size.

Histocompatibility effects: Several authors have proposed that when fetus and mother differ at major and/or minor histocompatibility loci, there is an enhancement of uteroplacental blood flow and hence fetal and placental size (BILLINGTON 1964; JAMES 1965; FINKEL and LILLY 1971; BEER, SCOTT and BILLINGHAM 1975; HAMILTON, HAMILTON and HAMILTON 1985). There was no clear indication of an influence due to histocompatibility loci, and this experiment neither refutes nor supports the contentions of other authors. It should be noted, however, that in the previous reports claiming an effect due to histologic incompatibility, no adjustment was made for differences in uterine litter size and sex of progeny and the influence of heterozygosity of the hybrid lines was not discounted. The comparison of syngenic inbred embryo transfers with transfers of inbred embryos into hybrid mothers suggests that effects attributed by some authors to histoincompatibility may in fact stem from heterozygosity of the mother. Furthermore, histocompatibility effects are not consistent with the progeny genotype by uterus genotype interactions observed here.

Cytoplasmic effects: This experiment has not addressed the potential of cytoplasmic effects contributing to progeny performance. However, there are several compelling reasons to believe cytoplasmic effects are not important contributors to progeny performance and that they have not biased our estimates of maternal uterine effects. First, consider that expression of cytoplasmic effects would be contained within the effects of progeny genotype. Because our estimates of uterine effects were obtained independently of progeny genotype effects, cytoplasmic effects are not a bias in their estimates. Second, recent studies have failed to find significant cytoplasmic effects among long-term selection lines of mice (PETTERS *et al.* 1988).

In current usage, cytoplasmic effects are not always synonymous with mitochondrial effects. One might argue that genetic differences between inbred strains might arise through differences in mitochondrial DNA. However, inbred lines of laboratory mice possess highly conserved mitochondrial DNA which lacks restriction fragment length polymorphisms (YONEKAWA *et al.* 1982; FERRIS *et al.* 1983). Therefore, it seems unlikely that cytoplasmic and/or mitochondrial

effects have contributed to the results reported herein.

Evolutionary considerations: The demonstration here of prenatal maternal effects which have a lingering influence on adult progeny phenotype is consistent with the model proposed by ATCHLEY and NEWMAN (1989) which suggests that prenatal as well as postnatal maternal effects may contribute to evolutionary divergence in mammals. The evolution of a quantitative trait under genetic maternal influence depends not only on the heritability of the trait but also the heritability of the maternal effect and the genetic covariance between the trait and the maternal effect. Models have been proposed which describe the evolution of traits under maternal influence (*e.g.*, KIRKPATRICK and LANDE 1989); however, these models rarely consider the distinction of prenatal versus postnatal maternal effects. Existence of both prenatal and postnatal effects further complicates predictions about mammalian evolution since the response to selection includes the heritabilities for the prenatal and postnatal maternal effects, the heritability of the trait in question, and the genetic covariances between the direct effects and prenatal effects, direct effects and postnatal effects, and prenatal and postnatal effects. Therefore, accurate predictions about mammalian evolution need to consider models which incorporate these distinct avenues of maternal influence on progeny phenotype.

In a natural population, one might speculate that factors comprising uterine genotype effects might be under some form of stabilizing selection. A negative maternal effect has been demonstrated to occur in mice (*e.g.*, FALCONER 1965). This negative maternal effect is such that a female of large body size will produce a larger litter than will a smaller female; however, the mice of the larger litter will be smaller in size on average than mice reared in a small litter. In second generation progeny, the maternal effect will be reversed so that small females reared from a large litter will produce smaller litters but ultimately larger daughters. If there exists an optimum body size, then an important control would be realized through stabilizing female reproductive capacity as mediated through her fertility as well as her ability to carry an optimum number of progeny to term.

Conclusions: The inbred SWR strain was more sensitive to the embryo transfer procedure than was the inbred C3H strain. However, strain differences were moderate on the continuously varying traits examined. Uterine effects were noted between the SWR and C3H strains, suggesting genetic differences between these isogenic lines. Uterine heterosis for postnatal body size was relatively small, but persisted through 63 days, indicating mostly additive gene action. Progeny heterosis was larger than uterine heterosis, particularly prior to weaning, indicating the importance of nonadditive gene effects. Presence of progeny × uterine interactions indicate specific growth responses of progeny genotypes which had developed in specific uterine genotypes. While the inbred lines differ at the *H2* MHC locus, progeny × uterine genotype interactions could not be explained by antigenic dissimilarity.

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