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Influence of Early Pregnancy on Reproductive Rate in Lines of Mice Selected for Litter Size*

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Summary. The influence of male-induced early puberty on female reproductive rate was determined in three lines of mice differing in litter size and body weight. The lines originated from a single base population and had undergone 20 generations of selection for the following criteria: large litter size at birth (L^+), large litter size and small 6-week body weight (L^+W^-), or small litter size and large 6-week body weight (L^-W^+). Females were paired with a mature intact male of the same line at 3, 5 or 7 weeks of age. Mean mating age, averaged over lines, was 26.5 ± .3, 38.3 ± .3 and 52.7 ± .3 days. Exposure to a mature male accelerated female sexual maturation in each line. When contrasted with their sibs mated at a later age, early-pregnant females from each line exhibited a decline in one or more component of reproductive performance, suggesting that the physiological state of the very young female was not optimum for normal pregnancy. In comparisons of early and later mating ages, all three lines showed a decreased littering rate at first mating, number born alive, and individual birth weight of progeny adjusted for litter size; L^+ and L^+W^- mice showed an increased perinatal mortality rate; L^+ and L^-W^+ had a reduction in litter size at birth. When the L^+, L^+W^- and L^-W^+ lines were compared with an unselected strain and a line selected for high postweaning gain in similar experiments, a genotype by environment interaction was apparent since all lines did not respond in a similar manner to early mating. The line ranking for litter size at birth for each age at male-exposure was $L^+ > L^+W^- > L^-W^+$, despite the significant line by age interaction. When litter size was adjusted by covariance for body weight at mating, the significant effects of age at male-exposure and line by age interaction were eliminated. All fertile females were remated after they had weaned their first litter to obtain information on litter size in parity two. Line differences in litter size at birth and number born alive were uniform across parities. An age by parity interaction was evident since the decreased fecundity at younger ages of male exposure in the L^+ and L^-W^+ litters of parity one was not evident in parity two. Litter feed efficiency during first parity gestation was defined as litter birth weight divided by either cumulative feed intake of the dam from mating to parturition (GEI) or cumulative feed intake from weaning to parturition (GEII). The ranking of lines for GEI and GEII was $L^+ > L^+W^- > L^-W^+$, but when feed efficiency was adjusted for littering rate, L^+W^- and L^-W^+ were not significantly different. With regard to age at mating, the ranking for GEI (7 wk > 5 wk > 3 wk) was reversed from GEII (3 wk > 5 wk > 7 wk) and these significant differences were maintained after adjustment for littering rate.

Key words: Mice – Early puberty – Litter size – Selection – Reproductive rate – Pheromone

Introduction

Acceleration of puberty in female mice can be accomplished by exposing them to mature intact male mice or their urine (Vandenbergh 1973; Vandenbergh et al. 1975). The early pubertal response is triggered by the action of a male urinary pheromone and tactile cues from the male, which stimulate luteinizing hormone release and increase synthesis of estrogen by the female (Bronson and Desjardins 1974; Drickamer 1974; Bronson, 1975; Bronson and

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Maruniak 1975; Vandenbergh et al. 1975). When females from a random bred unselected strain (ICR) were induced to reach puberty in the presence of a mature male, mating during their first estrus did not adversely affect fertility, litter size at birth or postnatal maternal performance (Vandenbergh et al. 1972; Eisen 1973, 1975; Eisen et al. 1977). In a line of mice (M16) selected for rapid growth rate and exhibiting a positive correlated response in litter size at birth, early pregnancy in male-induced precocious females resulted in a reduction in litter size at birth compared to primiparous females mated at about 7 to 8 weeks of age (Eisen 1977). The reduced litter size was the consequence of a smaller body weight at mating, possibly resulting in a reduced ovulation rate. These results are an example of a genotype by environment interaction and illustrate the need for caution in extrapolating results due to complex environmental stimuli from one genetic strain to another. They raise the immediate questions of what the effect of early mating is on litter size in a line selected directly for large litter size and to what extent, if any, early pregnancy in a highly fecund line influences litter size in the subsequent parity.

This paper examines the influence of male-induced early puberty on female reproductive rate in three selected lines of mice that differ markedly in litter size and body weight.

Materials and Methods

The three genetic stocks used in this experiment have undergone mass selection for 20 generations. The base population was a randombred substrain of ICR mice (Eisen and Hanrahan 1974). The L^+ line was selected directly for large litter size at birth. The other two lines were selected indirectly for litter size using a selection index. The L^+W^- line was selected for large litter size and small 6-week body weight while L^-W^+ was selected for small litter size and large 6-week body weight. Litter size at birth was defined as the total number of live plus dead pups born, and excluded litters of zero due to infertility. Only primiparous females mated at 8 to 10 weeks of age were used during the selection phase, and their litters were standardized to eight pups at one day of age. Further details of the selection experiment have been reported by Eisen (1978). Females used in the present experiment were reared by their own mothers in standardized litters of eight pups. At least two males were assigned to each litter because female mice reared in litters with zero or one male mature earlier than those reared with two or more males (Drickamer 1976). Litters were weaned at 21 days of age. Weekly body weights and feed intakes were recorded for the individually caged females until a mature male was

Table 1. Mean weekly body weights, feed intakes and feed efficiencies for virgin females of each line

	Body weight (g)				
Line[d]	3 wk	4 wk	5 wk	6 wk	7 wk
L^+	16.3[a]	24.3[a]	28.9[a]	29.7[a]	30.9[a]
L^-W^+	16.4[a]	25.2[a]	28.9[a]	30.3[a]	32.3[b]
L^+W^-	12.7[b]	18.9[b]	21.4[b]	22.5[b]	23.5[c]
S.E.[e]	0.3	0.3	0.3	0.4	0.4
	Feed intake (g/day)				
Line[d]	3 to 4 wk	4 to 5 wk	5 to 6 wk	6 to 7 wk	
L^+	4.9[a]	6.0[a]	5.8[a]	5.8[a]	
L^-W^+	5.1[a]	6.1[a]	5.8[a]	6.0[a]	
L^-W^+	4.4[b]	4.9[b]	4.8[b]	4.9[b]	
S.E.[e]	0.06	0.08	0.09	0.08	
	Feed efficiency (100 g/g)				
Line[d]	3 to 4 wk	4 to 5 wk	5 to 6 wk	6 to 7 wk	
L^+	23.5[a]	10.9[a]	2.7[a]	3.0[a]	
L^-W^+	24.8[a]	8.6[b]	3.0[a]	4.9[b]	
L^+W^-	20.2[b]	7.1[c]	3.1[a]	2.8[a]	
S.E.[e]	0.7	0.5	0.6	0.6	

[a,b,c] Column means under the same heading with no letters in common are significantly different at $P < 0.05$

[d] Ranges in sample size: 3 wk body wt, 134 to 138; 4 and 5 wk body wt, 3 to 4 and 4 to 5 wk feed intake and feed efficiency, 83 to 91; 6 and 7 wk body wt, 5 to 6 and 6 to 7 wk feed intake and feed efficiency, 39 to 43

[e] Approximate standard errors

placed in the cage. Since females were not grouped but were caged individually, the Whitten effect whereby grouped females become acyclic would not be expected. Full sisters were randomly allocated to be paired with a mature intact male of the same line at 3, 5 or 7 weeks of age. By using males of the same line it is possible that some differences may have arisen from male effects, but these are assumed to be negligible. Full and half sib matings were avoided. Male and female were continuously paired in a cage for 3 weeks or until a copulatory plug was detected or the female was obviously pregnant. Female mice were examined daily for a copulatory plug. When a plug was detected, the female was weighed and placed in a cage where total feed consumption during gestation was recorded. Beginning on day 18 of gestation, females were checked daily for evidence of littering. Litter size, number born alive and weight of the dam and total litter were recorded on the day of birth. For parturient females in which a copulatory plug was not detected, age at conception was estimated by subtracting 19 days from age at parturition. Dam feed efficiency during gestation was calculated as 100 X (dam weight gain)/(dam feed intake). Litter feed efficiency was calculated as either 100 X litter birth weight divided by dam feed intake during gestation or litter birth weight divided by an estimate of dam feed intake from weaning to parturition. Females having more than 16 pups were reduced to 16 at day of birth, while those with 16 or less were given the opportunity to rear all young that survived. Females reared their own litter until weaning at 21 days. Females were remated to obtain second parity information on litter size and number born alive. These matings were staggered at 2 week intervals to coincide with the differences in the initial age of exposure to a mature male. The mean and standard deviation of female age at their second successful mating for the groups originally exposed to a male at 3, 5 and 7 weeks were respectively 85.3 ± 4.6, 99.7 ± 4.0 and 113.3 ± 4.0 days. The mean number of days elapsed between weaning of the first litter and the second mating was 20. The experiment was conducted in a room maintained at 21 ± 1C, 50 to 60% relative humidity and a 12 hr light/12 hr dark cycle. Females were fed Purina Mouse Chow ad libitum until they weaned their first litter. They were then fed Purina Laboratory Chow until they were placed in a cage with a male to be remated, when Purina Mouse Chow was reintroduced as the ration. The response variables were analyzed by least squares methods for unequal subclass numbers (Harvey 1975). The statistical model included the fixed effects of selected line, age of male-exposure and line by age interaction, and the random effects due to among and within full sib family variation. The approximate F-tests in this split-plot analysis are as follows: (1) the among full sib family mean square is the error term used to test for the significance of line effects and (2) the within full sib family mean square tests for age and line by age interaction effects. Covariates which were added to the model for specific traits are discussed in the results section. Binomally distributed traits were analyzed by the maximum likelihood procedure described by Tallis (1964). Duncan's multiple comparison procedure tested the significance of mean differences, which were declared significant at $P < .05$.

Results

Growth and Feed Consumption of Virgin Females

Weekly body weights and feed intakes of L^+ and L^-W^+ virgin females were similar, apart from a slightly larger 7-week body weight for L^-W^+ females (Table 1). Both the L^+ and L^-W^+ lines were larger and consumed more feed than L^+W^-. Differences in weekly feed efficiency fluctuated among lines, but L^+ and L^-W^+ clearly were more

Table 2. Means for traits of the dam from mating to parturition by line and age at male-exposure

Line[d]	Exposure to mating (days)	Body wt. at mating (g)	Body wt at partur. (g)	Wt. gain mating partur. (g/day)	Feed intake mating to partur. (g/day)	Adj. feed intake mating to partur.[e] (g/day)	Dam feed eff. mating to partur (100 g/g)
L^+	3.8^a	27.6^a	43.4^a	0.84^a	7.6^a	7.3^a	11.0^a
L^-W^+	4.1^a	28.4^b	43.2^a	0.78^b	7.3^b	7.0^b	10.7^{ab}
L^+W^-	4.9^b	22.2^c	35.1^b	0.67^c	6.5^c	7.2^a	10.3^b
S.E.[f]	0.3	0.3	0.4	0.018	0.06	0.06	0.22
Age[d]							
3	5.5^a	21.7^a	39.5^a	0.93^a	7.3^a	7.7^a	12.7^a
5	3.3^b	27.1^b	40.8^b	0.72^b	7.1^b	7.1^b	10.1^b
7	3.7^b	29.4^c	41.9^c	0.66^c	7.1^b	6.8^c	9.3^c
S.E.[f]	0.3	0.2	0.3	0.013	0.05	0.04	0.16
F-test							
L × A	0.18^{NS}	4.78**	1.00^{NS}	6.64**	1.54^{NS}	3.38**	5.21**

NS Not significant; ** $P < 0.01$

a,b,c Column means under the same heading with no letters in common are significantly different at $P < 0.05$

d Ranges in N are 106 to 142 for lines and 97 to 148 for ages

e Adjusted by covariance for metabolic body size of the dam during gestation ($b = 83.7 \pm 6.1$; $R^2 = .41$; $P < 0.01$)

f Approximate standard error

efficient than L^+W^- from 3 to 4 and 4 to 5 weeks and over the entire 3 to 7 week period.

Mating Age and Time Between Exposure and Mating

Means for traits of the primiparous dam from mating to parturition are given in Table 2 by line and age of male-exposure. Only records on females that produced a viable litter following their first mating are included. Mean mating age, averaged over lines, was 26.5 ± .3, 38.3 ± .3 and 52.7 ± .3 days for females exposed to mature males and 3, 5 and 7 weeks of age, respectively. The number of days between male-exposure and first mating was significantly longer in females exposed at 3 weeks of age than at 5 or 7 weeks. This finding is consistent with the report that mean age at first estrus, as measured by the vaginal lavage criterion (Vandenbergh 1969), varied seasonally from 33 to 35 days in a substrain of ICR mice (Drickamer 1977). Female mice exposed to mature males at 3 weeks of age were definitely not sexually mature, and the combined influence of the male urinary pheromone and tactile cues would be expected to initiate the hormonal responses culminating in first estrus followed shortly thereafter by copulation. In contrast, females exposed to males at 5 weeks represent a mixture of pre- and post- first estrus mice. Since most of the former group probably are at the verge of first estrus, presence of a mature male would not be expected to increase the interval from male exposure to mating above that observed in the 7-week-old female group, most of whom would be expected to have had their first estrus prior to male exposure.

Number of days between male exposure and actual mating was significantly longer in L^+W^- females compared with L^-W^+ or L^+. When 3-week-old unselected ICR females were exposed to mature males, a negative phenotypic regression was observed for number of days from exposure to mating on 3-week body weight (Eisen 1975). Since L^+W^- mice were smaller at weaning than L^-W^+ or L^+, there is an indication of a negative genetic regression as well; i.e. the genetically smaller female requires a longer period of stimulation by the male urinary pheromone and/or male tactile cues prior to attaining its first ovulatory cycle. No significant line by age interaction was noted because a longer interval between male exposure and mating also occurred in L^+W^- mice at 5 and 7 weeks. A tendency for this interval to be increased due to selection was reported in L^+W^- females when exposed to males at 8 to 10 weeks during the first 12 generations of selection (Eisen 1978). The delay in the interval from male exposure to mating observed at later ages in L^+W^- females almost certainly involves other unexplored factors which may be associated with the female's estrous cycle or libido in the male.

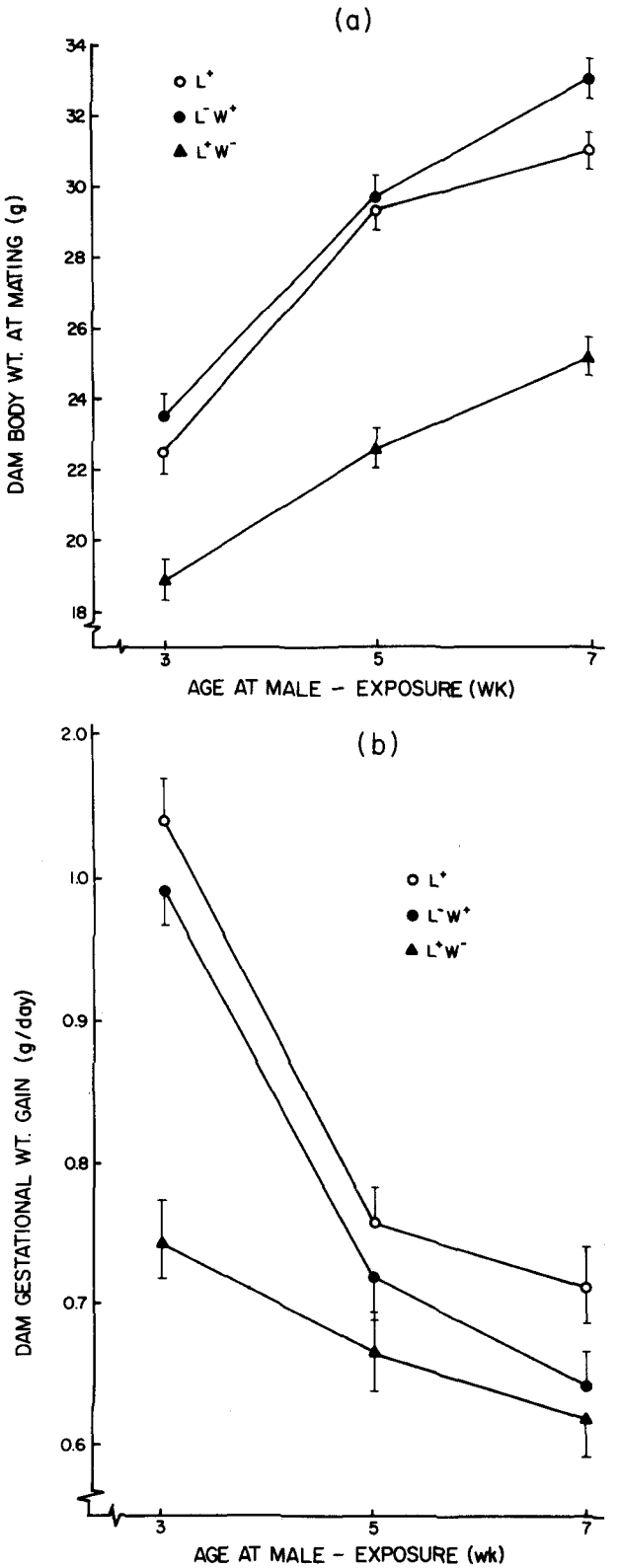

Fig. 1a and b. Line by age subclass means for (a) body weight at mating and (b) weight gain during gestation in first parity females. Vertical lines in this and subsequent figures represent standard errors

Female Growth and Feed Consumption During Gestation

The L^+ and L^-W^+ females had larger body weights at mating and parturition than L^+W^-(Table 2), which agrees with ranking of the lines for weekly body weights of virgin females. The significantly larger body weight of L^-W^+ dams at mating compared with L^+ was eliminated at parturition because the latter dams had a higher gestational weight gain. Body weight at mating increased quadratically with age at male exposure, there being a much higher weight increase at mating when comparing 3-week and 5-week male exposure than when comparing 5-week and 7-week means. A compensatory quadratic effect was observed for gestational weight gain where younger dams gained more weight. Consequently, age at male exposure differences in dam body weight at parturition were reduced, although the ranking of female weights with respect to age at male exposure was maintained. The significant line by age interactions for body weight at mating and gestational weight gain were not caused by a change in the ranking of lines at different ages of male exposure (Fig. 1a, b).

Differences in gestational feed intake among the lines (Table 2) reflect genetic differences in dam body weight and gain during gestation and total fetal mass. The major portion of the line differences due to the dams' requirements for maintenance were removed by adjusting for the dams' metabolic body size during gestation ($W^{\cdot 75} = (W_m^{\cdot 75} + W_p^{\cdot 75})/2$ where W_m and W_p are female body weights at mating and parturition, respectively). The higher adjusted feed intake in L^+ and L^+W^- dams compared with L^-W^+ probably reflects the greater nutritional demand for gestating a larger total fetal mass. However L^+W^- and L^-W^+ females exposed to males at 3 weeks did not differ in adjusted gestational feed intake, resulting in a significant interaction (Fig. 2a). Similar line differences were reported when females were mated at a later age after having been reared in litters of different size (Eisen and Durrant 1980). Only minor differences in unadjusted feed intake were observed among the three ages of male exposure and the line by age interaction was not important. When adjusted for metabolic body size, younger females had a higher feed intake than older females within each line. Perhaps the younger dams' greater feed intake when adjusted for metabolic body size indicates higher nutritional demands for their growth needs.

The line and age rankings for gestational dam feed efficiency show the same pattern noted for gestational weight gain (Table 2). Based on the marginal means, L^+ was more efficient than L^+W^-, whereas L^-W^+ was intermediate and did not differ from either of the extremes. These data would appear at first to disagree with the previous finding that L^+ and L^+W^- dams were equally efficient during pregnancy, and greater than L^-W^+ (Eisen and Durrant 1980). The significant line by age interaction for dam feed efficiency, however, readily explains the apparent discrepancy. When females were exposed to males at 7 weeks, which was nearest to the age at male exposure in the previously cited study, complete agreement in ranking of the line means was observed (Fig. 2b). Thus, when gestation transpires subsequent to the normal onset of sexual maturation, the more efficiently growing dams are those producing a large litter. In contrast, gestation following exposure to males at 3 weeks occurs when primi-

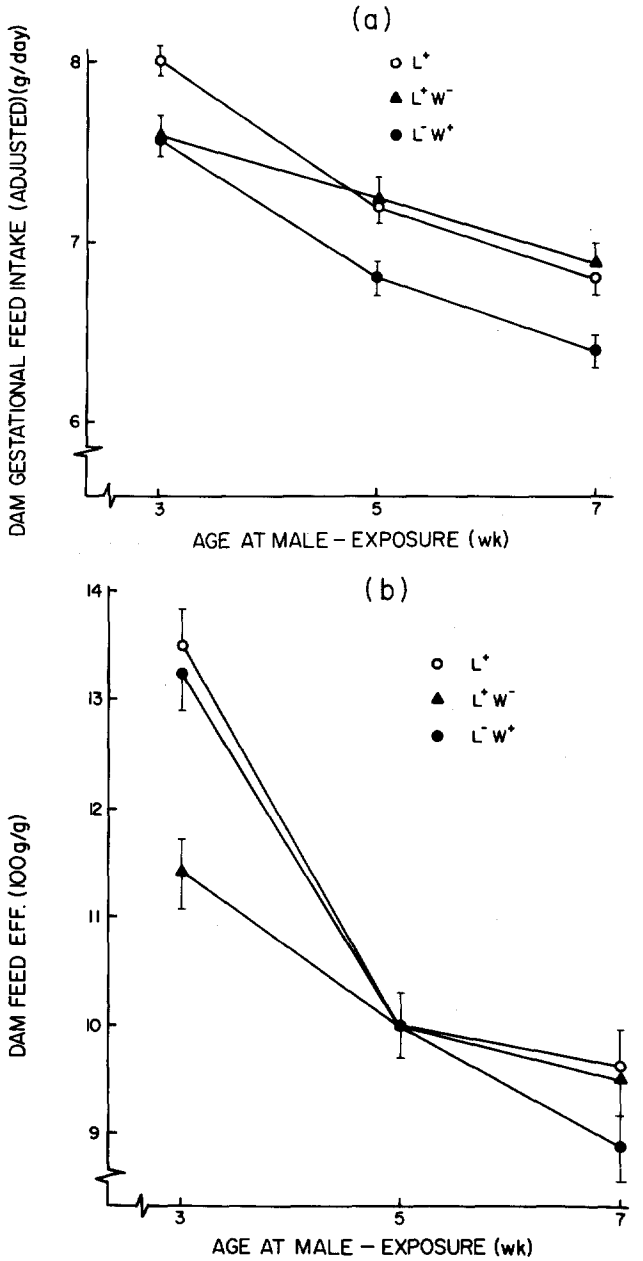

Fig. 2a and b. Line by age subclass means for (a) dam feed intake, adjusted for metabolic body size and (b) dam feed efficiency during gestation in first parity females

parous females are in their most rapid phase of postweaning growth (Monteiro and Falconer 1966), and dam feed efficiency favors the more rapidly growing lines, L^+ and L^-W^+, over the slower growing L^+W^- line. When gestation occurred following 5-week male-exposure there were no line differences in dam feed efficiency. Within each line, early pregnant females were more efficient than females gestating at a later age. Similar results were reported for an unselected ICR substrain (Eisen 1975) and a line selected for high postweaning gain (Eisen 1977); efficiency of both protein and fat deposition also was higher in the early-pregnant dams of these lines (Eisen and Leatherwood 1976, 1979).

Littering Rate, Fertility and Litter Size in First Parity Matings

Reproductive traits of primiparous females are given in Table 3. 'Littering' rate following first mating was higher in L^+ and L^-W^+ females relative to L^+W^-. Littering rate at first mating was defined as the percentage of females producing a litter following their first mating. Part of the lower littering rate in the L^+W^- line was due to a higher percentage of infertile females. This was determined by remating all females who failed to litter after their first mating to known fertile males. The fertility rate was quite high in all of the lines. The lower littering rate at first mating in L^+W^- females was found across all ages and may be associated with a delayed sexual maturation or the slower rate of development in this line, discussed earlier. Littering rate following first mating was about 11% less in females exposed to males at 3 weeks compared with 5 and 7 weeks, and this was not associated with any differential female fertility across the three age groups. These data suggest that in a significant proportion of young females that ovulated and copulated after exposure to mature males, the physiological environment essential for normal pregnancy to proceed was not completely functional. Whether the reduced function resulted in a lower conception rate or pre- or post- implantation loss of all embryos remains to be determined.

Differences in line means for primiparous litter size at birth (Table 3) were attributed to the effects of selection (Eisen 1978). Results for litter size and number born alive in parity two females are discussed at the conclusion of this section. At each age of male exposure, the L^+ line had the highest first parity litter size, while the L^+W^- and L^-W^+ lines diverged in the expected direction (Table 3, Fig. 3a). There was a significant line by age interaction. The L^+ and L^-W^+ lines increased in litter size with age at mating, whereas L^+W^- did not. Furthermore, first parity litter size in L^-W^+ females increased linearly with age at male exposure, whereas L^+ mice did not increase from 5 to 7 weeks. When first parity litter size was adjusted by covariance analysis for body weight at mating, the ranking of line differences was not altered, but age and line by age interaction effects were no longer significant. The pooled within line by age regression of first parity litter size on body weight at mating was .18 ± .08 ($P < .01$). The F-test

Table 3. Means for fecundity and reproductive rate of dams by line and age at male-exposure (first parity only)

Line[d]	Littering rate first mating (%)	Infertile females (%)	Litter size at birth	Adj. Litter size at birth[e]	Number born alive	Perinatal mortality (%)
L^+	93.5^a	1.6^a	17.1^a	16.8^a	16.3^a	4.4^a
L^-W^+	96.6^a	1.4^a	11.4^b	11.1^b	10.2^b	9.5^b
L^+W^-	84.7^b	5.3^b	14.5^c	15.5^c	13.2^c	9.7^b
S.E.[f]			0.4	0.4	0.4	
Age[d]						
3	83.9^a	2.9^a	13.7^a	14.5^a	12.5^a	8.7^a
5	94.8^b	2.8^a	14.5^b	14.5^a	13.6^b	8.8^a
7	95.0^b	2.6^a	14.9^b	14.5^a	14.0^b	5.8^b
S.E.[f]			0.3	0.3	0.3	
F-test						
L x A	NS[g]	NS[g]	3.31**	1.36^{NS}	0.89^{NS}	**[g]

NS Not significant; ** $P < 0.01$

a,b,c Column means under the same heading with no letters in common are significantly different at $P < 0.05$

d Ranges in N are 106 to 142 for lines and 97 to 148 for ages

e Adjusted by covariance for dam body weight at mating ($b = 0.18 \pm 0.08$; $R^2 = 0.02$; $P < 0.01$)

f Approximate standard error

g Ascertained by maximum likelihood method of Tallis (1964)

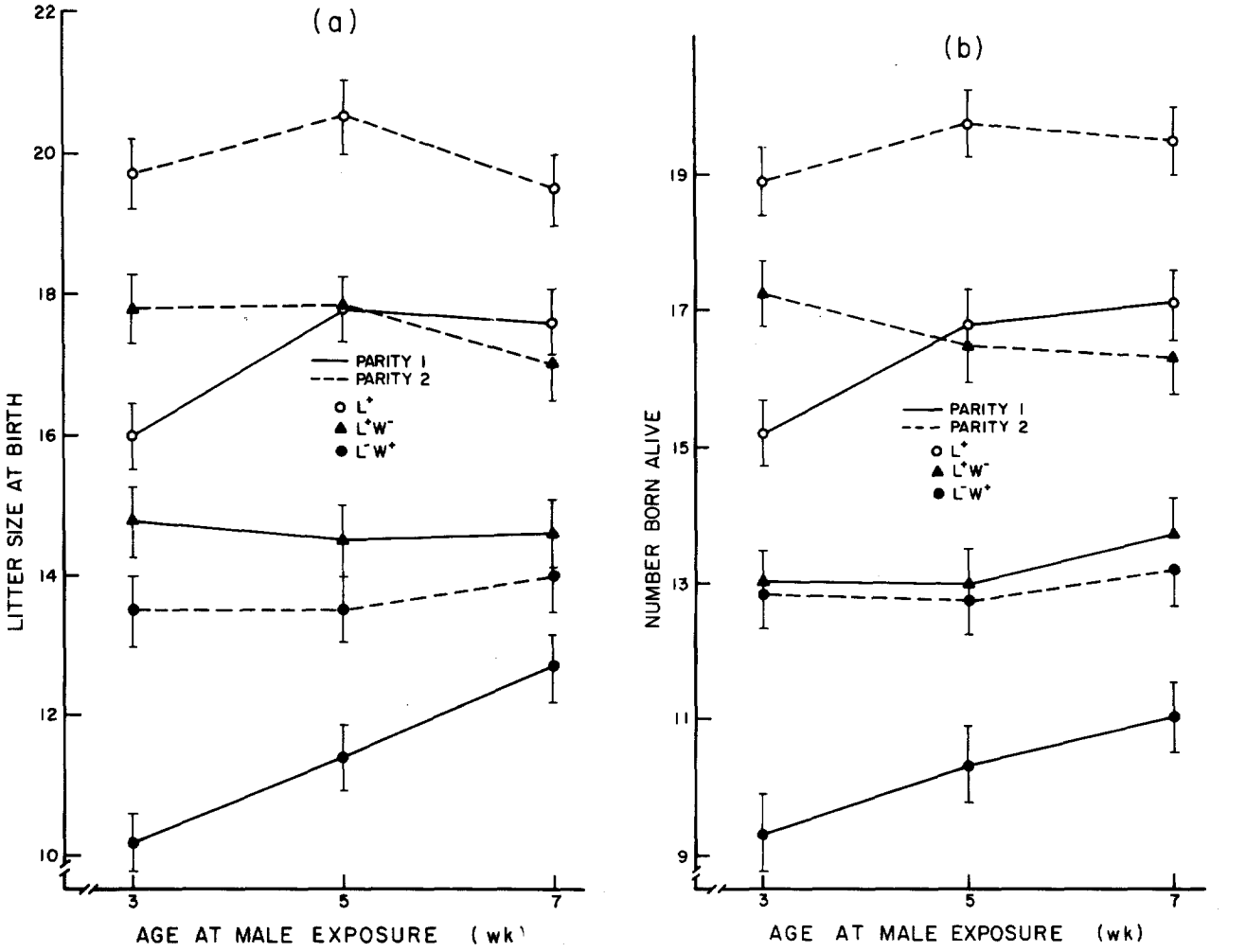

Fig. 3a and b. Line by age subclass means for (a) litter size at birth and (b) number born alive in parities one and two

for heterogeneity among the subclass regressions was not significant, nor was there any significant deviation from linearity.

Previously, Eisen (1975) found that females of the ICR substrain, from which the present lines were derived, showed no decrease in litter size at birth with early pregnancy following male-induced puberty. In contrast, a line selected for large postweaning weight gain (M16) from the same base did have a decreased litter size in early-pregnant females compared to controls, which was eliminated when early-pregnant and normal-pregnant females were adjusted for body weight at mating (Eisen 1977). In a related experiment, Machin and Page (1973) paired female and male mice at 6, 10 and 14 weeks of age and observed an increase in number born and female body weight at pairing with age at pairing. No attempt was made to compare females at a constant body weight. Kennedy and Kennedy (1972) concluded that age effects on number of corpora lutea and litter size in mice were due to differences in body weight at mating.

The covariance adjustment of litter size to a constant body weight at mating has eliminated differences in litter size which are primarily due to age affecting the females' body weight. Environmental manipulations other than age, however, can induce body weight differences at a constant age. Eisen and Durrant (1980) studied the influence of varying postnatal litter size (8, 12 or 16) on reproductive performance in the L^+, L^+W^-, L^-W^+, a large weight line (W^+) and an unselected control. Females of each line reared in smaller litters had larger body weights at mating and larger litter sizes at birth, but when compared at a constant body weight there was no significant difference in litter size among the three postnatal litter size classes. Similar results were reported by Falconer (1965). Female mice and pigs reared in large litters produced fewer corpora lutea than those reared in small litters (Nelson and Robison, 1976a, b). In an experiment where female mice were reared in standardized litters of 4, 8 or 12, dams reared in postnatal litters of 4 gave birth to more pups than dams reared in litters of 12, but, peculiar-

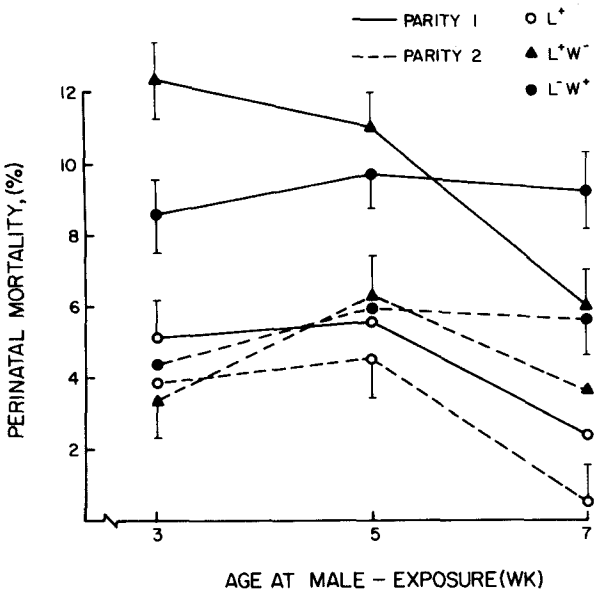

Fig. 4. Line by age subclass means for perinatal mortality in parities one and two

ly, dams reared in litters of 8 produced smaller litters than either the 4 or 12 litter size group (Machin and Page 1973).

Number born alive at first parity followed the same line and age trends as litter size at birth, except that there was no significant line by age interaction (Table 3, Fig. 3b). This is explained by the striking line by age interaction in perinatal mortality at first parity (Fig. 4). 'Perinatal mortality' includes still births and postnatal deaths due to cannabilism or unknown causes, all recorded on the day of parturition. Clearly, L^+ pups exhibited the lowest first parity mortality rate of the three lines at each age of male exposure. L^+ pups had similar first parity mortality rates at 3 and 5 weeks followed by a decline at 7 weeks. A similar age pattern of mortality rates was observed for L^+W^- pups, but a higher rate. In contrast, L^-W^+ pups showed no change in first parity mortality level with age. Previous studies had demonstrated a higher perinatal mortality rate in pups of early-pregnant females of the ICR substrain and the M16 line (Eisen 1975, 1977).

Table 4. The means for total litter weight and mean individual body weight at birth by line and age at male-exposure (first parity only)

Line[d]	Litter birth wt. (g)	Individual birth wt. (g)	Adj. individual birth wt.[e] (g)
L^+	25.7^a	1.58^a	1.65^a
L^-W^+	18.7^b	1.85^b	1.78^b
L^+W^-	20.5^c	1.57^a	1.58^c
S.E.[f]	0.5	0.02	0.02
Age[d]			
3	20.4^a	1.67^a	1.66^a
5	21.4^a	1.64^a	1.65^a
7	23.0^b	1.67^a	1.71^b
S.E.[f]	0.5	0.01	0.01
F-test			
L x A	0.33^{NS}	1.45^{NS}	1.23^{NS}

NS Not significant

a,b,c Column means under the same heading with no letters in common are significantly different at $P < 0.05$

d Ranges in N are 106 to 142 for lines and 97 to 148 for ages

e Adjusted by covariance for linear ($b_1 = -0.057 \pm 0.009$; $P < 0.01$) and quadratic ($b_2 = 0.0011 \pm 0.0003$; $P < 0.01$) effects of litter seize at birth ($R^2 = 0.39$; $P < 0.01$)

f Approximate standard error

Litter birth weight differences among the lines and age at male-exposure simply reflect differences already noted for number born alive (Table 4). Mean individual birth weight was higher in L^-W^+ mice compared to L^+ or L^+W^-. When line effects were adjusted for litter size, L^+ progeny were intermediate in birth weight to L^-W^+ and L^+W^- progeny. Unadjusted individual birth weight revealed no age at male exposure differences, but when adjusted for litter size, progeny born to females exposed to males at 7 weeks were heavier than progeny of those exposed at 3 and 5 weeks. Since there was no evidence of a line by age interaction, the marginal differences are representative of each line.

The slight detrimental influence of early pregnancy on adjusted individual birth weight was also apparent in the ICR substrain (Eisen 1975), but a reverse trend was observed in the M16 line selected for high postweaning gain (Eisen 1977). The smaller adjusted individual birth weights found in progeny of L^+, L^+W^-, L^-W^+ and ICR control females mated at an extremely early age may have been the result of incomplete development of the uterine circulatory system essential for supplying nutrients to the developing fetus.

Litter Feed Efficiency

Attention is now focused on the comparisons of litter feed efficiency. Line by age subclass means for the two definitions of litter feed efficiency together with cumulative feed intake of the dam from mating to parturition and from weaning to parturition are given in Table 5. Note that litter birth weight, the numerator of both definitions of litter feed efficiency, is determined to a large extent by number born alive. Cumulative feed consumption from weaning to mating was estimated from the data on virgin females in Table 1 under the assumption that feed intake of females paired with a male prior to mating does not differ from virgin females of a comparable age. As line by age interactions were not significant for the

Table 5. Means of cumulative feed intake of dams and litter feed efficiency by line, age at male-exposure and line x age subclasses

Line x Age	N	Cumulative feed intake of dams (g)			Litter feed efficiency (100 g/g)	
		21 days to mating	Mating to parturition (A)	21 days to parturition (B)	Litter birth wt/A	Litter birth wt/B
Line Age						
L^+ 3	35	25.5	147.4	172.9	16.5	13.9
5	48	97.7	144.6	242.3	17.7	10.5
7	40	177.2	140.0	317.2	19.8	8.6
L^-W^- 3	42	26.5	141.6	168.1	12.0	10.2
5	57	97.0	136.6	233.6	13.8	7.9
7	43	180.8	139.5	320.3	14.8	6.4
L^+W^- 3	23	26.4	125.8	152.2	15.7	13.1
5	43	83.3	124.6	207.9	16.0	9.7
7	45	155.1	123.3	278.4	17.3	7.8
Line						
L^+	123	100.1^a	144.0^a	244.1^a	18.0^a	11.0^a
L^-W^+	142	101.4^a	139.2^b	240.7^b	13.5^b	8.2^b
L^+W^-	111	88.3^b	124.6^c	212.8^c	16.3^c	10.2^c
Age						
3	100	26.1^a	138.3^a	164.5^a	14.7^a	12.4^a
5	148	92.7^b	135.3^b	227.9^b	15.8^b	9.4^b
7	128	171.0^c	133.3^b	305.3^c	17.3^c	7.6^c

^{a,b,c} Column means under the same heading with no letters in common are significantly different at $P < 0.05$

traits given in Table 5, emphasis is placed on describing the marginal means by line and age. L^+ and L^-W^+ females consumed approximately the same quantity of feed prior to mating but much more feed than L^+W^- females. Differences in feed intake during gestation have already been described. From 21 days of age to parturition cumulative feed consumption was significantly higher in L^+ than in L^-W^+, but the magnitude of the difference was relatively small, whereas L^+W^- females had a much lower cumulative feed intake than L^+ and L^-W^+. The ranking of lines was the same for each definition of litter feed efficiency, which agrees with an earlier experiment (Eisen and Durrant 1980). However, line differences in litter feed efficiency should be adjusted for the lower littering rate at first mating of the L^+W^- line compared to L^+ and L^-W^+ (Table 3). When this was done for each measure of efficiency, the difference between L^+W^- and L^-W^+ mice was eliminated.

Using the definition of litter feed efficiency based on feed consumption from mating to parturition, ICR females exposed at 3 and 7 weeks were equally efficient (Eisen 1975), whereas M16 females exposed at 3 weeks were less efficient (Eisen 1977). Again, this discrepancy between ICR and M16 mice was caused by the differential response in number born alive.

Turning to the effects of age at male exposure, the sizeable differences in cumulative feed consumption from weaning to parturition is due mainly to the difference in number of days from weaning to mating in the three age groups. For the definition of litter feed efficiency which only accounts for the dam's gestational feed intake, litters of females exposed at 7 weeks were most efficient, whereas those exposed at 3 weeks were least efficient. This was obviously due primarily to the mean differences in number born alive because differences in cumulative feed consumption were relatively small. The second definition of litter feed efficiency, which considers the period of food consumed by the dam from the time she is weaned until parturition, reverses the ranking of the age means, a result explained by the greater amount of feed consumed prior to mating in the females exposed at an older age. There is, however, a bias in these differences favouring the females exposed to males at three weeks since they had a lower littering rate following their first mating (Table 3), which is not taken into account in the calculation. An approximate adjustment for this bias was obtained by multiplying

the litter feed efficiency means at each age (12.4; 9.4; 7.6) by the respective mean littering rates (0.839; 0.948; 0.950) which still yielded significant age differences (10.4; 8.9; 7.2).

Litter Size in Second Parity Matings

Litter size at birth and number born alive were consistently higher in parity two compared with parity one for all line by age subclasses (Fig. 3a, b and Table 6). There was no evidence for a line by parity interaction for these measures of fecundity; i.e. line differences were fairly uniform across parities. An age by parity interaction was present, however, since the decreased fecundity at younger ages of male exposure observed in the L^+ and L^-W^+ lines in parity one was not evident in parity two. For each line there was a tendency for parity differences in litter size and number born alive to decrease with age at male exposure. This suggests a compensatory effect for early-pregnant L^+ and L^-W^+ females who produced fewer offspring in their first parity compared to females of the same lines mated at a later age. Fecundity summed over both parities still increased with age at male exposure in L^+ and L^-W^+ females (Table 6). As expected, no increase in total fecundity accrued in the L^+W^- line with age at male exposure because age at male exposure effects were absent in each parity. There were no line or age at male exposure differences in repeatability and the pooled repeatability estimates were 0.20 ± 0.05 for litter size at birth and 0.24 ± 0.05 for number born alive. The conclusion from this part of the experiment is that there was no adverse influence on fecundity in the second parity of female mice who were induced to have their first parity litters at an extremely

Table 6. The difference between and sum of parity one and two means for litter size at birth and numbers born alive

	Age at male-exposure		
Line	3	5	7
	Difference for litter size ± S.E.[c]		
L^+	3.7 ± 0.7**	2.8 ± 0.7**	1.9 ± 0.8*
L^-W^+	3.2 ± 0.7**	2.1 ± 0.5**	1.3 ± 0.6*
L^+W^-	3.0 ± 0.7**	3.3 ± 0.6**	2.6 ± 0.7**
Pooled	3.3 ± 0.4**	2.7 ± 0.4**	1.9 ± 0.4**
	Difference for number born alive ± S.E.[c]		
L^+	3.7 ± 0.7**	2.9 ± 0.7**	2.3 ± 0.8**
L^-W^+	3.6 ± 0.7**	2.4 ± 0.5**	2.2 ± 0.6**
L^+W^-	4.2 ± 0.7**	3.6 ± 0.6**	2.6 ± 0.7**
Pooled	3.8 ± 0.4**	3.0 ± 0.4**	2.4 ± 0.4**
	Sum for litter size ± S.E.[d]		
L^+	35.7 ± 0.8[a]	38.4 ± 0.8[b]	37.1 ± 0.9[ab]
L^-W^+	23.7 ± 0.8[a]	24.9 ± 0.7[a]	26.7 ± 0.8[b]
L^+W^-	32.6 ± 0.8[a]	32.3 ± 0.8[a]	31.6 ± 0.8[a]
	Sum for number born alive ± S.E.[d]		
L^+	34.1 ± 0.8[a]	36.5 ± 0.8[b]	36.5 ± 0.9[b]
L^-W^+	22.2 ± 0.8[a]	23.0 ± 0.7[ab]	24.2 ± 0.8[b]
L^+W^-	30.2 ± 0.8[a]	29.4 ± 0.8[a]	30.0 ± 0.8[a]

* $P < 0.05$; ** $P < 0.01$

[a,b] Row sums under the same heading with no letters in common are significantly different at $P < 0.05$

[c] Parity two minus parity one

[d] Sum of parities one and two

Table 7. Summary of effects of early mating and early pregnancy on reproductive characters in five lines of mice

	Line[ab]				
Character	ICR	M16	L^+	L^+W^-	L^-W^+
Mating age (days)	29.2	28.9	26.0	26.3	27.1
Time from exposure to mating (days)	+	+	+	+	+
Littering rate at first mating (%)	–	0	–	–	–
Litter size at birth	0	–	–	0	–
Individual birth wt. adjusted for litter size (g)	–	+	–	–	–
Perinatal mortality (%)	+	+	+	+	–
Lactational performance[c]	0	NA	NA	NA	NA

[a] Data on lines from other studies: ICR, an unselected line (Eisen 1973; 1975 Eisen et al. 1977); M16, a line selected for high 3 to 6 week weight gain (Eisen 1977).

[b] The symbols in the body of the table represent the direction of response to early mating relative to controls of the same line mated at 7 weeks: + indicates increase, – indicates decrease, 0 indicates no change and NA indicates data not available

[c] Litter growth and mammary gland DNA and RNA

young age by exposure to and subsequent mating with mature males. This conclusion holds for lines selected for large litter size (L^+), large litter size and small body weight (L^+W^-), and small litter size and large body weight (L^-W^+).

Discussion

A summary of the qualitative effects of early mating and early pregnancy on reproductive traits in five lines is given in Table 7. Mean mating ages in the L^+, L^+W^- and L^-W^+ lines are not directly comparable with the ICR and M16 lines since experiments were not contemporaneous. Females from all five lines responded positively in the presence of a mature male by attaining an accelerated rate of sexual maturation, culminating in copulation at an average age of about four weeks. When contrasted with their sibs mated at a later age, early-pregnant females from each line exhibited a decline in one or more of the components of reproductive performance, suggesting that the physiological environment essential for normal pregnancy was not fully functional. This was evidenced in some lines by a decreased littering rate at first mating, litter size at birth, number born alive and individual birth weight adjusted for litter size, or increased perinatal mortality. It is apparent that a genotype by environmental interaction was present because, for each reproductive trait measured, all lines did not respond in the same qualitative manner. Selection for litter size and/or body weight may have led to differential correlated responses which were responsible for the interactions. However, since replicate lines were not available, the possibility that genetic drift may be responsible for the interactions cannot be excluded (Falconer 1973).

All lines except M16 had a reduced littering rate following early mating. M16 mice have a lower fertility rate (Eisen et al. 1973) which may be associated with their obesity (Eisen et al. 1977). Consequently, early mating prior to the onset of adult obesity may have counteracted a portion of the negative effects of early mating on littering rate observed in the other lines.

Early mating yielded a reduction in litter size at birth in M16, L^+ and L^-W^+ females, whereas there was no reduction in ICR and L^+W^- females. While at present there is no direct evidence, it is possible that this interaction was caused by the young females of M16, L^+ and L^-W^+ ovulating fewer eggs at first estrus than at later periods. All three of these lines are larger than the ICR control from which they were derived. It is known, for example, that there are positive phenotypic and genetic correlations between litter size and ovulation rate or body weight (Land 1970; Eisen 1978). In contrast to the other selected lines, L^+W^-, which is the smallest of the five lines at all ages, did not exhibit a reduction in litter size. L^+W^- females have been shown to have a high evolation rate as a correlated response to selection, despite their small size (Durrant et al. 1980).

The failure to find an increase in perinatal mortality in L^-W^+ females may be associated with the higher level of perinatal mortality observed in this line, even when mated at older ages. In addition, the L^-W^+ line has a higher prenatal mortality rate than the other lines (Durrant et al. 1980).

M16 females subjected to early mating did not show the decline in adjusted individual birth weight that was observed in the other lines. The uterus of M16 females may be more fully developed at this young age. Another explanation is related to the fact that M16 females were mated to ICR males so that the crossbred progeny may have fared better than purebreds in the uterus of early-pregnant females. These interpretations must remain tentative since genotype of uterus and genotype of fetus effects were confounded.

The use of male-induced sexual maturation followed by early mating can extend the usefulness of the mouse as a model organism for research in problems related to animal breeding, quantitative genetics and reproductive physiology. These include studies on the following topics:

a) The mechanisms involved in early onset of female sexual maturation without the introduction of exogenous hormones;

b) The underlying basis for any genotype by environment interactions such as the ones already described;

c) The mechanisms causing increased protein deposition of the female during gestation at a time when she is already in a rapid phase of growth (Eisen and Leatherwood, 1976, 1978);

d) Evaluating early mating schemes as a method of maximizing reproductive efficiency of the female;

e) Selection for certain metric traits associated with the precocious female such as ovulation rate or litter size;

f) Reducing generation interval by selection for metric traits not associated directly with early puberty.

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