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Estrous Cycle Dynamics in Different Strains of Mice (41127)

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Abstract. Estrous cyclicity in the olfactory presence of males was studied in an unselected control line and in five selected lines which differ in fecundity. Improved reproductive performance (high embryo survival, large litters) is correlated with increased regularity of the cycle, while poor reproductive performance (small litters) is associated with prolongation of the cycle and anestrum. Strain variation in the duration of the proestrus/estrus phase of the cycle is associated with changes in ovulation rate and is indicative of substantial heritabilities for the physiological determinants of these traits.

Recurring 4- or 5-day estrous cycles are characteristic of the laboratory rat. In contrast, estrous cycle length (4–6 days) is variable in the laboratory mouse and regularly recurring cycles are rare. It was postulated that a genetic factor accounts for some of the variation in estrous cyclicity among inbred strains of mice (1). Recent studies have confirmed this hypothesis (2–4) and have provided evidence that hereditary factors also regulate the duration of estrus (3, 5).

Stimuli from the social environment can also affect the duration and occurrence of the mouse estrous cycle (6–8). Exposure to intact males results in shorter and more regular cycles (9, 10), while grouping of females in the absence of males leads to suppression of cyclicity (9, 11, 12). These effects are presumably mediated by olfactory stimulation, since prolonged diestrus in grouped females is overridden and estrus synchronized in response to male or male urine exposure (13, 14).

In the present study, estrous cyclicity was examined in lines of mice successfully selected for large litter size, high embryo survival, small litter size and rapid postweaning gain. The timing and magnitude of gonadotropin release was previously studied in several of these strains by grouping 15–20 females per cage prior to male olfactory contact (15). However, the pattern of spontaneous estrous cyclicity has not been studied except in an unselected control line (C).

Methods. *Animals.* Females were obtained from lines of mice derived from the same base population developed from a cross of four inbred strains (C57Bl/6J, AKR/J, C3H/J, DBA/2J). The selection criteria utilized to develop the lines studied were the following:

1. Line C: unselected control line.
2. Line CN-: small litters.
3. Line Sl: large litters.
4. Line E: high embryo survival.
5. Line G: rapid post-weaning gain.
6. Line G′: rapid post-weaning gain.

With the exception of G′, details of the selection procedure and response of the lines were reported (16–18). G′ is a subline of line G, separated from G at generation 22 and maintained as a separate population, also under continuous selection for rapid postweaning gain. Line G′ was subjected to two cycles of five generations each of full-sib mating of several subfamilies, followed by crossing of these families; hence, line G′ has a higher inbreeding coefficient than line G.

Litter size was determined on the day of birth. At 2 days of age pups were sexed and litters were reduced to 10 animals without augmentation of those with fewer newborn. Animals were weaned at 21 days of age, permanently identified by notching one ear, and caged by sex (4–6/cage). At 6 weeks of age, nulliparous females were randomly removed from the cages in which they were housed from weaning and at this time were housed 4–6/cage according to line. Due to space limitations, all animals were housed in the same colony room (6 × 8 m). Temperature (21–23°) and illumination (0500–

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1900 hr) were controlled. Food (white diet) and water were supplied *ad libitum*.

Estrous cyclicity. Adult females of a particular line were caged between males of the same line, so that on a given shelf every other cage contained two to four adult males. A 2-week period elapsed to permit the establishment of spontaneous cycles (19) before daily observations of vaginal fluid cell content were initiated. Vaginal smears were prepared between 1000 and 1100 hr and were examined in unstained wet preparations. The following criteria were used for identification of cycle stages: proestrus—nucleated or nucleated and cornified cells; estrus—cornified cells; metestrus—leukocytes and cornified cells; diestrus—leukocytes or leukocytes and some nucleated cells. The endpoint of the cycle was represented by the appearance of leukocytes in a smear which on the previous day had been fully cornified or contained nucleated and cornified cells; this corresponds to metestrus and represents the most precise cellular change (19).

With the exception of line G, which was studied for 25 consecutive days, all lines were observed for a minimum of 35 days. Six parameters were measured: (i) the number of complete estrous cycles during 25 (or 35) days; (ii) the length of each complete cycle; (iii) the length of each cycle of short duration (<7.0 days); (iv) the frequency of either short cycles (<7.0 days), prolonged cycles (7.0–9.5 days), pseudopregnancies (10.0–13.5 days), or periods of anestrus (≥14.0 days); (v) the number of days spent in either proestrus, estrus, metestrus, or diestrus during each complete cycle (expressed for each cycle stage as a percentage of the cycle length); and (vi) the number of days spent in either proestrus, estrus, metestrus, or diestrus during each short cycle (expressed for each stage as a percentage of the cycle length).

Data analysis. With the exception of parameter iv, data were subjected to analyses of variance by the hierarchical (or nested) model

$$Y_{ijk} = l_i + c_{ij} + e_{ijk},$$

where

Y_{ijk} = the observation of the kth female within the jth cage within the ith line,
= the overall mean,
l_i = effect of the ith line,
c_{ij} = effect of the jth cage within the ith line,
e_{ijk} = error (remainder).

For parameters ii, iii, v, and vi, the datum for each animal in the nested analysis of variance was the average value for each of the respective measurements taken (e.g., the average length of complete cycles in the case of parameter ii).

With one exception (discussed under results), the cage within line effect was not significant. Consequently, the effect of the jth cage within the ith line (c_{ij}) was ignored to increase the power of the test for lines. Data were subsequently subjected to a one-way analysis of variance by the reduced model

$$Y_{ij} = + l_i + e_{ij}.$$

For each analysis yielding a significant F, comparisons among group means were made using Duncan's new multiple range test. Differences between lines in the proportion of cycles of varying length (parameter iv) were assessed by means of the χ^2 statistic.

Results. *Reproductive performance.* Typical reproductive performance levels (based on composite data obtained from animals other than those utilized in the present study) are summarized in Table I. As a correlated response to selection for large litters and rapid post-weaning gain, ovulation rate has increased in lines S1, G, and G′. Prenatal survival has decreased in lines G and CN-, but it has increased in lines S1 and E.

Occurrence and duration of the cycle. The effect of strain on the number of complete estrous cycles during 25 days is shown in Table II. Analysis of variance failed to reveal a cage within line effect, but did indicate a strain influence on estrous cyclicity ($P < 0.001$). Individual comparisons among means showed that the line selected for high embryo survival (E) completed more cycles than did any of the other lines (P's <

TABLE I. OVULATION RATE, PRENATAL SURVIVAL, AND LITTER SIZE OF FIVE SELECTED LINES AND AN UNSELECTED CONTROL LINE DEVELOPED FROM A COMMON BASE POPULATION

Line	Selection criterion	Mean number ± SE of: Ova or corpora lutea (CL)[a]	Mean number ± SE of: Normal fetuses at 16 days or number born (N)[b]	Prenatal survival (N/CL)
C	Unselected	10.1 ± 0.29	7.8 ± 0.21	0.77
CN-	Small litters	10.1 ± 0.30	5.8 ± 0.27	0.57
Sl	Large litters	17.4 ± 0.43	15.3 ± 0.31	0.88
E	High embryo survival	11.6 ± 0.20	10.2 ± 0.21	0.88
G	Rapid 21–42 day gain	16.0 ± 0.55	8.1 ± 0.34	0.51
G′	Rapid 21–42 day gain	16.3 ± 0.27	8.3 ± 0.27	0.51

[a] In females mated at 8 to 10 weeks of age; N = 24–58 animals per line.
[b] Based on approximately 100 females per line also mated at 8 to 10 weeks of age.

0.01). Both the control line (C) and the lines selected for rapid growth (G and G′) completed more cycles than did the line selected for small litters (CN-). Examination of the number of cycles completed during 35 days substantiated the observations that (i) line E had more regular cycles compared to all the other lines, and (ii) line C completed more cycles than did line CN- ($P < 0.05$).

Length of the estrous cycle is summarized in Tables IIIa and b. Variation among the lines was seen in cycle length inclusive of prolonged (7.0–9.5 days) and long (≥10 days) cycles (Table IIIa) ($P < 0.001$). The strains also differed with respect to normal (4–6 days) cycle length (Table IIIb) ($P < 0.05$). Line CN- had longer cycles than did any of the other selected lines (P's ≤ 0.05). The mean length of all cycles in line E (5.62 ± 0.30 days) was shorter than observed in line C (7.54 ± 0.55 days) ($P < 0.05$). During 35 days, the average cycle length in line E was shorter (5.88 ± 0.24 days) than in all the other lines (P's ≤ .05). The average length of the 4–6 day cycle was longer in line G′ (5.20 ± 0.13 days) than in either line C or line Sl (P's < 0.05).

Distribution of cycles. An examination of the distribution of cycle lengths for each line (Fig. 1) showed a major peak at 4 to 5 days in lines C, CN-, and Sl; 5-day cycles were predominant in lines E and G; and 5- to 6-day cycles were most frequent in line G′. A secondary peak at 10 to 12 days was also evident in lines C and CN-. A third peak corresponding to cycle lengths suggestive of anestrus (≥14 days) was more pronounced in line CN- than in the control

TABLE II. EFFECT OF LINE ON THE NUMBER OF COMPLETE ESTROUS CYCLES DURING 25 DAYS

Line	N	Mean number of cycles ± SE
C	30	2.89 ± 0.18
CN-	31	2.13 ± 0.16[a]
Sl	34	2.50 ± 0.13
E	27	3.78 ± 0.17[b]
G	36	2.64 ± 0.15
G′	22	2.86 ± 0.14

[a] Significantly different from line C, $P < 0.05$.
[b] Significantly different from line C, $P < 0.01$.

TABLE III. EFFECT OF LINE ON THE LENGTH OF COMPLETE ESTROUS CYCLES DURING 25 DAYS

Line	N	Mean length ± SE
(a) All cycles		
C	30	7.54 ± 0.55
CN-	31	8.83 ± 0.62
Sl	34	6.39 ± 0.50
E	27	5.62 ± 0.30[a]
G	36	6.34 ± 0.34
G′	22	6.78 ± 0.27
(b) Short (<7.0 days) cycles[b]		
C	27	4.75 ± 0.10
CN-	25	4.87 ± 0.11
Sl	30	4.77 ± 0.09
E	26	5.05 ± 0.09
G	34	4.90 ± 0.12
G′	20	5.20 ± 0.13[a]

[a] Significantly different from line C, $P < 0.05$.
[b] Animals exhibiting at least one short cycle were included in the analysis.

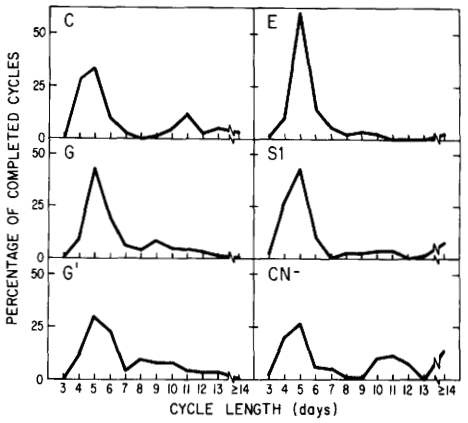

FIG. 1. Percentage distribution of estrous cycles completed by an unselected control line (C), and by mice selected for rapid post-weaning gain (G, G'), high embryo survival (E), large litters (Sl) or small litters (CN-).

or other selected lines, with the exception of line Sl. Cycle lengths of 7 to 9 days were most frequent in the rapid growth lines, particularly in line G'.

The distribution of normal cycles is shown in Table IV. Between-line differences in the frequency of 4-, 5-, and 6-day cycles were indicated by the χ^2 test. A predominance of 4- and 5-day cycles was seen in the control line, as well as in the lines selected for litter size (CN- and Sl). In line E, 5-day cycles recurred regularly (they were more frequent in line E than in all the other lines except G; (P's $\leq$ 0.01). More 6-day cycles were present in lines G and G' than in lines C, CN- and Sl (P's $\leq$ 0.05).

Division of long cycles into the categories of "pseudopregnancy" (10.0–13.5 days) and "anestrus" ($\geq$14.0 days) resulted in the distribution summarized in Table V. This categorization showed that the pronounced irregularity of estrous cyclicity in line CN- was not related to an increased incidence of pseudopregnancy (as compared to the control line) but to more frequent periods of anestrus ($P < 0.025$). Spontaneous pseudopregnancy was more frequent in both lines CN- and C than in either line Sl or line E (P's $\leq$ 0.01). Cycles 10.0–13.5 days in duration were also more frequent in line CN- than in line G ($P < 0.01$). However, lines G and G' were more subject to pseudopregnancy than was line E.

Components of the cycle. The effect of line on stages of the cycle (diestrus, proestrus, estrus, and metestrus) is shown in Tables VIa and b. As indicated by hierarchical analyses of variance, no significant effect of cage within line on the proportion of days spent in diestrus, proestrus, or estrus was noted (Table VIa). However, with respect to metestrus, a cage/line effect was present ($P < 0.01$). Consequently, each line was subjected to a hierarchical analysis of variance to determine the source of the cage/line effect. The results showed that within line G, the variance attributable to females/cages was greater than in the other lines, thus producing a significant cage/line effect on the proportion of days spent in metestrus. A closer inspection of the line G data revealed that one cage of females had metestrus smears 34% as compared to the line average of 21% of all cycles. Females in this cage also spent less time in diestrus than other line G females (35% as compared to the line average of 50%). Since the cage/line effect was confined to only one line (G), the variance associated with this effect (largely attributable to one cage) was negligible relative to the variation associated with the main effect of line. Thus, the data in Table VIa were subsequently subjected to a one-way analysis of variance (as explained under Methods). The results

TABLE IV. PERCENTAGE DISTRIBUTION OF CYCLES 4, 5, OR 6 DAYS IN DURATION[a]

Cycle length (days)	Line							
	C	CN-	Sl	E	G	G'	χ^2	P
4.0	39	39	34	12	13	18	41.79	<0.001
5.0	47	50	52	71	60	46	18.43	<0.001
6.0	14	11	14	17	27	36	29.36	<0.001

[a] Based on the total number of 4-, 5-, and 6-day cycles for each line.

TABLE V. PERCENTAGE DISTRIBUTION OF "PSEUDOPREGNANCIES" (10.0–13.5 DAYS) AND PERIODS OF "ANESTRUS" (≥14 DAYS)

Cycle length (days)	Line							
	C	CN-	Sl	E	G	G′	χ^2	P
10.0–13.5	22	28	8	2	12	17	35.53	<0.001
≥14.0	4	14	7	2	0	0	31.76[a]	<0.001

[a] The expected frequencies of observed cycles were not less than 4.2/cell.

indicated that the number of days spent in each phase of a complete cycle differed among the lines (P's < 0.001). Lines C, Sl, and CN- had longer periods of diestrus than did lines E, G, and G′ (P's < 0.01). Lines C and CN- spent proportionately less time in proestrus than did lines E, G, and G′ (P's ≤ 0.05), and line CN- had relatively fewer proestrus smears than did line Sl (P < 0.05). With respect to estrus, lines C and CN- exhibited proportionately fewer smears of fully cornified cells than did lines E, G, and G′ (P's ≤ 0.05), and line CN- had fewer estrus smears than did line Sl (P < 0.05). Correspondingly, lines E, G, and G′ exhibited longer periods of metestrus than did lines C, Sl, and CN- (P's < 0.01). The effect of line on the proportion of days spent in the various cycle stages during complete cycles was identical throughout a 35-day observation period to that observed over 25 days (excluding line G).

The proportion of days spent in each phase of the short cycle is shown in Table VIb. Analyses of variance showed that the effect of strain was significant with respect to diestrus (P < 0.001) and metestrus (P < 0.001). The effect of line on the proportion of estrus days was only marginally significant (P = 0.07). Lines G and G′ spent less time in diestrus and more time in metestrus than did the control line (P's < 0.01). In contrast, line E did not differ from line C with respect to the proportion of days spent in any phase of the spontaneous cycle. Lines G and G′ also had longer periods of metestrus than did the other selected lines (Sl, E, and CN-, P's < 0.01). Of the two lines under continuous selection for rapid post-weaning gain, G′ spent more time in metestrus than did line G (P < 0.05). Al-

TABLE VI. EFFECT OF LINE ON ESTROUS CYCLE COMPOSITION[a]

Line	N	Mean proportion ± SE			
		Diestrus	Proestrus	Estrus	Metestrus
		(a) During 25 days			
C	30	0.63 ± 0.02	0.11 ± 0.01	0.10 ± 0.01	0.16 ± 0.01
CN-	32	0.65 ± 0.02	0.11 ± 0.01	0.08 ± 0.01	0.16 ± 0.01
Sl	36	0.61 ± 0.03	0.13 ± 0.02	0.12 ± 0.01	0.14 ± 0.01
E	28	0.50 ± 0.02[b]	0.15 ± 0.01[b]	0.14 ± 0.01[b]	0.21 ± 0.05[b]
G	36	0.50 ± 0.02[b]	0.15 ± 0.01[b]	0.14 ± 0.01[b]	0.21 ± 0.02[b]
G′	22	0.47 ± 0.03[b]	0.14 ± 0.01[c]	0.16 ± 0.02[b]	0.23 ± 0.02[b]
		(b) During cycles <7 days			
C	25	0.41 ± 0.03	0.22 ± 0.02	0.15 ± 0.01	0.22 ± 0.02
CN-	20	0.43 ± 0.02	0.22 ± 0.01	0.17 ± 0.01	0.18 ± 0.01
Sl	30	0.41 ± 0.02	0.22 ± 0.01	0.19 ± 0.01[c]	0.18 ± 0.01
E	26	0.45 ± 0.01	0.21 ± 0.01	0.16 ± 0.01	0.18 ± 0.01
G	32	0.30 ± 0.03[b]	0.22 ± 0.01	0.19 ± 0.01[c]	0.29 ± 0.03[b]
G′	20	0.27 ± 0.04[b]	0.21 ± 0.01	0.17 ± 0.02	0.35 ± 0.04[b]

[a] Based on percentage of days spent in each phase of the cycle.
[b] Significantly different from line C, P < 0.01.
[c] Significantly different from line C, P < 0.05.

though the effect of line on the proportion of days estrus occurred was only marginally significant, differences between means were found using Duncan's new multiple range test: estrus smears were observed more often in lines Sl and G than in the control lines (P's < 0.05).

Discussion. Length of the estrous cycle in the mouse (*Mus musculus*) varies from 3 to 9 days, with a mode from 4 to 6 days (20). The same variation in estrous cyclicity is characteristic of the strains of mice we examined. Cycles of varying length occurred in every line, but the normal cycle averaged 5 days in duration. Repeated examination of certain of these lines (C, CN-, Sl and E) confirmed these observations and established that strain-specific characteristics are retained. Although length of the spontaneous cycle was fairly consistent, mean cycle length inclusive of all cycles was variable because the proportion of females in which aberrant cycles recurred differed among the control and selected lines.

The most dramatic increase in cycle regularity occurred in the line selected for high embryo survival. Line E females completed more cycles than any other line and their cycle length was markedly consistent. Normal cycles also predominated in the line selected for large litters (Sl), which has an ovulation rate higher than that of line E, but is equivalent in rate of prenatal survival (Table I). In contrast, nearly half of the cycles completed by females selected for small litters were aberrant in nature. Consequently, the average duration of the cycle in line CN- was lengthened to approximately 9 days.

Two types of irregular cycles have been described in mice: those in which the formation of deciduomata is possible (pseudopregnancy), and those characterized by a prolonged diestrum and a rapid return to estrus upon pairing with a male (anestrus). Both spontaneous pseudopregnancy (7) and periods of anestrus (9) occur when female mice are grouped together in the absence of a male. Parks and Bruce (11) suggested that differences in cage density promote the appearance of the two types of aberrant cycles; pseudopregnancies intervene if the groups are small, while periods of anestrus predominate if the groups are large. However, Bronson (21) is cautious of this assumption, and suggests that differences in genetic background or early experiential factors may also be causative. In the present study, females were housed in small (four to six per cage) groups in the olfactory presence of males. Under these conditions, diestrus intervals of 10–13 days in duration were generally more frequent than periods of diestrus $\geq$14 days in length. Whether cycles $\geq$14 days in duration represent pseudopregnancies rather than periods of anestrus cannot be determined from our data. True pseudopregnancies (approximately 11 days in length) occurred in 25% of all cycles in female mice housed together in groups of four (7), which is comparable to the incidence of cycles 10–13 days in duration in lines C (22%) and CN- (28%). However, in line CN-, one-third of all aberrant cycles were $\geq$14 [$\bar{X} = 17.83 \pm 0.57$ (SE)] days long, which supports Bronson's (21) suggestion that strain differences may influence the expression of one or the other types of cycle irregularity. It is not known at this time if the between-line differences in cycle regularity result, at least in part, from strain variation in responsiveness to male pheromonal stimulation. The lack of a cage within line effect for any of the parameters measured is interesting and suggests that females respond uniformly to pheromonal signals produced by males of their own line.

Further support for the suggestion that genetic (in addition to environmental) factors regulate estrous cyclicity is provided by females selected for rapid growth. The normal cycle has been lengthened in lines G and G′, wherein 6- and 7- to 9-day cycles recur. As a result, cycles of relatively short duration appear less often, a phenomenon that probably accounts for the longer interval to mating following the introduction of males in line G (22) and line G′ (unpublished observation). Contrary to what might be expected, an increase in the diestrus interval cannot account for the prolongation of the cycle. Actually, the proportion of time females selected for rapid growth

spend in metestrus has increased relative to other lines (C, CN- and Sl). This increase in the duration of metestrus is most noticeable in cycles <7 days long in which lines G and G′ also show the shortest periods of diestrus. In line G′, prolonged metestrus intervals explain the relative increase in length of the spontaneous cycle (5.20 as compared to 4.7 days in line C).

Of particular interest are the proportionately longer periods of proestrus and estrus which are characteristic of females in which selection has produced a correlated response potentially augmentative of litter size, i.e., increased ovulation rate or high embryo survival. Relative to the small litter size line, the other selected lines show lengthened proestrus (lines E, G, and G′) and/or estrus intervals (lines E, G, G′, and Sl). During normal cycles, vaginal smears indicative of estrus are most often observed in females with a high ovulation rate (lines Sl and G), with the exception of line G′ in which length of the metestrus interval has increased.

In addition to confirming a hereditary factor for the physiological determinants of estrus duration (3, 5), the present study has extended our knowledge of the ways in which selection for one component of reproduction affects other components. The exceptional uniformity of estrous cycle length in line E females, successfully selected for high embryo survival, is particularly interesting. The uniformity in litter size of this strain (23) is an expected result of the reduction in prenatal loss; the uniformity in estrous cycle length described here indicates a generally more precise regulation of reproduction in this line. Studies of the hormonal control of pregnancy in these mice (24) and several of the other lines (25) suggest that improved fecundity following selection for reproductive traits could be mediated by changes in endocrine function. The marked strain variation in estrous cyclicity may also be associated with alterations in the hormonal regulation of reproductive processes. Studies beyond the scope of this report indicate that the preovulatory surge of luteinizing hormone (LH) is delayed in females with a lengthened estrus and a high ovulation rate (line Sl). The positive correlation between increased ovulation rate and the duration of estrus has not been reported previously in the mouse, but it has in sheep (26, 27). A delay in the ovulatory discharge of LH relative to the onset of estrus is also correlated with an increased ovulation rate in ewes (27, 28). This finding of a similar association of traits in mammalian species as different as sheep and mice is intriguing and illustrates the efficacy of using genetic variation to elucidate physiological processes inherent in the control of reproduction.

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