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Social Influences on Reproductive Maturation in Female White-Footed Mice (Peromyscus leucopus noveboracensis)

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College of William & Mary - Arts & Sciences

SOCIAL INFLUENCES ON REPRODUCTIVE MATURATION IN FEMALE WHITE-FOOTED MICE (PEROMYSCUS LEUCOPUS NOVEBORACENSIS)

A Thesis

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In Partial Fulfillment

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Master of Arts

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Michelle Lee Mabry

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of

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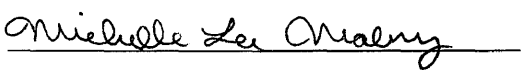

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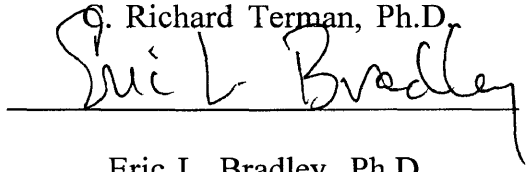

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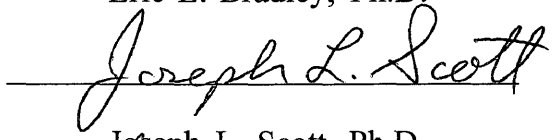

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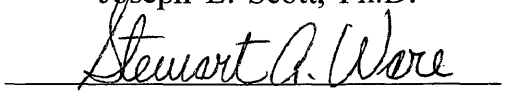

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ABSTRACT

Studies on the house mouse, (*Mus musculus*) have demonstrated that when juvenile females are housed with other juveniles or adult females, reproductive maturation of the juveniles is delayed, as shown by a significantly later mean age of vaginal introitus and first estrus. This delay has been shown to be caused by a urinary chemosignal released by the grouped females.

This study examined whether or not the white-footed mouse (*Peromyscus leucopus noveboracensis*) shows a similar delay in reproductive maturation. Experiments were designed to replicate the original experiments with *Mus musculus*. Juvenile females housed one, two or five per cage in Experiment I showed no significant difference in mean age of vaginal introitus, first estrus, selected organ weights or body weights. Juvenile females housed one, two or eight per cage in Experiment II showed no significant difference in mean age of vaginal introitus, first estrus, ovary, uterine horn or body weights. Juvenile females housed singly had significantly larger adrenal glands than those housed two or eight per cage. Juveniles housed in contact with one adult female, four adult females, or separated from contact with adult females in Experiment III showed no significant difference in men age of vaginal introitus, first estrus, selected organ weights, or body weights.

Analyses of cumulative percentage curves of females with open vaginae versus age showed that juveniles housed with four adults had a significantly slower rate of reproductive maturation than juveniles housed with four juveniles. In Experiment III, the proportion of juvenile females with open vaginae at 38-40 days of age was significantly less than juveniles housed with one adult female. There was no significant difference in the proportion of females with open vaginae present at any other age.

The data indicate that *Peromyscus leucopus noveboracensis* responds differently than *Mus musculus* under similar social situations. Juvenile white-footed mice are not delayed in reproductive maturation when housed with other juvenile females. Juvenile females are delayed in reproductive maturation when housed with four adult females. This delay is not permanent, and juveniles spontaneously recover at approximately 42 days of age. This delay may have an effect on population regulation by delaying juvenile female's first reproduction. However, grouped female induced delay in reproductive maturation of juveniles is probably not a significant mechanism for population regulation in natural populations of *Peromyscus leucopus noveboracensis*.

SOCIAL INFLUENCES ON REPRODUCTIVE MATURATION IN FEMALE WHITE-FOOTED MICE (PEROMYSCUS LEUCOPUS NOVEBORACENSIS)

INTRODUCTION

Population size in animals is affected in part by the age at which individuals become reproductively mature. The earlier an animal matures, the more likely it is to produce more offspring, which will increase the population size. Studies on the house mouse (*Mus musculus*) have shown that when juvenile females are grouped with juvenile or adult females, the reproductive maturation of the juveniles is delayed compared to juveniles housed singly, as measured by mean age of vaginal introitus and first estrus (Drickamer, 1974, 1977). In the house mouse, this delay has been shown to be caused by a urinary chemosignal produced by the grouped females (Drickamer, 1982a, 1982b, 1984a, 1984b, 1990). Drickamer (1982c) has described this delay phenomenon as a part of a "feedback model for population control via chemosignals and related social cues". Experiments with feral house mouse populations support Drickamer's idea of urinary chemosignals regulating population size, confirming that this delay in reproductive maturation is not an artifact of domestication of laboratory strains (Drickamer, 1990).

The purpose of this study was to determine if the white-footed mouse (*Peromyscus leucopus noveboracensis*) shows a similar delay in reproductive maturation due to female grouping as shown by Drickamer (1974). A similar response in the white-footed mouse might help explain the mid-summer reproductive inhibition seen in natural populations (Terman, 1993; Wolff, 1986). This reproductive shut-down is seen in the field as a drastic reduction in percentage of

females found in reproductive condition (pregnant, lactating, vagina open or closed) in May, June, and July (Terman, 1993). This reproductive inhibition is not affected by addition of supplemental food (Wolff, 1986; Terman, 1992). The cause of this shut-down is still unclear, but perhaps some element of delay in reproductive maturation of female juveniles caused by other juvenile females or adult females may partially explain this phenomenon.

MATERIALS AND GENERAL METHODS

Animal Care

Animals used in this study were female white-footed mice (*Peromyscus leucopus noveboracensis*) born into a laboratory colony at the Laboratory of Endocrinology and Population Ecology at the College of William and Mary, Williamsburg, Virginia. The laboratory colony is maintained as outbred with no matings between animals more closely related than first cousins, and with addition of wild-caught mice yearly. All experimental animals were born into and raised in litters with at least three pups, with both sexes represented. Young were weaned at 21 days of age and maintained with same-sex siblings until used in the experiments. Experiments were begun in May 1993 and concluded in April 1994. Wood shavings were used as bedding, and food (Rat, Mouse, Hamster Agway 3000) and tap water were available *ad libitum*. Cages and bedding were changed every two weeks, and cage tops and water bottles were changed every month. Experiments were conducted in rooms approximately five meters square, lit by four 40 watt florescent light bulbs in a 14L:10D cycle with lights on from 0700 h to 2100 h. Temperature was maintained at 23 $\pm$ 3C throughout the year. Air was exchanged with outside air from five to eight times per hour. Ambient humidity was maintained except during

the summer, when a portable dehumidifier was used to help reduce food mold.

Animals were sacrificed at the end of each experiment using diethyl ether and/or chloroform, vaginal introitus was noted, they were weighed, the abdominal cavity opened, and they were placed in 10% buffered formalin. The carcass remained in the formalin for a minimum of two weeks until selected organs were cleaned of fat, lightly blotted on paper toweling, and weighed.

Sexual Maturation Criteria

Experimental animals were examined daily between 0700 h and 1100 h for vaginal introitus (an approximate indicator of reproductive maturity, Rogers and Beauchamp, 1974). Once perforate, the experimental animals were examined by vaginal lavage for characteristics of their estrous cycle. The vaginal lavage was performed by gently washing the vagina with approximately 0.1 ml of distilled water using a smoothed syringe, and then placing the water onto a clean glass slide. The syringe was cleaned between individuals by at least five rinses of approximately 1 ml of distilled water. The slide was allowed to air dry, and cell types determined by examination under a light microscope (100 x) using the criteria of Clark (1936) and Bradley and Terman (1979). Slides were not stained before examination. Interpretation of vaginal smears was based on the relative abundance of leukocytes, nucleated epithelial cells, and squamated epithelial found in daily smears. Stages and cell types in the estrous cycle were as follows: proestrus (nucleated epithelial

cells), estrus (squamated epithelial cells), metestrus (nucleated epithelial cells and leukocytes), and diestrus (leukocytes). Each perforate experimental animal was examined by vaginal lavage for five consecutive days, following which the treatment of animals varied by experiment.

Data Recorded

Data recorded on each experimental female were: age at vaginal opening, age at first estrus or metestrus, initial and final body weight (to 0.1 g), and adrenal, ovarian and uterine horn weights (to 0.1 mg).

Statistical Analysis

Each experiment consisted of fifteen replicates. Mean age of vaginal opening, age at first estrus or metestrus, adrenal, ovarian, and uterine weights, initial body weight, final body weight, and weight gain were compared by one-way Analysis of Variance. Only females showing vaginal opening or an estrus/metestrus smear were included in the analyses of mean age of vaginal opening and mean age at first estrus. Multiple comparisons between groups were made by Student-Newman-Keuls test. Kolmogorov-Smirnov two-sample tests (Sokal and Rohlf, 1981; Rohlf and Sokal, 1981) were used on cumulative percentage curves of females showing vaginal opening versus age at vaginal opening. G-tests of independence

were also used on number of females perforate between treatments (Sokal and Rohlf, 1981; Rohlf and Sokal, 1981). Regressions were used to compare final body weight versus age at vaginal opening. Analyses of Variance and regressions were performed using the SPSS/PC 5.0.1 program. In all cases, $p<0.05$ was considered statistically significant.

DETAILED METHODS AND RESULTS

Experiment I

Purpose

Drickamer (1974) demonstrated that when juvenile female house mice (*Mus musculus*) are housed in groups, reproductive maturation is delayed compared with juvenile females housed singly. This experiment was designed to replicate Drickamer's original experiment, and determine if a delay in reproductive maturation can be seen in the white-footed mouse (*Peromyscus leucopus noveboracensis*). Because cage size used here differed slightly from Drickamer (1974), area per animal was used to replicate as closely as possible the original densities. Drickamer found no significant difference in mean age of vaginal opening comparing one juvenile female per cage (420 cm^2/mouse) and two juvenile females per cage (240cm^2/mouse), but animals housed at five juveniles per cage (84cm^2/mouse) had significantly later mean age of vaginal opening than those housed at one or two juvenile females per cage. Age at first estrus was significantly earlier at one juvenile female per cage (420cm^2/mouse) than at two juveniles (240cm^2/mouse) or five juveniles per cage (84cm^2/mouse) (Drickamer, 1974).

Methods

Experiment I consisted of three treatments which varied the density of 21 to

25 day old juvenile females by housing them as follows: (a) one juvenile; (b) two juveniles; and (c) five juveniles housed per 17.3 by 28.2 by 12.3 cm plastic opaque cage. This corresponded to densities of 488cm^2, 244cm^2, and 98cm^2/mouse, respectively. All animals were toe-clipped for individual identification. One experimental animal was chosen randomly (coin flip) per cage, and ear-bobbed for easier identification. Animals housed singly were also ear-bobbed. The experimental animals were examined daily for evidence of vaginal introitus as described above. Once perforate, vaginal smears were obtained for five days, following which each animal was sacrificed and gravimetric analyses were conducted. Experimental animals not perforate by 90 days of age were sacrificed, and underwent the same gravimetric analyses.

Results

The results of Experiment I (Table 1) showed no significant difference between the three treatment groups in mean age of vaginal opening, mean age at first estrus/metestrus, mean adrenal weights, mean ovary weights, mean uterine horn weights, mean initial body weight, mean final body weight, or mean weight gain. There was no significant differences in cumulative percentage curves of vaginal opening versus age (Figure 1) between the three treatment groups. Regression showed no relation between final body weight and age at vaginal opening.

TABLE 1.- Mean age of vaginal introitus, first estrus (days); adrenal, ovary, and uterine horn weights (mg); initial weight, final weight, weight gain (g) and standard errors for females housed at different densities in Experiment I.

Density	Vaginal Introitus	First Estrus	Adrenal Weight	Ovary Weight	Uterine Horn Weight	Initial Weight	Final Weight	Weight Gain
(a) 1 JV/cage	43.0 (3.7)[a]	43.4 (3.8)[a]	8.0 (0.9)[a]	5.9 (0.4)[a]	32.2 (4.5)[a]	10.0 (0.3)[a]	15.3 (0.6)[a]	5.2 (0.5)[a]
(b) 2 JV/cage	36.0 (2.3)[a]	38.7 (1.7)[a]	8.3 (0.6)[a]	6.6 (0.4)[a]	40.7 (4.3)[a]	9.8 (0.4)[a]	15.1 (0.5)[a]	5.3 (0.4)[a]
(c) 5 JV/cage	39.7 (3.5)[a]	37.2 (1.2)[a]	7.2 (0.5)[a]	6.3 (0.5)[a]	41.5 (4.7)[a]	10.7 (0.5)[a]	16.3 (0.6)[a]	5.6 (0.6)[a]
df	2/ 42	2/ 41	2/ 42	2/ 42	2/ 42	2/ 42	2/ 42	2/ 42
F	1.1885	1.5890	0.7070	0.7074	1.3110	1.2919	1.1668	0.1614
p	0.3147	0.2165	0.4989	0.4987	0.2803	0.2854	0.3213	0.8515
n (a, b, c)	(15, 15, 15)	(15, 15, 14)	(15, 15, 15)	(15, 15, 15)	(15, 15, 15)	(15, 15, 15)	(15, 15, 15)	(15, 15, 15)

The F-ratios from 1-way analysis of variance and associated probabilities are given at the bottom of each column. Within a vertical column those means with different superscripts are significantly different at the 0.05 level (SNK Multiple Comparison).

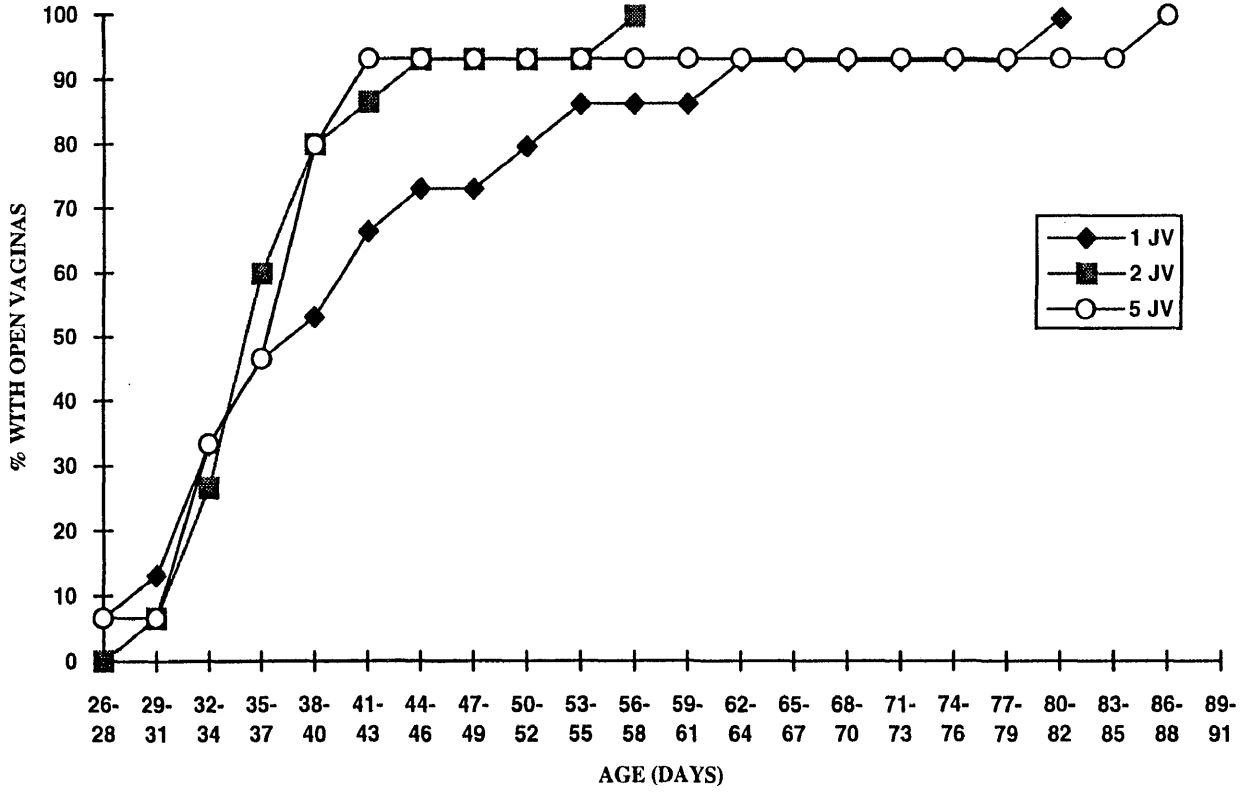

Figure 1. Experiment I: Influence of grouping juveniles on cumulative percentage of female *Peromyscus leucopus noveboracensis* having open vaginae.

Experiment II

Purpose

Since Experiment I showed no significant differences in mean age of vaginal opening or age of first estrus between females housed singly, in pairs, or groups of five, Experiment II was designed to test whether increased density would produce delay in reproductive maturation as seen in Drickamer (1974). Drickamer (1974) found that females housed at five juveniles per cage ($84cm^2$/mouse) had significantly later ages of vaginal opening and first estrus than females housed at one juvenile per cage ($420cm^2$/mouse), and juvenile females housed at seven females per cage ($60cm^2$/mouse) had significantly later ages of vaginal opening and first estrus than those housed at five females per cage ($84cm^2$/mouse).

Methods

Experiment II consisted of the following three treatments which increased the density of 21 to 25 day old juvenile females by housing them as follows: (a) one juvenile; (b) two juveniles; and (c) eight juveniles housed per 17.3 by 28.2 by 12.3 plastic opaque cage. This corresponded to densities of $488cm^2$, $244cm^2$, and $61cm^2$/mouse, respectively. All animals were toe-clipped for individual identification. One experimental animal was chosen randomly (coin flip) per cage and ear-bobbed for easier identification. Animals housed singly were also ear-bobbed. The experimental animals were examined daily for evidence of vaginal

introitus as described above. Once perforate, vaginal smears were obtained for five days, following which each animal was sacrificed and gravimetric analyses were conducted. Experimental animals not perforate by 60 days of age were sacrificed and underwent the same gravimetric analyses. Sixty days of age was chosen as the end point of Experiment II instead of 90 days as in Experiment I since 92% of the experimental females were perforate by 60 days of age in Experiment I (Figure 1).

Results

Experimental females in Experiment II (Table 2) did not differ significantly between three treatment groups in the mean age of vaginal opening, mean age at first estrus/metestrus, mean ovary weights, mean uterine horn weights, mean initial body weight, mean final body weight, or mean weight gain. Mean adrenal weight showed that juveniles housed singly had significantly larger adrenal glands than those housed two or eight juveniles per cage (F df 2/42=5.3585, p=0.0085). There was no significant difference in the cumulative percentage curves of vaginal opening versus age (Figure 2) between the three treatments. Regression showed no relation between final body weight and age at vaginal opening.

Experiment III

Purpose

Since Experiments I and II provided no significant evidence for juvenile

TABLE 2. - Mean age of vaginal introitus, first estrus (days); adrenal, ovary, and uterine horn weights (mg); initial weight, final weight and weight gain (g) and standard errors for females housed at increased density in Experiment II.

Density	Vaginal Introitus	First Estrus	Adrenal Weight	Ovary Weight	Uterine Horn Weight	Initial Weight	Final Weight	Weight Gain
(a) 1 JV/cage	38.8 (2.0)[a]	40.4 (2.0)[a]	9.9 (0.6)[a]	6.4 (0.5)[a]	36.9 (5.7)[a]	9.7 (0.4)[a]	15.8 (0.4)[a]	6.2 (0.5)[a]
(b) 2 JV/cage	37.5 (1.6)[a]	38.6 (1.7)[a]	7.7 (0.2)[b]	5.8 (0.4)[a]	36.3 (3.8)[a]	10.1 (0.4)[a]	15.4 (0.5)[a]	5.3 (0.6)[a]
(c) 8 JV/cage	41.5 (2.1)[a]	42.1 (2.1)[a]	8.4 (0.5)[b]	6.0 (0.7)[a]	31.2 (4.9)[a]	10.6 (0.4)[a]	17.2 (0.5)[a]	6.6 (0.6)[a]
df	2/ 38	2/ 33	2/ 42	2/ 42	2/ 42	2/ 42	2/ 42	2/ 42
F	1.1860	0.7431	5.3585	0.3650	0.4167	1.2576	3.1704	1.2475
p	0.3165	0.4834	0.0085	0.6963	0.6619	0.2948	0.0522	0.2976
n (a, b, c)	(13, 15, 13)	(13, 11, 12)	(15, 15, 15)	(15, 15, 15)	(15, 15, 15)	(15, 15, 15)	(15, 15, 15)	(15, 15, 15)

The F-ratios from 1-way analyses of variance and associated probabilities are given at the bottom of each column. Within a vertical column those means with different superscripts are significantly different at the 0.05 level (SNK Multiple Comparison).

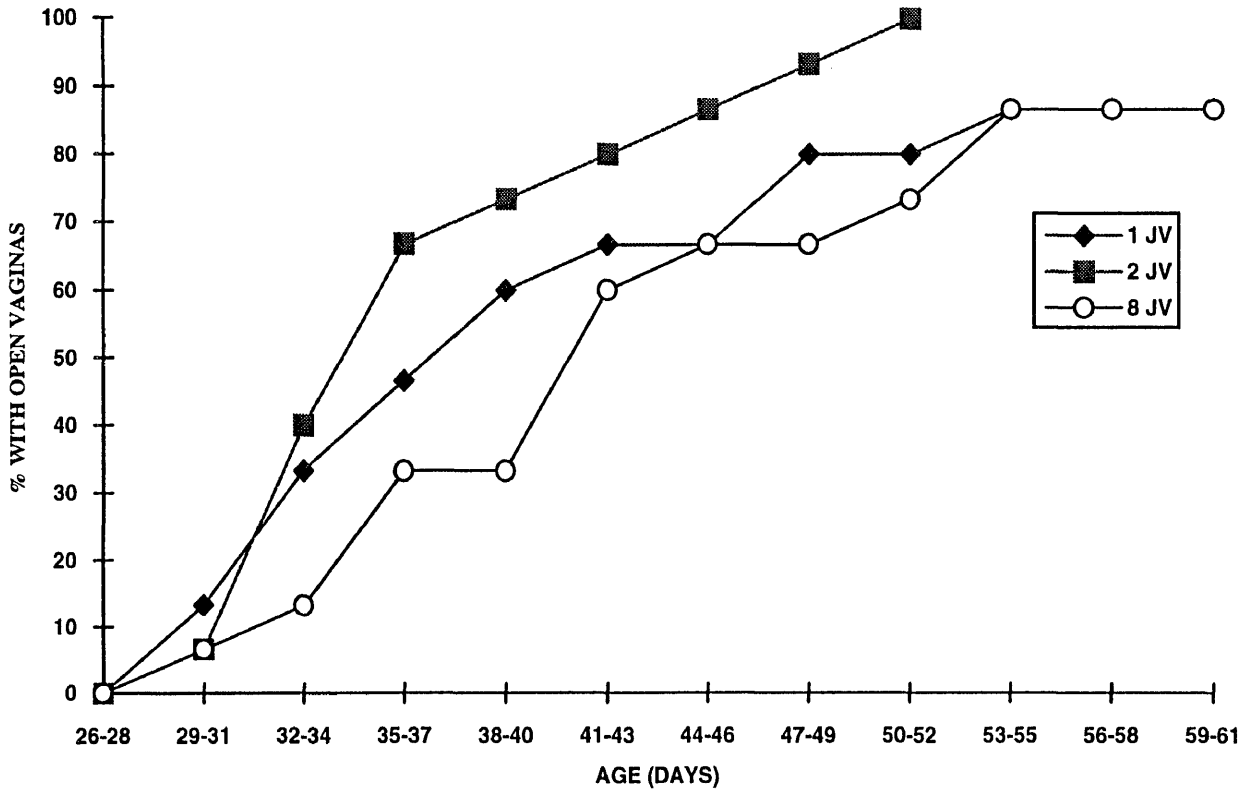

Figure 2. Experiment II: Influence of increased density of juveniles on cumulative percentage of female *Peromyscus leucopus noveboracensis* having open vaginae.

maturational delay by density of other juveniles, this experiment was designed to test whether the presence of adult females delay reproductive maturity in juvenile females. Drickamer (1974) showed that urine from grouped (4 per cage) adult females can also delay reproductive maturation in juvenile females, as evidenced by later mean ages of vaginal opening and first estrus.

Methods

This experiment used wooden cages with a double layer of 1/4" hardware cloth partition to separate the cage into two compartments (28 by 15 by 14 cm), each with three solid wooden walls and floor, and with a wire lid fitting on each compartment. This allowed for exchange of visual, auditory, and olfactory signals across the partition, but tactile contact was prevented. Experiment III consisted of the following four treatments: (a) one juvenilefemale housed with one adult ($\geq$ 60 days old) nulliparous female on one side of the wire partition, and (b) one juvenile on the opposite side of the same cage. In treatment (c) one juvenile female was housed with four adult ($\geq$ 60 days old) nulliparous females on one side of the partition, and (d) one juvenile on the opposite side of the same cage. Whenever possible (77% of cages), sibling juvenile females were used on the opposite sides of the same cage, to reduce the amount of variability possibly due to differences between litters. The experimental animals were examined daily for evidence of vaginal introitus. Once females were perforate, vaginal smears were obtained by lavage for five days. Animals were maintained in each treatment until juveniles

reached 60 days of age, following which they were sacrificed and underwent gravimetric analyses as above.

Results Experiment III

The results of Experiment III (Table 3) showed no significant difference between the four treatment groups in the mean age of vaginal opening, mean age at first estrus/metestrus, mean adrenal weights, mean ovary weights, mean uterine horn weights, mean initial body weight, mean final body weight or mean weight gain. There was no significant difference in the cumulative percentage curves of vaginal opening versus age (Figure 3) between the four treatments by Kolmogorov-Smirnov analyses. Regression showed no relationship between final body weight and age at vaginal opening. Treatments (a) and (c) were significantly ($p<0.001$) different in proportion of females perforate at 38-40 days of age, but not so at other ages.

Additional analyses of the cumulative percentage curves between experiments (Figures 1, 2, and 3) showed that Experiment I, treatment (c) of five juveniles per cage had a significantly ($0.01>p>0.005$) faster rate of maturation than juveniles in Experiment III, treatment (c) of one juvenile in contact with four adult females.

TABLE 3. - Mean age of vaginal introitus, first estrus (days); adrenal, ovary, and uterine horn weights (mg); initial weight, final weight and weight gain (g) and standard errors for juvenile females housed in contact with adult females, or separated from adults by wire partition Experiment III.

Treatment	Vaginal Introitus	First Estrus	Adrenal Weight	Ovary Weight	Uterine Horn Weight	Initial Weight	Final Weight	Weight Gain
(a) 1 JV + 1 AD	41.5 (2.2)[a]	42.1 (2.4)[a]	10.6 (0.5)[a]	9.9 (1.0)[a]	39.6 (5.2)[a]	9.9 (0.5)[a]	18.5 (0.7)[a]	8.6 (0.6)[a]
(b) 1 JV	44.6 (2.4)[a]	44.4 (2.3)[a]	11.2 (2.6)[a]	11.1 (1.2)[a]	41.1 (6.5)[a]	9.5 (0.6)[a]	18.1 (0.5)[a]	8.6 (0.5)[a]
(c) 1 JV + 4 AD	47.8 (2.0)[a]	47.9 (1.9)[a]	11.0 (2.5)[a]	7.6 (1.1)[a]	25.6 (5.7)[a]	10.4 (0.4)[a]	19.1 (0.6)[a]	8.7 (0.6)[a]
(d) 1 JV	46.5 (1.8)[a]	47.6 (1.9)[a]	12.8 (1.0)[a]	9.4 (1.1)[a]	36.9 (5.8)[a]	9.8 (0.5)[a]	17.2 (0.6)[a]	7.4 (0.6)[a]
df	3/ 46	3/ 43	3/ 56	3/ 56	3/ 56	3/ 56	3/ 56	3/ 56
F	1.5468	1.5459	1.7317	1.8162	1.4651	0.5003	1.5978	1.2784
p	0.2151	0.2164	0.1709	0.1547	0.2339	0.6836	0.2001	0.2907
n (a, b)	(13, 14)	(12, 13)	(15, 15)	(15, 15)	(15, 15)	(15, 15)	(15, 15)	(15, 15)
(c, d)	(10, 13)	(9, 13)	(15, 15)	(15, 15)	(15, 15)	(15, 15)	(15, 15)	(15, 15)

The F-ratios from 1-way analyses of variance and associated probabilities are given at the bottom of each column. Within a vertical column those means with different superscripts are significantly different at the 0.05 level (SNK Multiple Comparison).

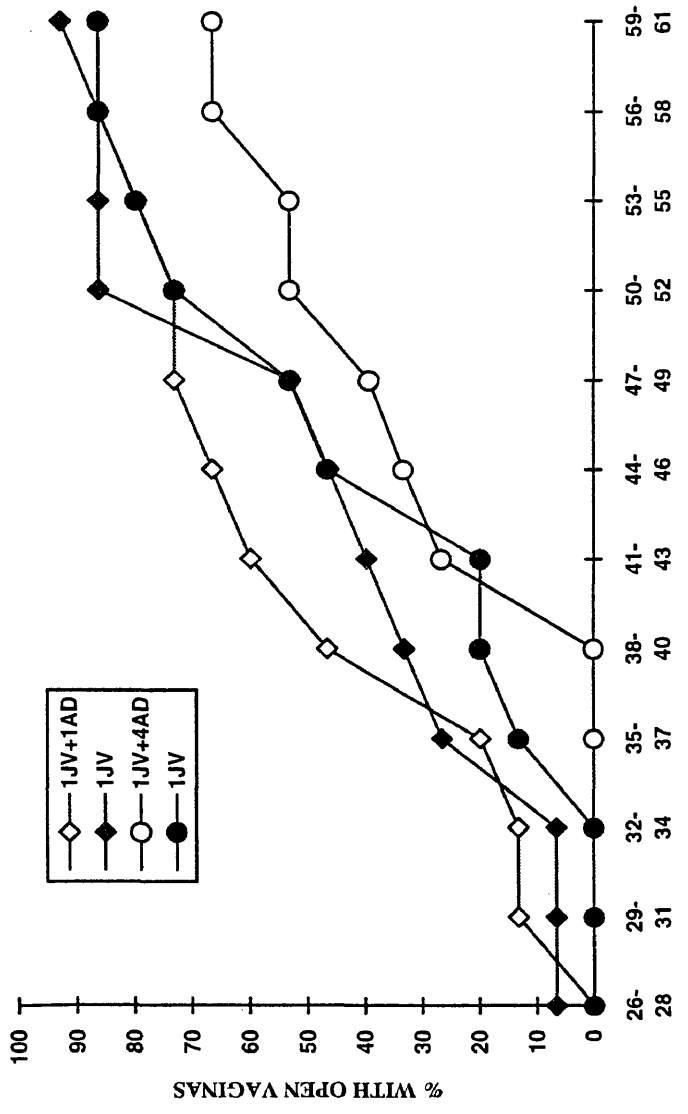

Figure 3. Experiment III: Influence of adult female contact or separation on cumulative percentage of juvenile female *Peromyscus leucopus noveboracensis* having open vaginae.

DISCUSSION

The results of these experiments showed a difference in the response to grouping in *Peromyscus leucopus noveboracensis* compared to the house mouse *Mus musculus* (Drickamer, 1974). Juvenile females housed at similar densities used by Drickamer (1974) showed no difference in mean age of vaginal introitus or first estrus, with increased density, contrary to what was seen in the house mouse. Juvenile females housed with adult females also did not show a significant difference in mean age of vaginal introitus or first estrus, compared to juveniles separated from the adult groups by a hardware cloth partition.

There were no significant differences in selected organ weights between treatments in each experiment, with the exception of Experiment II, where juvenile females housed alone had significantly larger adrenal glands. Adrenal hypertrophy is seen in rodents in crowded or other stressful conditions (Christian, 1970, 1975; Welch, 1964). Reasons why this significant difference was seen in juvenile females housed in isolation in Experiment II, but not Experiment I are unclear. Experiments I and II were conducted at different times of the year, and perhaps seasonality had an effect on the adrenal glands.

There were no significant differences in rate of maturation, as shown by the graph of cumulative percentage of females with open vaginae versus age (Figures 1 and 2), with differing juvenile densities. There were no significant differences in

rate of juvenile maturation with adult female contact, or separation by a hardware cloth partition (Figure 3). Experiment III was interesting in that no juvenile females in contact with four adult females were perforate until 41 days of age, whereas 47% of the juveniles in contact with one adult were perforate by this age. The number of females perforate at age 38-40 days in Experiment III, showed that there were significantly ($p<0.001$, G-test of Independence) fewer females perforate when juveniles are housed with four adult females versus being housed with one adult female. This difference is no longer significant when the number of females perforate in each treatment is compared at 41-43 days of age. This confirms a delay in reproductive maturation produced by grouped adult females in Experiment III, the effects of which are not permanent. These data suggest that adults inhibit maturation until approximately 42 days of age, at which time rate of reproductive maturation increases to approximately the level seen in isolated juveniles or those in contact with one adult.

A comparison between Experiment I and Experiment III yielded an interesting finding, in that juveniles housed with other juveniles had a significantly faster rate of reproductive maturation than those housed with adult females. Experimental design differed between Experiments I and III, in that though the number of animals per cage was the same (five), there were five juveniles in Experiment I, and one juvenile and four adults in Experiment III. Further, the cage size was smaller in Experiment III, increasing the density in Experiment III to 84 cm^2/mouse, from 98 cm^2/mouse in Experiment I. The social environment was also

different in Experiment III in that another juvenile female was on the opposite side of the same cage as the juvenile housed with four adult females. These changes in area/animal and social stimuli may have had an effect on the rate of maturation. Cumulative percentage curves were not significantly different between the Experiments I and II, or Experiments II and III.

A noticeable difference between *Peromyscus leucopus noveboracensis* and *Mus musculus* is the white-footed mouse's variability in response to these experimental manipulations. The individual variation in each treatment in Experiment I was surprising; all experimental animals in groups of two were perforate by 58 days of age, while one individual housed alone was not perforate until 80 days of age, and one experimental juvenile housed with four other juveniles was not perforate until 86 days of age. This sort of variability of age of vaginal opening was not seen in studies of *Mus musculus*, but has been observed in studies of the prairie deermouse, *Peromyscus maniculatus bairdi* (Bradley and Terman, 1979). Comparing coefficients of variation of age of vaginal opening in juvenile females housed one, two or five per cage, resulted in CV=33.0, 25.1, and 33.9, respectively for *P. l. noveboracensis*, and CV=9.8, 7.7, and 8.9, respectively for *M. musculus*. The original experiments with the house mouse (Drickamer, 1974) were conducted using inbred laboratory strains, whereas the white-footed mice used in these experiments were no more than three generations removed from the wild, and inbreeding is prevented in colony maintenance. The variability of response of *P. l. noveboracensis* may be responsible for the lack of significance in the differences of

rate of vaginal opening that was seen.

Questions have been raised as to the reliability of vaginal introitus as an indicator of reproductive maturation, with the suggestion that first estrus be used as a more reliable indicator. This study suggested that vaginal introitus is a good indicator of an estrus stage, in that 93% of all females examined showed an estrus or metestrus stage within five days of vaginal opening. The criteria of estrus or a metestrus stage was used because the progression of stages were somewhat irregular. The estrous cycle of *Peromyscus leucopus* is approximately five days (Dewsbury et al, 1977), but the interval between stages varied in this study, so the criteria of an actual estrus, or a metestrus smear was used to determine potential female receptivity. In *Peromyscus maniculatus bairdi*, an estrus smear was found not to be an indicator of female receptivity, since 30% of females observed to copulate did not show an estrus stage when a vaginal smear was taken immediately after copulation (Bradley and Terman, 1979). Data presented here cannot support estrous cyclicity, since the smearing period was too brief to show a complete estrous cycle. Most animals did show a progression of cell types, which would indicate establishment of an estrous cycle, with the following exceptions. Three females in Experiment II, treatment (b) showed evidence of a change in cell types, with proestrus and diestrus stages, but no estrus or metestrus stages were seen. It is possible that an estrus or metestrus stage might have been detected if the period of vaginal lavage was extended past five days. Seven females, one each in Experiment I (a) (c), Experiment II (b) (c), and Experiment III (a) (b) (c), showed no evidence of a

change in cell types, and remained in diestrus throughout the five day smearing period.

Another common indicator of reproductive maturation is body weight. In this study, I found no significant relation between body weight at death and age at vaginal opening. In Experiments I and II, the ages of the animals at time of death was directly related to age at vaginal opening, since the animals were sacrificed five days after vaginal opening. This gave an approximation of body weight at age of vaginal introitus, assuming that the rate of change in body weight before and after vaginal introitus is the same, which may not be true. The lack of a relation between body weight and age at vaginal opening in Experiments I and II may be due to the *ad libitum* laboratory diet fed to the animals. Wolff (1986 and others) used $\leq$15 g as being the criteria for a juvenile animal. In field studies, a smaller weight is still probably reliable, since younger animals may be out-competed for food, and not put on weight as quickly. Wolff (1986) also used pelage color as an indicator of maturity, with gray pelage being juvenile, and brown pelage being adult. Pelage change was not noted in these experiments, but pelage appeared to consistently brown with little or no gray fur when the animals were sacrificed in all three experiments (pers. obs.).

In summary then, *Peromyscus leucopus noveboracensis* showed no delay in mean age of reproductive maturation when juveniles are grouped together, unlike *Mus musculus* (Drickamer, 1974). These findings do not support a grouped juvenile induced delay in female reproductive maturation in white-footed mice as is seen in

house mice. However, juvenile females respond differently to being housed with adult females versus being housed with juveniles. The phenomenon of grouped female induced reproductive maturational delay is significant when adult females comprise the social group in *Peromyscus leucopus noveboracensis*, but not when juvenile females comprise the social group. Contact with several adult females causes only a temporarily delay in reproductive maturation of juvenile females, from which the juveniles are able to recover. Given that no juveniles in contact with four adults showed vaginal introitus until 41 days of age in Experiment III (Figure 3), these individuals may be delayed in opportunities to reproduce.

Future experiments involving grouping adult females and juvenile females would further elucidate differences described here. If this delay in reproductive maturation seen in Experiment III can be replicated using a larger sample size, and an experimental design consistent with Experiments I and II, then the mechanisms behind the responses could be examined. This could involve the use of no-contact cages, exchange of soiled bedding, or urine collected from grouped adult females. Thus, adult female induced reproductive maturational delay may have an effect on population regulation by temporarily suppressing juvenile female reproduction. This suppression is not permanent however, and juveniles will recover from this delay spontaneously. Data presented in these experiments do not support the idea that female induced reproductive delay is a major component of the mid-summer reproductive inhibition seen in natural populations of *Peromyscus leucopus noveboracensis*.

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