OESTROUS FREQUENCY AND INCIDENCE OF PREGNANCY IN MICE HOUSED SINGLY AND IN GROUPS AT 4, 21, AND 33°C

By Pamela R. Pennycuik*

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Abstract

Oestrous cycle frequency was measured in mice housed singly, in mice housed in groups, and in grouped females in olfactory and tactile contact with a male. The animals were kept at 4, 21, or 33°C. When the mice were housed singly, all groups had between 2·2 and 3·5 cycles in the 16 days period of observation. The small differences between the groups were not significant. Grouping (15 mice per cage) caused a significant reduction in oestrous frequency at all three temperatures, but the differences between the temperature groups were not significant. Introduction of a caged male into the group of females had no effect on oestrous frequency at 4 and 21°C. At 33°C frequency increased and the difference between the temperature groups became significant. When the males were released into the cages of grouped females the incidence of pregnancy in the group at 33°C was seven times greater than that at 4 or 21°C.

The results suggest that environmental temperature may modify the breeding performance of grouped females by altering their sensitivity to the presence of males.

I. Introduction

In the field, mice usually cease breeding in the winter months (Southern and Laurie 1946; Breakey 1963; Pearson 1963; Lidicker 1966; DeLong 1967; Berry 1968; Newsome 1969). In at least two instances, this cessation of breeding has been found to be associated with an increase in the frequency of anoestrous females in the population (DeLong 1967; Newsome 1969). One of the environmental factors thought to play a part in suppressing reproduction in winter is low environmental temperature (Pearson 1963; DeLong 1967).

In freely growing populations housed in pens, breeding usually declines as population numbers increase (Strecker and Emlen 1953; Crowcroft and Rowe 1957; Lidicker 1965; Kessler 1966; Newsome 1967). This decline too, has been found to be associated with an increase in the frequency of anoestrous females in the population (Crowcroft and Rowe 1957; Newsome 1967). Several mechanisms have been suggested to explain this cessation of breeding and the increase in the frequency of anoestrous females (see Whitten and Bronson 1970). One of these is that female

* Division of Animal Genetics, CSIRO, P.O. Box 90, Epping, N.S.W. 2121.

primer pheromones may suppress oestrus when the number of females in the group is high (Whitten and Bronson 1970). However, these authors considered that this possibility was unlikely because of the relative ease with which this suppression could be broken by male odour (Whitten 1959).

Low temperatures have been found to have little effect on the reproductive capacity of mice housed as pairs in cages exposed to outdoor conditions (Pennycuik 1972). In pens, where mice are able to determine the group size in which they live, the numbers in the deme are usually larger than this, and females usually outnumber males (Reimer and Petras 1967). If environmental temperature affected the sensitivity of females to the presence of other females or to the presence of males, this could perhaps explain the marked breeding depression in field populations during the winter months. This experiment was designed to test this possibility. The temperatures used were 4, 21, and 33°C and the reproductive characters studied were the frequency of oestrus and the incidence of pregnancy.

II. Materials and Methods

(*a*) *The Environments*

Three different environments were used: 4, 21, and 33°C. The 4°C room was a cold room in which lighting was regulated to 14 hr light and 10 hr darkness. The 21°C environment was an air-conditioned laboratory lit by large windows. The 33°C environment was a chicken incubator standing in a room exposed to natural day length. Humidity was not controlled in the 4 and 33°C environments; in the 21°C environment it was prevented from rising above 60% by means of a dehumidifier. All three environments contained the experimental animals only, i.e. animals were not exposed to smells from the main mouse colony.

(*b*) *Cages*

The cages used were of solid metal with a wire mesh lid. Single mice were housed in cages with a floor area 15 by 27 cm, groups of mice were housed in cages with a floor area of 28 by 43 cm. All cages had approximately 2 cm sawdust in the bottom. This was changed at intervals of approximately 4 weeks. Mice at 4 and 21°C were given wood-wool for nests.

The cages used for males introduced into the large cages of females were cylindrical treacle tins 10 cm in diameter. These had 1-cm holes drilled at intervals round the sides and in the lids, i.e. it was possible for the mice to touch one another.

(*c*) *Food*

The animals were fed *ad libitum* on a pelleted diet supplied by Allied Feeds. The composition of this diet is as follows: wheatmeal 54%, coconut meal 6%, milk powder 8%, lucerne meal 10%, meat meal 19·5%, dried yeast 1%, bone flour 1%, salt 0·5%, cod liver oil 12·5 ml/kg, vitamin A 8800 i.u./kg, vitamin D_3 2200 i.u./kg, vitamin E 22 i.u./kg, butylated hydroxytoluene 108 mg/kg.

(*d*) *Animals*

The animals (*Mus musculus*) used were from a random-breeding stock formed by crossing mice from our wild colony with a laboratory stock, R70.

(*e*) *Procedures*

Ninety adult female mice were divided into three groups of 30 animals by splitting litters from 18 mothers. Each group was moved to one of the experimental temperatures described above.

The treatments, numbers of animals, and measurements carried out are summarized for the group at 4°C in the following tabulation:

Day	Treatment and numbers of animals		Measurements
0	To 4°C, mice housed singly (30)		Weighed
19			Vaginal smears
34	Mice divided into two groups:		
	"Singles" (15)	"Grouped" (15)	
47			Weighed, Vaginal smears
62			
69		Caged male into female cage	
75			Weighed, Vaginal smears
96			
103		Male released	Weighed
104			Vaginal smears
119	Females killed	Male removed	
125		Females moved to separate cages and watched for birth of litter	
137		Last litter born	
138			Weighed, Vaginal smears
153		1 male placed in cage with each female	
168		Males removed and females watched for birth of litters	
171		Last litter born	
		Females killed	

The groups at 21 and 33°C were treated in the same way. Essentially the treatments were designed to show:

(1) Whether exposure to different temperatures affected the number of oestrous periods observed in 16 days in mice housed singly.
(2) If exposure to an extreme temperature did affect the number of oestrous cycles, whether these returned to normal as the animals became acclimatized.
(3) Whether the depression in the number of oestrous cycles in females housed in groups was greater at one temperature than at another.
(4) Whether exposure to a caged male affected the number of oestrous cycles in grouped females more at one temperature than another.
(5) Whether temperature affected the number of females becoming pregnant when the male was released into the group of females.
(6) Whether number of oestrous cycles in 16 days returned to normal when the mice were again housed singly.
(7) Whether temperature affected the number of females which became pregnant when each female was paired with one male.
(8) Whether any of the treatments affected the growth rate of the animals.

Vaginal smears were made for 16 consecutive days during each period of measurement. They were carried out at approximately the same time each day by the same observer throughout the experiment. For each 16-day period of observation the number of times that each mouse exhibited an oestrous smear was recorded. An oestrous smear was considered to be one in which all the cells were fully cornified and which was followed next day by one containing leucocytes typical of metoestrus (Whitten 1966).

Mice were counted as pregnant if they had a litter or, if they died before the end of the experiment, if they were found to have foetuses *in utero*.

III. Results

Table 1 shows the effects of exposure to 4, 21, or 33°C on the frequency of occurrence of oestrus in mice housed singly. During the first period of measurement the mice at 4°C had fewer cycles than those at 21 or 33°C but the difference between the groups was not significant. At all temperatures there was a small increase in frequency with time but in no instance was the change significant.

Table 1

Frequency of occurrence of oestrus during four 16-day periods (mean ± s.e.) in mice living singly at 4, 21, and 33°C

At each temperature group size was 14

Time from start of experiment (days)	Environmental temperature (°C)			$F_{4,21,33}$
	4	21	33	
19–34 (1)	2·21±0·27	2·57±0·37	3·00±0·32	1·47
47–62 (2)	3·21±0·26	2·64±0·44	3·50±0·13	4·22*
75–96 (3)	2·93±0·19	2·79±0·37	3·43±0·22	3·17
104–119(4)	2·71±0·33	3·14±0·32	3·21±0·33	0·69
$F_{1,2,3,4}$	2·52	0·13	0·75	

* $0{\cdot}05 > P > 0{\cdot}01$.

Table 2

Effects of grouping and exposure to a male on the number of oestrous cycles observed during four 16 day periods (mean ± s.e.) in mice at 4, 21, and 33°C

At each temperature group size was 15, with the exception of period 4 at 4°C where group size was 14

Time from start of experiment (days)	Treatment	Environmental temperature (°C)			$F_{4,21,33}$
		4	21	33	
19–34 (1)	Housed singly	2·33±0·26	2·80±0·27	3·27±0·24	3·35*
47–62 (2)	Grouped	1·07±0·24	0·60±0·21	1·33±0·23	2·75
75–96 (3)	Grouped with male	1·07±0·24	0·53±0·23	1·93±0·36	6·28**
138–153(4)	Housed singly	2·71±0·26	3·27±0·24	3·40±0·31	1·78
$F_{1,2}$		12·62***	42·17***	35·21***	
$F_{2,3}$		0·00	0·05	2·01	
$F_{3,4}$		21·61***	68·73***	9·61***	
$F_{1\,4}$		1·04	1·72	0·11	

* $0{\cdot}05 > P > 0{\cdot}01$. ** $0{\cdot}01 > P > 0{\cdot}001$. *** $0{\cdot}001 > P$.

Table 2 shows the effects of grouping and of the presence of a male on the frequency of oestrus in mice at 4, 21, and 33°C. At all three temperatures grouping caused a reduction in the number of times the mice came into oestrus during a 16-day period. The difference between the three groups was not significant. Introduction of a

caged male had no effect on the animals at 4 and 21°C but his presence increased the frequency of oestrus at 33°C. This treatment caused the difference between the three temperature groups to become significant. When the females were housed singly again in period 4 the frequency of oestrus returned to the levels at the start of the experiment.

TABLE 3

FREQUENCY OF PREGNANCIES AMONG FEMALES CAGED WITH MALES FOR 16 DAYS

Animals were caged in the proportion of one male to 14 or 15 females, or one male to one female, and were kept at temperatures of 4, 21, or 33°C

Treatment	4°C		21°C		33°C		χ^2
	No. of females	No. of pregnancies	No. of females	No. of pregnancies	No. of females	No. of pregnancies	
One male with 14 or 15 females	14	1	15	1	15	7	9·605**
One male with one female	14	11	15	12	14	12	0·263
χ^2	14·583***		16·425***		4·896*		

* $0{\cdot}05 > P > 0{\cdot}01$. ** $0{\cdot}01 > P > 0{\cdot}001$. *** $0{\cdot}001 > P$.

Table 3 shows the frequency of pregnancies among females caged with males for 16 days. In one group one male was placed in a cage of 14 or 15 females, in the second the animals were housed as pairs. At 4 and at 21°C only one female became pregnant when the ratio of males to females was 1 : 15. At 33°C seven females

TABLE 4

WEIGHTS (MEAN±S.E.) AT THE START OF THE EXPERIMENT (DAY 0) WHEN ALL MICE WERE AT 21°C AND AT 103 DAYS WHEN THEY WERE HOUSED SINGLY OR IN GROUPS AT 4, 21, OR 33°C

Time from start of experiment (days)	4°C		21°C		33°C	
	Housed singly	Grouped	Housed singly	Grouped	Housed singly	Grouped
0	22·5±1·2	23·0±0·6	23·2±0·8	22·7±0·8	23·3±1·1	23·1±0·6
103	28·9±1·1	27·8±0·7	27·7±0·9	28·4±0·8	28·8±1·6	28·6±0·8

became pregnant and the difference between this group and the two at the lower temperatures was significant. When the animals were housed as pairs approximately 80% of the females became pregnant in all three groups.

Growth rates were very similar in "single" and "grouped" mice at all three temperatures (Table 4).

IV. Discussion

In the mice used in this experiment, the incidence of pregnancy in females grouped together with one male was undoubtedly affected by environmental temperature. The frequency of oestrus in grouped females exposed to a male was also singificantly different in the three temperature groups. These results suggest that one possible explanation for breeding depression in field populations in the winter months is that grouping of females tends to suppress oestrus and that this suppression is not broken by the presence of males when the temperatures are low. If these results are confirmed in mice of other strains it may be necessary to reassess the importance of the part played by female primer pheromones in the control of population size (Whitten and Bronson 1970). It will also be necessary to examine the data collected in field studies for evidence of this method of controlling population size.

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