REPRODUCTIVE PERFORMANCE AND BODY WEIGHTS OF MICE MAINTAINED FOR 12 GENERATIONS AT 34°C

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Summary

The reproductive performance and body weights of mice exposed to 34°C for 12 generations were compared with those of mice maintained at 21°C over the same period.

During a mating period of 12 weeks the mice at 34°C reared only six pups to 6 weeks of age compared with 23 pups reared by pairs at 21°C. This decline in numbers was due to reduction in the number of matings which produced litters, reduction in the number of litters born, reduction in litter size at birth, and increase in the numbers of pups dying between birth and 6 weeks of age. Maintenance of the stock at 34°C for 12 generations had little or no effect on these parameters, but did affect the time from the setting up of the mating to the birth of the first litter, the length of gestation, and the length of the interval between birth and the first feed.

Three- and 6-week-old pups at 34°C were lighter than pups of corresponding ages at 21°C. During exposure to 34°C, body weight increased between generations 2 and 4, but declined again gradually over the next eight generations.

I. Introduction

It is known that reproduction is impaired in both invertebrates and vertebrates by exposure to temperatures towards the upper end of the range compatible with adult survival. The degree of impairment appears to be related to the temperature employed, ranging from total infertility at sublethal temperatures to reduced fecundity at temperatures closer to the optimum for the species (Brun 1965; Pennycuik 1967). At the lower end of this zone of reproductive impairment, it should be possible to breed animals for improved performance at high environmental temperatures. This has been done for the hermaphroditic nematode, *Caenorhabditis elegans*, by Brun (1965) who showed that it was possible to breed this worm at 22°C for an indefinite number of generations and that reproductive output improved following an initial decline in fecundity over the first two or three generations. At 23°C this initial decline led to extinction of the stock in five or six generations. Similar experiments of shorter duration have been carried out with mammals. Those of Sundstroem (1922, 1930) suggest that mice and rats, like *C. elegans* at 22°C, can be bred for an indefinite number of generations at high temperatures, for this author observed no decline in litter size as generation number increased to four. However, Stieve (1923) and Chevillard and Cadot (1963) found that their stocks of mice and rats died out in about four generations as a result of a reduction in the yield of each mating, reduced litter size, and reduced pup survivals.

The experiments of Sundstroem (1922, 1930) were not carried out for sufficiently long to establish whether the fecundity of his animals could be improved by selection, or to follow the physiological and morphological changes which occurred

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prior to, or during, improvement. In this experiment and in that reported in the following paper (Pennycuik 1969*a*) an attempt was made to answer some of these questions.

II. Materials and Methods

(*a*) *The Constant-temperature Room and the Incubator*

The control temperature (21°C) was that at which the animal room was maintained. Humidity was prevented from rising above 70% with a de-humidifier. The experimental temperature (34°C) was achieved by using a chicken incubator which stood in the animal room.

(*b*) *The Diet*

The animals were fed *ad lib.* on a pelleted diet prepared by Drug Houses of Australia (wheatmeal 54%, coconut meal 5·6%, meat meal 18·8%, milk powder 8%, yeast 1%, sterilized bone flour 0·8%, salt 0·5%, lucerne 10%, cod-liver oil 1·3%). In addition, they were given approximately 2·0 g of fresh lucerne once a week.

(*c*) *Animals and Procedures*

The mice were from a random-breeding strain R70, formed by crossing a multiple recessive stock from Harwell with inbred strains 101, C_3H, and CBA. For two generations (−2 and −1) this stock was kept at 21°C. The litters of animals belonging to generation −1 were split at weaning and half the males and half the females were transferred to 34°C. These animals became generation 0 of the R95 stock. The remaining mice were kept at 21°C and became generation 0 of the R70 stock.

From this point on no selection was practised on the R70 stock but the R95 stock was selected on the basis of the number of pups reared to 6 weeks of age following a 12-week mating period, i.e. on parental performance. The selection procedure was as follows. All matings were ranked according to the number of pups reared to 6 weeks of age. Following the exclusion of those which reared no pups, the productivity of the best mating relative to that of the least productive was calculated. Using this index as a basis, 26 pups were selected for the next generation; the least productive mating contributed one pup only, the other matings contributed the appropriate multiple of this figure. In some generations the number contributed by the best mating was as high as 8. This occurred when the number of productive matings was low and when the gradient from the most to the least productive was steep. In setting up these matings, sib matings were avoided and mating was delayed until the mice were 10 weeks old or older. Selection was continued in this manner for 12 generations.

Throughout the experiment, cages were examined for litters at least three times a week but usually more frequently. The date of birth was estimated from the degree of maturity of the pups; this date was recorded and so too were the numbers born, the numbers alive, and the numbers of each sex. Because checks for new-born litters were not made every day or several times a day, litter size at birth and the mortality among the new-born pups were almost certainly underestimated.

At 3 weeks the number of pups in the litter was again recorded. Litters were usually weaned at this time but if pups were small, weaning was sometimes delayed until the next litter was born. Final counts of litter size were made when pups were 6 weeks of age. All pups born to the R95 stock were weighed to the nearest 0·5 g at 3 and 6 weeks; in the R70 stock, generations −2 to 10, only the first litter was weighed, in later litters only the numbers of animals alive at each age were recorded. In generations 11 and 12 all pups from all litters were weighed at 3 and 6 weeks.

From the date on which the matings were set up and the date on which the litters were born, it was possible to establish the length of the interval between mating and the birth of the first litter and of the interval between litters. By assuming that between-litter intervals of 37 days or less indicated successful post-partum matings and that intervals of 38 days or greater indicated post-lactation matings, it was possible to estimate the percentage of all second and subsequent pregnancies which resulted from successful post-partum matings.

In addition to these measurements made each generation, mice of selected generations were examined for the ability of males to sire litters, the ability of females to become pregnant following service, the length of gestation, and the length of the interval between birth and the first feed. The methods employed in making these measurements are described elsewhere (Pennycuik 1967). Male fertility measurements were carried out at generations 8 and 9, female fertility measurements on generations 1–6, gestation length measurements in generations 1, 2, 4, 5, 8, and 9, and the length of the birth to feeding interval in generations 8 and 9.

(*d*) *Inbreeding Coefficients*

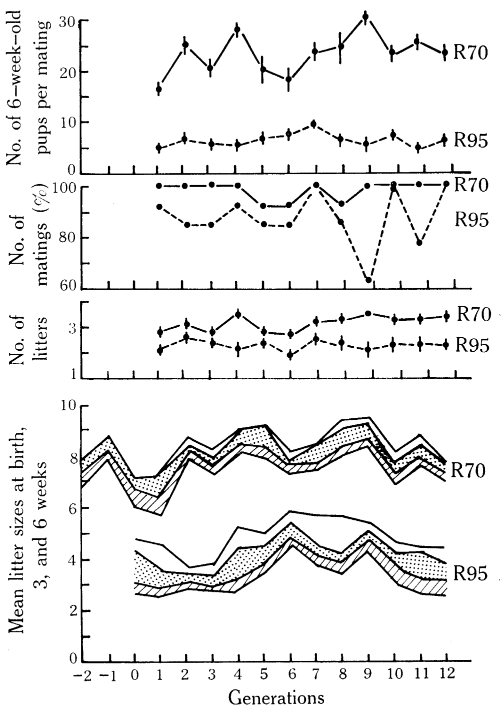
Because the number of matings contributing to the next generation was small in the R95 stock, it was feared that these animals might have become very inbred and that this could have affected the results. Inbreeding coefficients were therefore calculated for both groups. In the R70 stock there was a steady increase in the value of the coefficient from generation 0 to generation 10. From this point onwards it remained relatively constant at about 11%. In the R95 stock the increase in the coefficient was more rapid and there was no sign of a constant level being reached with increasing generation number. The degree of inbreeding at the thirteenth generation in the R95 stock was approximately double that in the R70 stock (19%).

III. Results

(*a*) *Productivity*

Figure 1(*a*) illustrates the differences, between mothers at 21 and 34°C, in the numbers of pups reared to 6 weeks of age during a 12-week mating period. Exposure

Fig. 1



(*a*) Number of pups (mean ± S.E.) reared successfully to 6 weeks of age during a 12-week mating period by R70 mice at 21°C and R95 mice at 34°C. All pairs were included in these calculations, irrespective of whether they had any offspring or not.

(*b*) Number of pairs which produced one or more litters expressed as a percentage of the total number of pairs.

(*c*) Number of litters (mean ± S.E.) born to each pair which produced one or more litters.

(*d*) Litter sizes (means) at birth and number of pups alive at birth, 3, and 6 weeks. Unshaded area indicates losses during the first 24 hr, the area delineated by stippling the losses between birth and 3 weeks, and the oblique shading the losses between 3 and 6 weeks.

to the higher temperature reduced these numbers to approximately one-quarter of those at 21°C and there was little change in performance between generations 1 and 12.

Figures 1(*b*), 1(*c*), and 1(*d*) show that the reduced productivity at 34°C was due to several causes: to an increase in the number of unproductive matings, a reduction in the number of litters born to each productive mating, a fall in litter size at birth, and an increase in the mortality of pups between birth and 6 weeks of age. The relative importance of these four factors was of the order of 1 : 2 : 2 : 1. These figures also show that there was little change, with increasing generation number, in the relative importance of the factors which contributed to reduced productivity.

TABLE 1

INTERVALS FROM SETTING UP OF MATING PAIRS TO BIRTH OF FIRST LITTER AND FREQUENCY OF POST-PARTUM PREGNANCIES IN R70 MICE MAINTAINED AT 21°C AND R95 MICE MAINTAINED AT 34°C

Generation No.	Dates when Matings were Set Up		Mean Time to Birth of First Litter (days ± S.E.)		Post-partum Pregnancies as % of Second and Subsequent Pregnancies	
	R70	R95	R70	R95	R70	R95
−2	12.ix.61		23·8±0·9			
−1	4.i.62		22·9±1·1			
0	20.iii.62	21.iii.62	21·6±0·4	51·2±5·5		
1	3.vii.62	28.vi.62	21·9±0·3	51·8±6·2	66·6	69·2
2	12.xii.62	12.xii.62	23·8±1·7	30·9±4·9	62·9	55·6
3	7.v.63	7.v.63	22·3±1·0	38·9±3·8	60·8	86·7
4	11.xi.63	25.x.63	22·6±1·4	36·1±4·3	90·6	69·2
5	7.iv.64	6.iv.64	23·5±0·9	27·5±2·5	61·9	60·0
6	25.viii.64	25.viii.64	21·8±0·5	24·9±1·7	60·9	30·0
7	13.i.65	13.i.65	22·2±1·2	21·2±0·4	82·1	44·4
8	10.vi.65	10.vi.65	24·4±2·2	28·4±2·5	89·2	64·3
9	20.x.65	20.x.65	23·4±1·4	29·6±5·7	84·8	55·5
10	30.iii.66	30.iii.66	20·9±0·3	26·2±1·8	86·6	64·7
11	18.viii.66	17.viii.66	23·0±1·9	27·0±2·1	96·7	46·2
12	4.i.67	4.i.67	21·8±0·5	23·3±0·9	96·8	64·7

TABLE 2

NUMBER OF SUCCESSFUL SERVICES (AS A PERCENTAGE OF THE TOTAL) WHEN R70 MALES WERE CROSSED TO R70 FEMALES OR R95 FEMALES, AND WHEN R95 MALES WERE CROSSED TO R70 FEMALES

The numbers in parenthesis indicate the numbers of animals investigated in each group

	Generations Investigated	R70 Stock (21°C)	R95 Stock (34°C)
Number of females pregnant following service by R70 males at 21°C (%)	1−6	86·1 (166)	63·5 (189)
Numbers of males which sired one or more litters when mated to R70 females at 21°C (%)	8, 9	100·0 (15)	81·3 (16)
Number of successful services following mating of fertile males to R70 females at 21°C (%)	8, 9	87·8 (15)	57·0 (13)

Examination of Table 1 shows that exposure to 34°C increased the length of time elapsing before the birth of the first litter and that selection caused a rapid decline in the duration of this interval. This could have led to an increase in the number of litters produced during a 12-week mating period. However, litter numbers remained relatively constant throughout 12 generations [Fig. 1(*c*)]. Table 1 also shows that, although the number of pregnancies resulting from post-partum matings showed

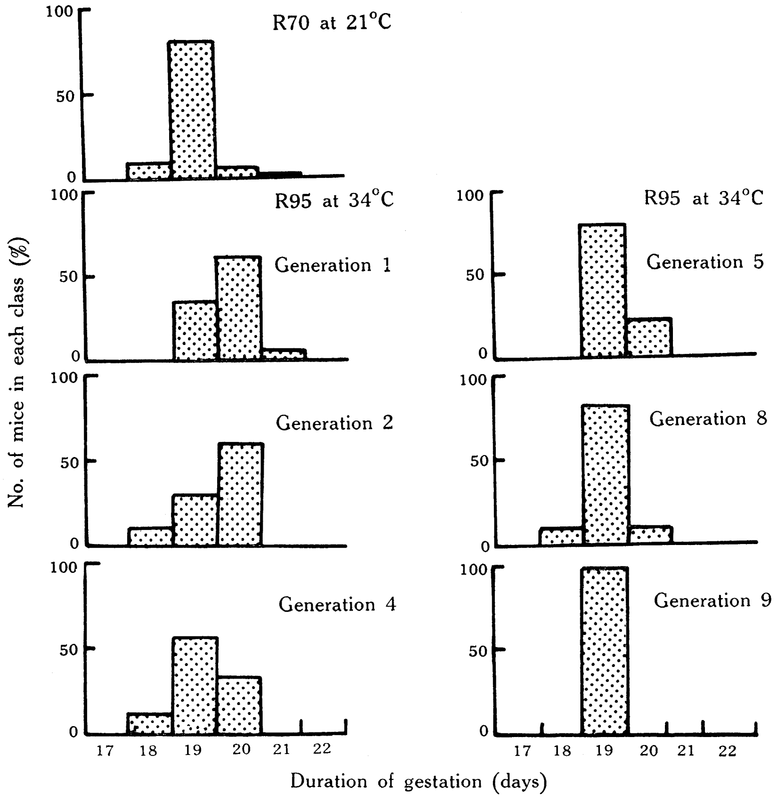


Fig. 2.—Gestation periods of mice at 21°C and of various generations of mice at 34°C. The number of animals in each group were as follows: R70 at 21°C, 149 mice; R95 generation 1, 23 mice; generation 2, 10 mice; generation 4, 9 mice; generation 5, 9 mice; generation 8, 11 mice; generation 9, 10 mice.

Table 3

LENGTH OF THE INTERVAL BETWEEN BIRTH AND THE FIRST FEED FOR R70 MICE AT 21°C, R70 MICE AT 32·7°C, AND R95 MICE AT 34°C

Group	Temp. (°C)	Generations	No. of Mice/ Group	Time to First Feed (min)	
				Mean	Range
R70	21	1–12	7	75	35–110
R70	32·7	1–12	12	129	45–200
R95	34	8 and 9	6	100	60–160

marked fluctuations at 34°C, there was no consistent trend with increasing generation number. More detailed examination of the fate of litters born as a result of post-partum and post-weaning matings shows that the former have a smaller chance of survival than the latter (Pennycuik 1969*b*). Animals which mated successfully at the post-partum oestrus, therefore, may have been at a disadvantage.

Table 2 suggests that both male and female infertility contributed to the reduction in the number of matings which produced litters and to the reduction in the number of litters produced by each mating. When R70 males were crossed with

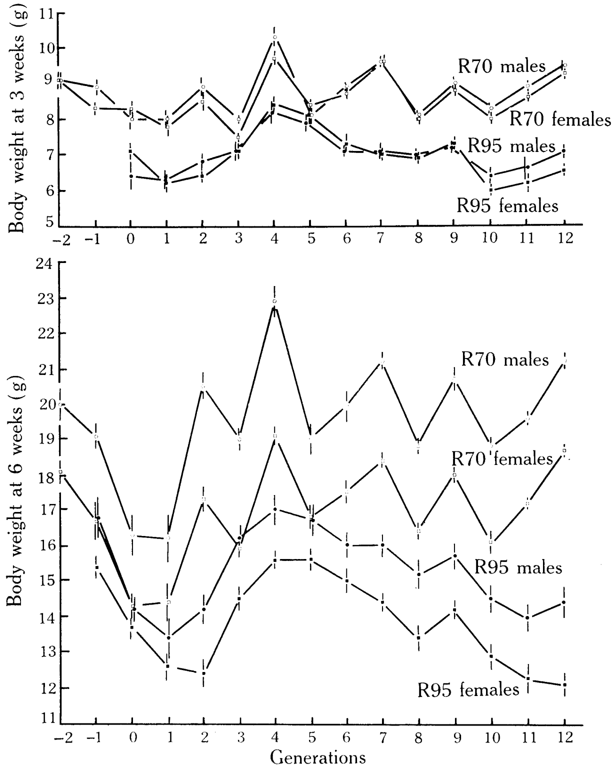


Fig. 3.—Body weights (means ± S.E.) at 3 and 6 weeks of age of R70 and R95 pups reared at 21 and 34°C respectively.

R70 females the percentage of pregnancies following service was higher than that resulting from R70 male by R95 female crosses or from R95 male by R70 female crosses. Comparison of the results obtained for both sexes at 34°C with those obtained for R70 mice exposed to 32·7°C from weaning age (Pennycuik 1967) suggests that maintenance of these mice at 34°C for several generations had little effect on the success of service in either sex.

Exposure to 34°C caused an increase in the length of gestation but this became shorter as selection proceeded (Fig. 2) and so too did the interval between the birth of the litter and the first feed (Table 3). Although both these changes could be expected to reduce mortality among pups during the first 24 hr of extra-uterine life no significant change was observed in the 12 generations [Fig. 1(*d*)].

(*b*) *Weights of Pups at 3 and 6 Weeks of Age*

The weights of male and female pups reared by mothers at 21 and 34°C are illustrated in Figure 3. Exposure to 34°C caused a significant fall in body weight at both ages. Results of mice at 21°C showed marked fluctuations which were found to be associated with season of birth rather than with generation number [cf. dates when matings were set up (Table 1) and weights at 3 and 6 weeks (Fig. 3)]. Results of mice at 34°C also showed changes during the course of the experiment but these were associated with generation rather than with season. Weights at both ages fell for 1–2 generations after the mice were introduced into the incubator, but rose again between generations 2 and 4. Weights at 3 weeks showed a small gradual decline between the fourth and twelfth generation, and weights at 6 weeks showed a more marked decline over the same period. The differences between weights at generation 4 and 12 were significant, as shown in the following tabulation:

Age of Mice (weeks)	*T* Values	
	Female	Male
3	$6 \cdot 143$ ($P < 0 \cdot 001$)	$3 \cdot 052$ ($0 \cdot 001 < P < 0 \cdot 01$)
6	$8 \cdot 141$ ($P < 0 \cdot 001$)	$3 \cdot 906$ ($P < 0 \cdot 001$)

IV. Discussion

These results show that although the fecundity of mice is depressed by exposure to 34°C, these animals can be maintained at this temperature for at least 12 generations. This is in agreement with the result of Sundstroem (1922, 1930) but in disagreement with those of Stieve (1923) and Chevillard and Cadot (1963). Unlike Brun's (1965) results with *C. elegans*, 12 generations at this elevated temperature was not sufficient to produce any improvement in the number of pups reared to 6 weeks of age. This is not unexpected, for reproduction in mammals is obviously more complex than that in nematodes.

The reduction in the number of pups reared to 6 weeks of age was due to four major factors: to a reduction in the number of pairs which produced litters, in the number of litters born to each pair which did produce litters, in the size of the litter at birth, and to an increase in pup mortality. These findings agree with those of Stieve (1923) and Chevillard and Cadot (1963). The relative contributions of these components of productivity (viz. 1 : 2 : 2 : 1) suggest that disturbances at various stages in the female reproductive cycle were as important as male infertility in limiting pup numbers, for litter size at birth and pup survivals reflect the efficiency of the mother rather than that of the father. Comparison of the number of successful services following R95 male × R70 female crosses with those following R70 male × R95 female crosses also suggests that female failure contributes significantly to the reduction in pup numbers at 34°C. These results are at variance with the thesis put forward by Cowles (1965).

In addition to its effects on productivity and its components, exposure to 34°C caused an increase in the time from the setting up of the mating to the birth of the first litter, the length of gestation, and in the length of the interval between the birth of the litter and the first feed. Following several generations at 34°C, all three of these intervals showed signs of returning to levels characteristic of mice at 21°C. It is possible that these changes may have measurable effects on productivity in later generations of mice at 34°C.

Exposure to 34°C caused the expected reduction in body weights at 3 and 6 weeks of age (Pennycuik 1967). The improvement in growth rate observed during the first four generations may have been due to a cumulative maternal effect [cf. the improvement in nesting mortality in inbred mice exposed to cold (Barnett 1962)]. The steady decline in weights at 3 and 6 weeks during the next eight generations was probably genetic in origin (Pennycuik 1969*a*). Apparently selection for productivity at 34°C favoured a decrease in size and the associated improvement in the capacity of the animal to lose heat rather than an increase in body size and an associated increase in the number of eggs shed at ovulation.

These results suggest that although a weak selection pressure for increased productivity at 34°C had no measurable effect on the selected character itself, it did affect both the body weight of the stock and the duration of events in the reproductive cycle.

V. Acknowledgments

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