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Selection of Laboratory Mice for Improved Reproductive Performance at High Environmental Temperature

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Abstract

When mice are transferred from a 'temperate' to a hot environment their reproductive output is reduced. To test whether this reduction could be countered by selection, three mouse lines, housed permanently as 32°C, were selected for increase in the numbers of young reared to 3 weeks when pairs were allowed to remain together for 12 weeks. In one line (R95) in which all fertile pairs contributed to the next generation, no improvement was observed in 19 generations. In two other lines (W21 and W34) in which only half the pairs in one generation contributed to the next, productivity returned, in about 12 generations, to almost the same levels as those of controls housed at 21°C.

At 32°C reduced productivity was due almost exclusively to a reduction in litter size at birth resulting from high intra-uterine mortality. Selection in the W21 and W34 stocks reduced intra-uterine losses but growth rate and resting oxygen consumption at 32°C were unaffected. However, selection line animals reared at 21°C were significantly lighter than controls at 21°C.

It is concluded that the depressed reproductive productivity at 32°C can be countered by selection, but that changes in characters usually associated with adaptation to hot environments do not necessarily accompany such improvement.

Introduction

Exposing mammals to suboptimal diets or extreme temperatures usually leads to lowered growth rate and impaired fertility. Although the depression in growth rate in such environments can be overcome by selection (Falconer 1960; Park *et al.* 1966; Dalton 1967; Baker and Cockrem 1970; Bateman 1971), efforts to improve reproductive productivity in unfavourable environments have met with more mixed success. Barnett (1965*a*) reported an improvement over 12 generations in the reproductive performance of randomly bred laboratory mice exposed to −3°C, but Pennycuik (1969*a*) was unable to demonstrate a change in the fertility of mice selected for 12 generations at 34°C.

The success of programs to improve fertility in unfavourable environments could depend upon the severity of the environment, the ability of the strains used to respond to selection, and the strength of the selection pressure. Failure to demonstrate an improvement in fertility in the mouse line exposed to 34°C could have been due to one or all of these factors. It was therefore decided to repeat the experiment at 32°C with mice of ancestry more varied than those used in the first experiment and with more severe selection criteria than before. This paper reports the results. In addition to describing the changes in reproductive performance as selection proceeded, this paper also presents data on the response of several unselected characters and on the effects of selection at 32°C on performance at 21°C.

Materials and Methods

Animal Room and Incubators

Control lines were housed in an animal room in which temperature was maintained at 21±1°C, humidity was not allowed to rise above 70% (but was often below this level), and the light:dark ratio was 14:10.

The selection lines were kept in incubators which stood in the animal room. For the first 14 generations of the original line (1961–1967) and for one generation of the two new lines the incubators used were commercial chicken incubators set at 34±1°C. From generation 15 to generation 32 (original selection line) and from generation 2 to generation 19 (new lines) the incubators used were heated cupboards set at 32±2°C.

Diets

The mice were fed on a pelleted diet throughout the experiment. From 1961 to 1968 (generations 1–13, original lines) the pellets were supplied by Drug Houses of Australia, Sydney; from 1968 to 1970 (generations 14–20, original line) they were supplied by Crest Mills, Sydney; and from 1970 to 1976 (generations 21–32, original line) they were supplied by Allied Feeds, Sydney. The composition of the diet supplied by Drug Houses of Australia is described elsewhere (Pennycuik 1969*a*). The compositions of the diets supplied by the other two firms were very similar to that supplied by Drug Houses of Australia.

Cages

Cages were of the metal shoe box type (27 by 15 by 13 cm). Bedding was sawdust and wood-wool or shredded paper. Food and water were supplied *ad libitum*.

Management of the Mice

In all lines mice were paired at 10 weeks of age and pairs were allowed to remain together for 12 weeks. Cages were examined for litters at least three times a week but usually more frequently. When the first litters reached 3 weeks of age all (or, in the case of large litters, some) of the pups were kept in groups of like sex, of not more than six mice. Animals were identified individually by ear markings. Second and subsequent litters were usually killed at 3 weeks of age.

Mouse Stocks

The starting stock (R70) was formed by crossing a multiple recessive stock from Harwell with inbred strains 101, C3H and CBA. The first selection line (R95) was established by transferring R70 mice to the incubator where they were selected (see below).

The 'new' lines (two controls and two selection lines) were formed by crossing R70 and R95 females from the 10th selection generation to males from an outdoor enclosure. These males were descendants of crosses between feral mice and mice carrying the Brachyury gene (T) (Pennycuik 1969*b*). The strain names used, the parental lines used to establish the strains, and the temperatures to which each line was exposed were as follows:

Strain	Parental lines	Environmental temp. (°C)
W70	W×R70	21
W21	W×R70	32
W95	W×R95	21
W34	W×R95	32

Method of Selection

No selection was practised on the three lines at 21°C, i.e. R70, W70 and W95.

The method of selecting parents in the R95 stock has been described in detail elsewhere (Pennycuik 1969*a*). Briefly, the procedure was as follows: when all litters born as a result of 12 weeks of pairing had reached 6 weeks of age, the 13 matings set up in each generation were ranked according to the number of 6-week-old pups reared (from generation 24 to generation 32 this was changed to the number of 3-week-old pups reared). All matings which reared offspring contributed to the next generation, but high ranking pairs contributed more offspring than low ranking pairs.

For stocks W21 and W34 the procedure was as follows: in each generation 20 pairs were mated, pairs remained together for 12 weeks, and then the males were removed. When the last litter born reached 3 weeks of age the 20 matings were ranked on the basis of the number of young reared to 3 weeks of age. Parents for the next generation were animals from the first litters of the 10 pairs with the highest rankings. Each of these pairs contributed four offspring to the next generation.

Transfer Mice

To examine the effects of exposure to 21°C (or 32–34°C) on performance at 32–34°C (or 21°C), mice from the selected lines and from the control lines were transferred between environments. The generations at which transfers were made were as follows: R95 generations 10 and 20; W70 generation 12; W21 generation 12; W95 generation 16; W34 generation 16. In all transfer lines two generations were reared at the new temperature (generations 1 and 2), but no selection was practised in the new environment.

Measurements, Methods of Measurement, and Generations in which these Measurements were Carried Out

(i) *Numbers of young at birth, 3 and 6 weeks and number of eggs shed*

Litter sizes at birth and numbers alive at birth were recorded when the litters were found. Because checks for newborn litters were not made every day, both total litter size and the number alive at birth were probably underestimated. Numbers alive at weaning were counted as close as possible to 3 weeks after the estimated date of birth and numbers alive at 6 weeks as close as possible to 6 weeks after the estimated birth date. Numbers reared to 3 weeks (or 6 weeks) by each pair were estimated by adding the numbers surviving to this age in each litter born to that pair.

Numbers born, numbers alive at birth and numbers alive at 3 weeks were counted in all generations in all lines, including transfer lines. Numbers alive at 6 weeks were counted for generations 0–23 (R70 and R95) and generations 0–3 (W stocks).

The ovulation rates of two selection lines, their controls and transfer lines were measured in females from the 14th generation (W70 and W21) and the 18th generation (W95 and W34). Ova released from the ampulla of the oviduct on the day of appearance of the vaginal plug were counted under a binocular microscope.

(ii) *Body weights*

Both males and females were weighed to the nearest 0·5 g when the mice were 3 and 6 weeks of age (or as close as possible to these ages). Weights were recorded in some generations only (see Fig. 6 and Table 3).

(iii) *Oxygen consumption*

The method of measuring oxygen consumption has been described elsewhere (Pennycuik 1972*b*). Basal oxygen consumptions [oxygen consumption (ml) per gram of tissue at 32°C] were used to examine the effects of selection for reproductive productivity on metabolic rates. In the R70 and R95 stocks basal consumptions were measured on mice from a number of different generations (see Fig. 7). In the W stocks basal measurements were carried out only on mice from the base stocks from which the lines were derived and on mice from the 14th generation (W70 and W21) or on mice from the 18th generation (W95 and W34).

The effects of selection on oxygen consumption were also examined by comparing the shape of the oxygen consumption curves (between 26 and 34°C) of mice from control stocks and of mice from selection lines, and by comparing the curves of control and selection line mice with those of transfer stocks reared at the same temperatures. The generations examined were: R70 and R95, generation 22; W70 and W21, generation 14; W95 and W34, generation 18.

(iv) *Tail lengths*

Tails were measured from tail base to tip with a centimetre rule on 6-week-old males from one pair of lines (W95 and W34) and the corresponding transfer lines after 18 generations of selection.

(v) *Hair cover*

After the entire animal had been clipped the hair was weighed and the quantity present was expressed as milligrams per square centimetre of surface area [surface area = $13 \cdot 2$ weight$^{0 \cdot 667}$ (Brody 1964)]. Measurements were carried out on adult males from one pair of lines and the corresponding transfer lines after 18 generations of selection.

(vi) *Nest weights*

A single adult female was placed in a pen (floor area $0 \cdot 14$ m^2) with one nesting box (floor area 1600 cm^2). Wood-wool was supplied *ad libitum* in the open area of the pen and the amount transported to the nesting box over a 3-day period was weighed. These measurements were also carried out on only one pair of lines after 18 generations of selection.

(vii) *Serum cholesterol levels*

Blood samples for estimates of serum cholesterol were from adult males from the W70 and W21 lines and from the corresponding transfer stocks. The method used was that of MacIntyre and Ralston (1954).

Statistical Analysis

Student's *t*-test (two-tailed) was used to test the significance of differences between groups.

Results

The Selected Character—Reproductive Productivity

(i) *Changes in the selected character and its components with increasing generation number*

Fig. 1 illustrates the responses of the three selection lines plotted against the cumulated selection differentials of the three lines. Two measures of response were examined—the total numbers of young reared to 3 weeks and the differences between the selection lines and their controls.

Throughout the experiment a small but steady selection pressure was applied to the R95 line, but the line did not respond to selection; realized heritabilities of total numbers were positive but small, and realized heritabilities of the differences between the selection line and the control line were negative at both temperatures. In the W lines the selection pressure applied was about double that applied to the R95 line. In both lines there was a fairly steady increase in numbers reared, but realized heritabilities of both total numbers and between-line differences although positive were low.

Fig. 2 illustrates the changes in the numbers of young reared by the three selection lines and their controls. Figs 3, 4 and 5 show the changes in the three major components contributing to the numbers reared, namely the percentage of fertile pairs in each generation, the numbers of litters born to each pair, and the litter sizes at birth and at 3 weeks.

Two of the three control lines (R70 and W70) produced about 24 pups per pair throughout the experiment (Fig. 2). In the third control line (W95, Fig. 2*c*) there was a small increase with time in the numbers reared to 3 weeks, but the linear regression coefficient of numbers weaned on generation number was not statistically significant ($0 \cdot 183 \pm 0 \cdot 160$, $P > 0 \cdot 05$). The number of young weaned by mice from this line (mean for all generations) was slightly higher than the number weaned by mice from the R70 and W70 lines [$27 \cdot 1 \pm 0 \cdot 94$ *v.* $24 \cdot 6 \pm 0 \cdot 58$ (R70) and $24 \cdot 4 \pm 0 \cdot 54$ (W70)].

In all three selection lines exposure to 34°C reduced the numbers of pups reared to about one-quarter of the numbers reared by controls at 21°C (Fig. 2). This reduction in numbers was due to four factors: a decline in the number of fertile pairs (Fig. 3), a fall in the number of litters born to each pair which produced young (Fig. 4), a reduction in litter size at birth, and an increase in pup mortality between birth and weaning (Fig. 5).

After transfer to 32°C numbers weaned increased to between 15 and 20 pups per pair. This increase was due to a return to control levels in the number of fertile

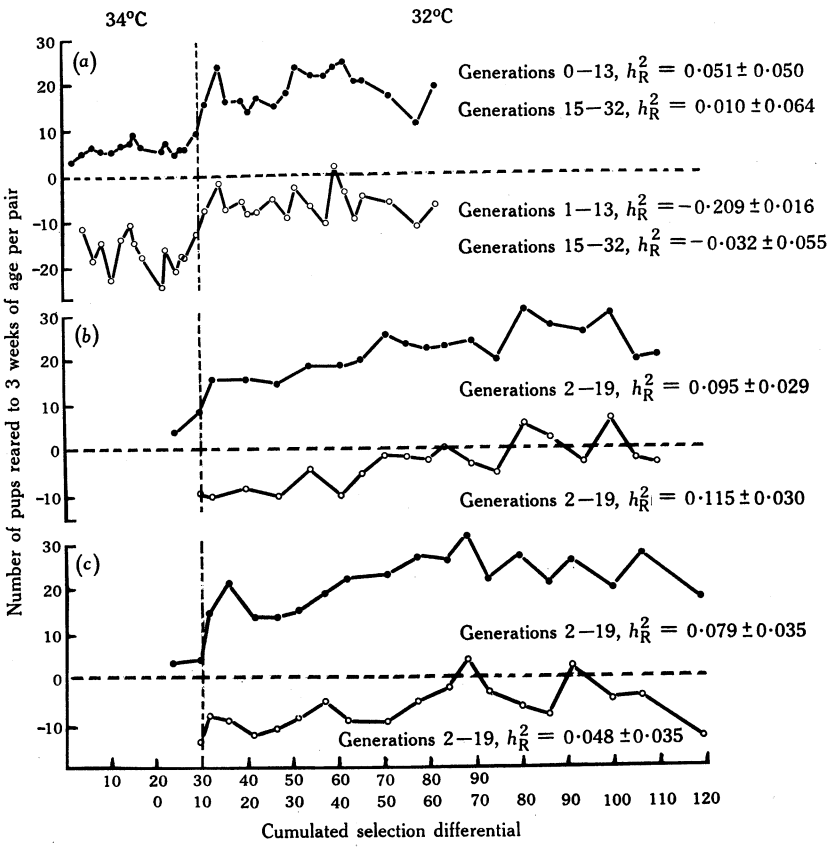

Fig. 1. Response to selection plotted against the cumulative selection differentials of the three selection lines. (*a*) R95 line, (*b*) W21 line, and (*c*) W34 line. ● Numbers of pups reared to 3 weeks of age. ○ Depression in the numbers reared to 3 weeks of age relative to the numbers reared by the corresponding control lines. Realized heritability estimates (h_R^2) are shown for each line.

pairs and the number of litters born per pair (Figs 3 and 4) and to larger litters at birth and lower pup mortality (Fig. 5). Selection for improved productivity was successful in only two of the selection lines, W21 and W34, when they were kept at 32°C (Fig. 2). Fig. 5 shows that this success was due to a fairly steady improvement in litter size at birth which led in turn to an increase in the numbers reared to 3 weeks. The improvement was most marked in the early generations of selection; after about generation 12 the response of both lines appeared to slow down. Orthogonal poly-

nomials were therefore fitted to the litter size data points against generation number for generations 2–19 to see whether the W lines were still responding to selection when the experiment ended. In both the W21 stock and the W34 stock the linear term was found to be significant [W21, $b_1 = 0{\cdot}134 \pm 0{\cdot}0300$ ($P < 0{\cdot}001$); W34, $b_1 = 0{\cdot}164 \pm 0{\cdot}0307$ ($P < 0{\cdot}001$)] but the quadratic term, although negative, was not significant (W21, $b_2 = -0{\cdot}00935 \pm 0{\cdot}00650$; W34, $b_2 = -0{\cdot}00502 \pm 0{\cdot}00665$). It is therefore possible that litter sizes were still increasing when the experiment ended.

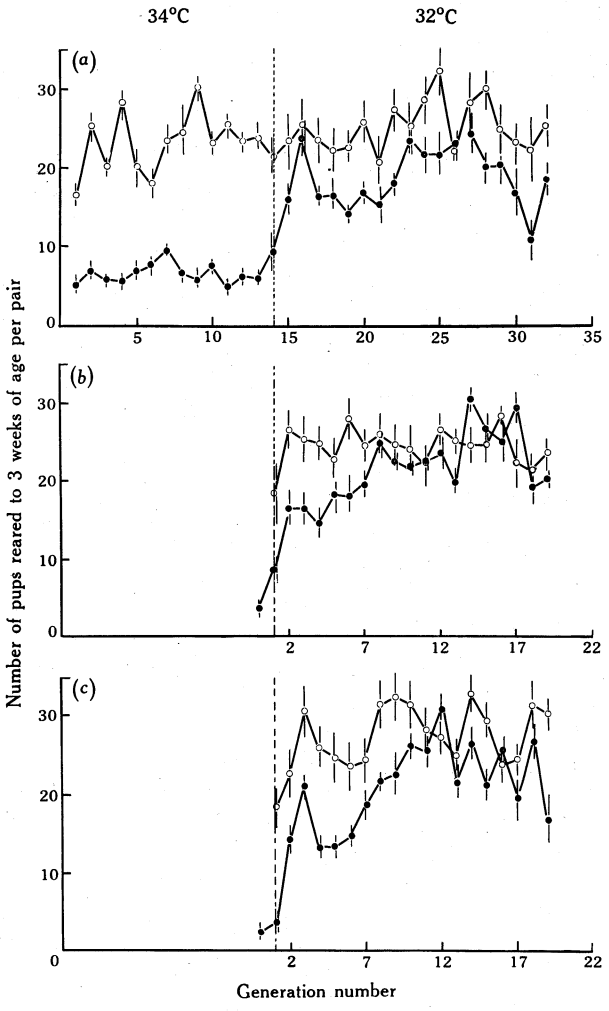

Fig. 2. Changes, with increasing generation number, in the mean number of pups per pair reared to 3 weeks of age by all pairs mated. (*a*) R70 and R95 lines, (*b*) W70 and W21 lines, and (*c*) W95 and W34 lines.
○ Control lines at 21°C.
● Selection lines at 34 or 32°C.

(ii) *Effects of selection on egg numbers and pup numbers at birth when selected and control mice were reared at 21 and 32°C*

In two of the three selection lines (W21 and W34) reproductive productivity improved as a result of selection. This improvement was due almost exclusively to an improvement in only one of the four major components of productivity, namely litter size at birth. The effects of selection on this latter character could have been due to two possible mechanisms: (1) an increase in the number of ovulations after

an initial depression resulting from exposure to 32°C, or (2) an increase in the number of conceptuses surviving between ovulation and birth.

Table 1 shows the numbers of eggs ovulated by mice from both pairs of W lines at 21 and 32°C. In both the W70 and W21 lines neither the differences between the selection line and the control line nor the differences between the mice exposed to 21 or 32°C were statistically significant. Exposure of the W95 mice to 32°C caused a decline in egg numbers ($0{\cdot}001 < P < 0{\cdot}01$) but the numbers shed by W34 mice at 32°C did not differ from those shed by W95 mice at 21°C or by W21 mice at 21°C. These results suggest that although exposure of unselected mice to 32°C may cause a slight depression in ovulation rate, this depression is usually small (cf. Sod-Moriah and Bedrak 1976). They also suggest that selection for productivity had little effect on the number of eggs shed either at 32 or at 21°C.

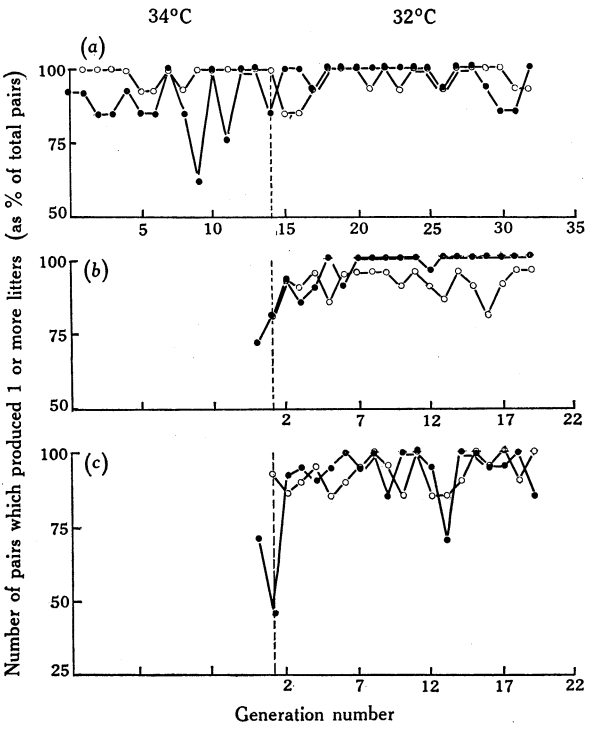

Fig. 3. Changes, with increasing generation number, in the frequency of fertile pairs among all pairs in the generation. (*a*) R70 and R95 lines; (*b*) W70 and W21 lines; and (*c*) W95 and W34 lines.
○ Control lines at 21°C.
● Selection lines at 34 or 32°C.

Table 2 illustrates mean litter sizes of mice from the W selection lines and their controls at 21 and 32°C. In both generations of the W70–W21 line and one generation of the W95–W34 line the difference between the litter sizes of the control and the selection line mice reached the 0·05 level or higher. When W70 mice were transfered to 32°C litter sizes decreased relative to those of controls (generation 12, $P < 0{\cdot}001$; generation 13, $P < 0{\cdot}05$). When W21 and W34 mice were transferred to 21°C litter sizes returned to, but did not exceed, those of control mice maintained at the same temperature and, with one exception (W34, generation 16), differences between the litter sizes of the animals at 21 and 34°C reached the 0·01 level or higher. The fact that litter sizes of mice from the W selection lines never rose *above* those of mice from the control lines suggests that the improved litter sizes resulting from selection were

achieved largely by overcoming the deleterious effects of high temperatures on pup survivals between conception and weaning.

Unselected Characters

(i) *Body weights at 3 and 6 weeks of age*

Changes with increasing generation number. Fig. 6 illustrates the fluctuations in 3- and 6-week body weights in the three selection lines and their controls.

In the control lines some of the variations in weight were due to seasonal fluctuations (discussed elsewhere by Pennycuik 1969*a*, 1972*a*) and some to changes in diet.

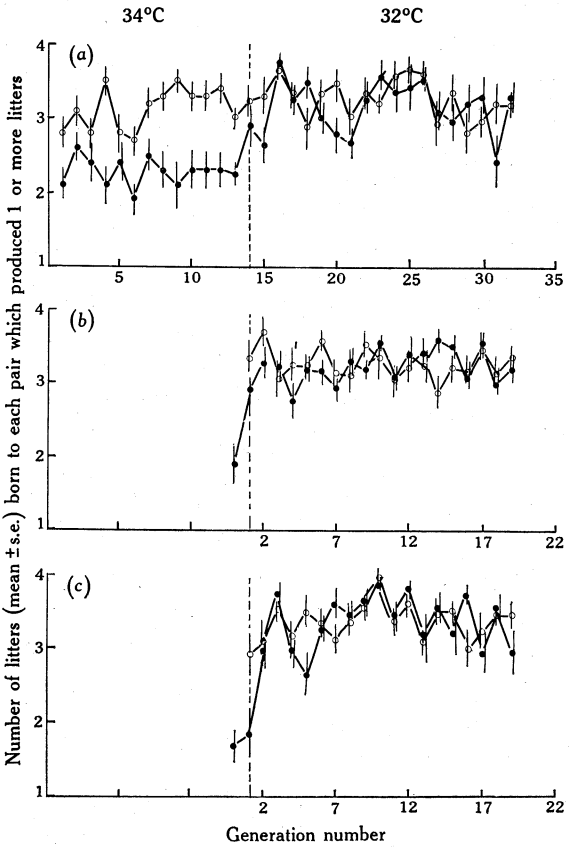

Fig. 4. Changes, with increasing generation number, in the mean number of litters born to those pairs which were fertile. (*a*) R70 and R95 lines, (*b*) W70 and W21 lines, and (*c*) W95 and W34 lines. ○ Control lines at 21°C. ● Selection lines at 34 or 32°C. Vertical bars represent standard errors.

Seasonal variations were most noticeable in the R70 stock during the first 12 generations of observation. At generation 14 in the R70 stock (generation 1 of the W70 and W95 stocks) the mice were transferred from the diet supplied by Drug Houses of Australia to the diet supplied by Crest Mills. In the R70 stock this change in diet caused an increase of about 3 g in the body weights of 6-week-old males. The change to the third diet at generation 20 had no further effect.

Weights of selection line mice were consistently lower than those of mice from the control lines. Transfer of the stocks from 34 to 32°C was accompanied by an increase in body weight in all lines, but as this coincided with transfer from the Drug Houses

of Australia diet to the Crest Mills diet it is not possible to say whether the increase was due to temperature, diet or to both factors. If the change-over period is ignored there is little evidence that selection for productivity affected body weight in any of

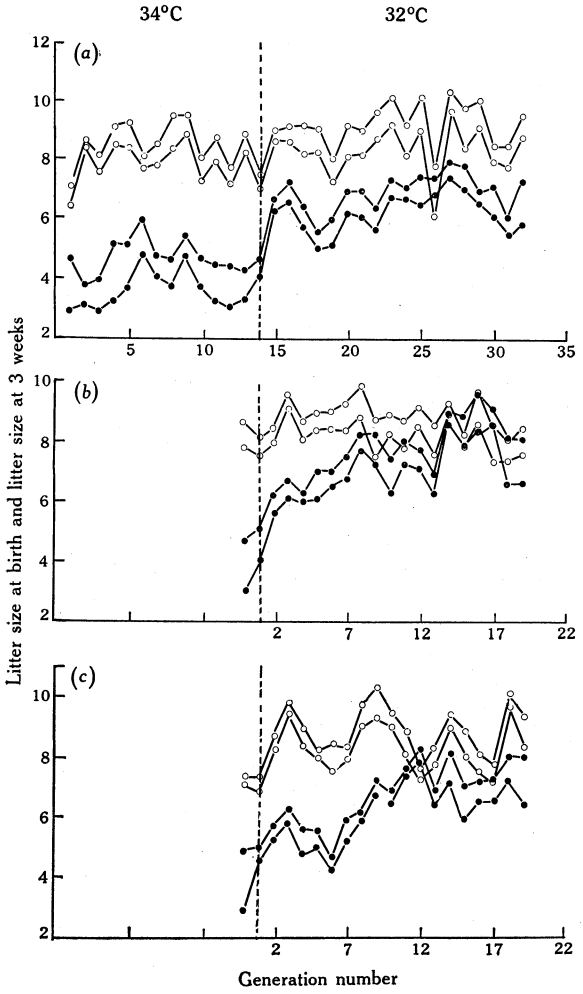

Fig. 5. Changes, with increasing generation number, in mean litter size at birth and mean litter size at 3 weeks. (*a*) R70 and R95 lines, (*b*) W70 and W21 lines, and (*c*) W95 and W34 lines. ○ Control lines at 21°C. ● Selection lines at 34 or 32°C.

Table 1. Number of eggs shed by mice from the control lines W70 and W95 at 21 and 32°C and by mice from the selection lines W21 and W34 at the same two temperatures

Strain	Generation	Temp. (°C)	No. of mice	Body weight (g) (mean±s.e.)	No. of eggs shed (mean±s.e.)
W70	14	21	15	24·7±0·6	10·1±0·5
	Transfer 2	32	13	24·8±0·2	8·9±0·5
W21	14	32	14	23·9±0·8	9·1±0·7
	Transfer 2	21	13	23·4±1·1	9·0±0·7
W95	18	21	15	27·0±1·2	9·0±0·5
	Transfer 2	32	17	23·5±1·0	7·1±0·4
W34	18	32	15	23·1±0·5	7·9±0·6
	Transfer 2	21	16	24·8±0·9	9·4±0·6

the lines at 32°C other than the R95 stock in which weights declined steadily from generation 5 to generation 13 (Pennycuik 1969*a*).

Effects of selection on body weights when selected and control mice were reared at 21 and at 32°C. In all selection lines 3- and 6-week body weights changed little when the mice were kept at 32°C but when mice from the W selection lines were moved to 21°C and allowed to rear young at that temperature their offspring tended to be lighter than young born to control mice reared at the same temperature. In the W21 stock this tendency was apparent at 3 weeks ($P<0{\cdot}001$ for both sexes) (Table 3); in the W34 stock body weights at 3 weeks did not differ from those of mice from the control lines, but by 6 weeks pups born to mice from the selection line were lighter than those born to mice from the control lines ($P<0{\cdot}001$ for both sexes) (Table 3). In the R95 stock, too, animals reared at 21°C were lighter at 6 weeks of age than R70 mice reared at the same temperature.

Table 2. Mean sizes of litters born to mice from control line mice W70 and W95 and to mice from the selection lines W21 and W34 for the generations when control animals (transfer controls) were maintained at 32°C and selected animals (transfer selected) were maintained at 21°C, together with mean litter sizes of both types of transfer mice

Strain	Generation	Temp. (°C)	No. of litters	Litter size (mean±s.e.)
		(*a*) W70–W21		
W70	12	21	58	8·95±0·32
	Transfer 0	32	47	6·55±0·39
W21	12	32	65	7·54±0·33
	Transfer 0	21	60	8·87±0·39
W70	13	21	58	8·38±0·27
	Transfer 1	32	60	7·40±0·31
W21	13	32	65	6·72±0·32
	Transfer 1	21	58	8·83±0·32
		(*b*) W95–W34		
W95	16	21	58	8·36±0·38
	Transfer 0	32	61	8·84±0·38
W34	16	32	72	7·39±0·25
	Transfer 0	21	55	7·60±0·36
W95	17	21	66	8·12±0·37
	Transfer 1	32	61	7·72±0·29
W34	17	32	57	7·46±0·29
	Transfer 1	21	53	8·91±0·36

(ii) *Oxygen consumption*

Changes at thermal neutrality (32°C) with increasing generation number. Fig. 7 illustrates the changes in oxygen consumption at 32°C in the selection lines and their controls. In the R70–R95 lines, which were the only ones in which oxygen consumptions were measured in more than two generations, there was no evidence of a trend over the generations or of differences between experimental animals and the corresponding controls. In the W70–W21 lines oxygen consumptions of selection line animals appeared to decline sharply over the generations but this fall was probably due to an error in measurement of the consumptions of base stock mice, for con-

sumption by the control mice declined over the generations in a manner similar to that of the selection line mice. In addition, the hourly consumptions of the base stock mice lay well outside the range of all other groups (1·4–1·8 ml/g). In the W95–W34 lines, like the R70–R95 lines, there was no evidence of a trend over the generations or of differences between experimental animals and the corresponding controls.

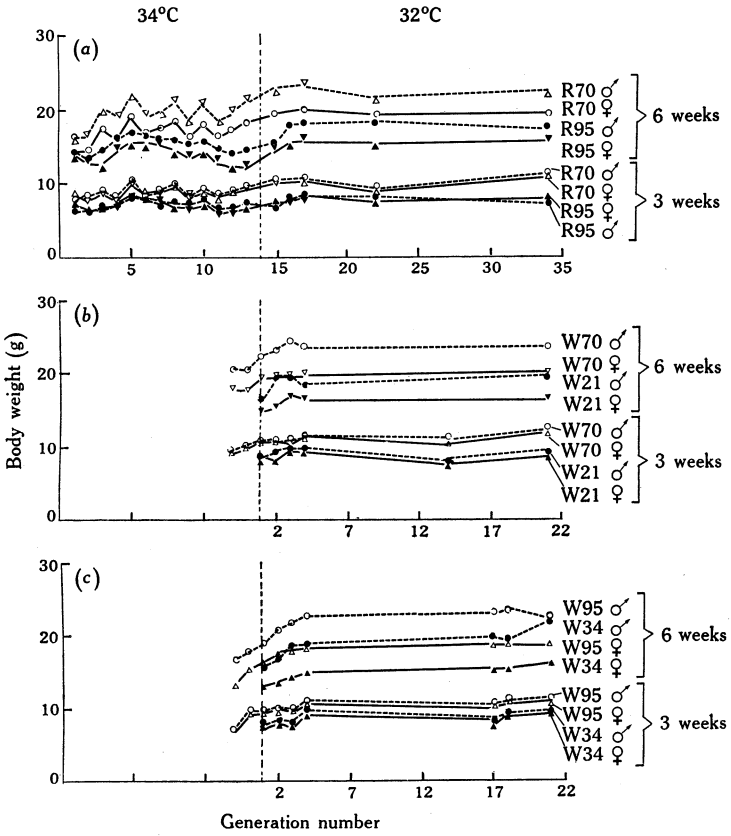

Fig. 6. Changes, with increasing generation number, in body weights of males and females at 3 and 6 weeks of age. (*a*) R70 and R95 lines, (*b*) W70 and W21 lines, and (*c*) W95 and W34 lines.

There was, therefore, no evidence that selection for productivity was associated with a change in metabolic rate at thermal neutrality.

Effects of selection on oxygen consumptions between 26 and 34°C. Oxygen consumptions at temperatures between 26 and 34°C, of mice from the W selection and control lines, were examined in adult males from the 14th generation of the W70–W21 stocks and from the 18th generation of the W95–W34 stocks. In each case some animals were reared at 21°C and others at 32°C (Fig. 8). Table 4 summarizes the significance of differences between the oxygen consumptions of the animals examined.

At temperatures below thermal neutrality (26–30°C) oxygen consumptions of selection line animals reared at 32°C tended to be higher than those of controls reared at 21°C. At temperatures above 30°C there was no evidence of differences between the lines.

Oxygen consumptions of the selection line mice reared at 21°C tended to be higher than those of controls reared at the same temperature, but differences between the two groups were statistically significant only for the W95 and W34 stocks at 26°C ($P<0{\cdot}01$). Oxygen consumptions of selection line mice reared at 32°C tended to be slightly greater than those of control line mice reared at the same temperature, but differences were significant only at 34°C for the W34–transfer W95 comparison ($P<0{\cdot}05$).

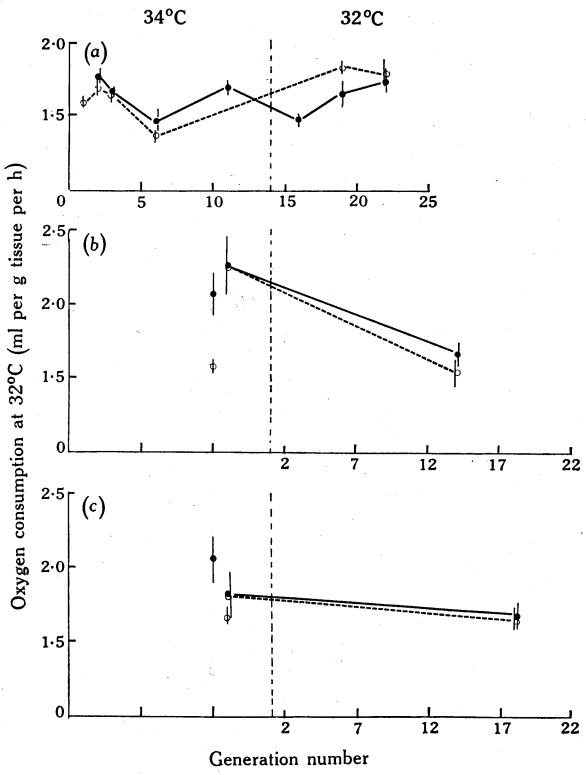

Fig. 7. Changes, with increasing generation number, in oxygen consumptions of adult male mice at 32°C. (*a*) R70 and R95 lines, (*b*) W70 and W21 lines, and (*c*) W95 and W34 lines. The consumptions of the two lines used as parents of the W stocks are also indicated in (*b*) and (*c*). ○ Control lines at 21°C. ● Selection lines at 34 or 32°C.

Evidently selection for productivity had no significant effect on the oxygen consumption of mice from the selection lines, but when selection line animals were adapted to 32°C their oxygen consumption at temperatures below thermal neutrality tended to be greater than that of control mice reared at 21°C.

Other characters. Table 5 summarizes the results of measurements made on tail length, hair cover, ability to collect nesting material and serum cholesterol levels. These features are thought to be related to the ability of mammals to adapt to temperature extremes [Harrison 1958 (tails); Bigham and Cockrem 1969 (tails); Barnett 1959 (hair); Dawson and Webster 1967 (hair); Wolfe and Barnett 1977 (nests); O'Kelly 1973 (plasma cholesterol)].

Tail length is thought to be of importance in temperature adaptation because of its effect on surface area:weight ratios (Harrison 1958) and because vasodilation in tail vessels at temperatures above 28–30°C greatly increases the amount of heat lost through this organ in animals adapted to temperatures below 28°C (Rand *et al.* 1965). Because of the possible importance of the tail as a site of heat loss at high environ-

Table 3. Body weights, at 3 and 6 weeks, of pups born to females from the selection lines W21 and W34 at 21 and 32°C, and of pups born to females from the control lines W70 and W95 at 21 and 32°C

Values for body weights are means±s.e.

Strain	Generation	Temp. (°C)	3 weeks				6 weeks			
			Females		Males		Females		Males	
			No. of mice	Weight (g)	No. of mice	Weight (g)	No. of mice	Weight (g)	No. of mice	Weight (g)
W70	14	21	67	10·5±0·3	63	11·2±0·2				
	Transfer 2	32	61	8·9±0·2	73	9·4±0·2				
W21	14	32	74	7·9±0·2	69	8·0±0·2				
	Transfer 2	21	70	9·9±0·1	68	10·2±0·2				
W95	18	21	65	10·0±0·2	56	10·6±0·3	41	18·7±0·3	35	23·1±0·4
	Transfer 2	32	60	8·2±0·2	58	8·0±0·2	40	15·7±0·4	35	18·5±0·4
W34	18	32	53	8·9±0·2	65	9·0±0·2	34	15·0±0·3	39	18·7±0·5
	Transfer 2	21	49	10·4±0·2	67	10·6±0·2	33	16·7±0·4	29	19·6±0·6

mental temperatures, it was thought that selections for productivity might be accompanied by an increase in tail length. However, although tails of mice from lines selected at 32°C were slightly longer (0·7 mm) than those of controls, the tails of

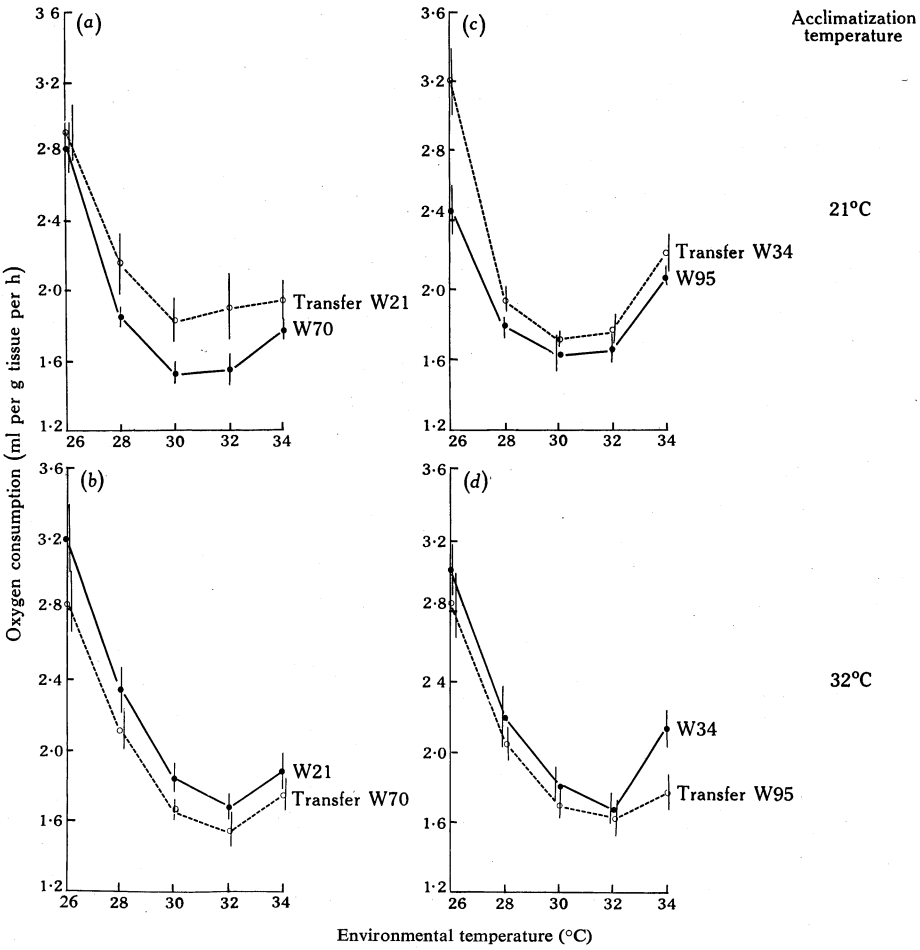

Fig. 8. Resting oxygen consumptions at temperatures between 26 and 34°C of adult males from the two selection lines W21 and W34, their controls W70 and W95, and of transfer mice from the four lines. (*a*) W70 mice (gen. 14) and transfer W21 mice (gen. 2) acclimatized to 21°C. (*b*) W21 mice (gen. 14) and transfer W70 mice (gen. 2) acclimatized to 32°C. (*c*) W95 mice (gen. 18) and transfer W34 mice (gen. 2) acclimatized to 21°C. (*d*) W34 mice (gen. 18) and transfer W95 mice (gen. 2) acclimatized to 32°C. ○ Selection lines reared at 32°C and control lines reared at 21°C. ● Selection lines reared at 21°C and control lines reared at 32°C (transfer lines). Vertical bars represent standard errors.

control line mice at 32°C were also longer (0·5 mm) than those of mice from the same lines reared at 21°C. Hence, the long tails of W34 mice were probably due to the direct action of the environment rather than to selection (cf. Harrison *et al.* 1959; Noel and Wright 1970). Unfortunately tail lengths were not measured in the base stock so it is not possible to determine whether tail lengths increased in both lines during the generations in captivity (cf. Barnett 1965*b*; Barnett *et al.* 1975).

There was no evidence of an effect of selection on hair growth, nest building or serum cholesterol levels.

Discussion

Like the R95 line at 34°C, the two new selection lines, W21 and W34, declined in productivity when first transferred to 32°C but, unlike the R95 line, productivity of the W lines improved in later generations at elevated temperatures. Therefore, given the necessary variations in the base stock, fertility at elevated temperatures can be returned by selection to levels comparable with those observed in favourable environments.

Table 4. Values of *t* for comparisons between oxygen consumptions of adult males from control and selection lines, for comparisons between control and transfer selection line animals, and for comparisons between selection line and transfer control animals

Strains compared		Temp. (°C) at which oxygen consumptions were measured:				
		26	28	30	32	34
W70	W21	1·64	3·44**	3·01*	1·74	0·85
W95	W34	2·99**	2·30*	1·49	0·26	0·64
W70	Transfer W21	0·45	1·61	2·03	1·87	0·81
W95	Transfer W34	3·04**	1·85	0·85	1·04	1·12
W21	Transfer W70	1·54	1·66	1·15	1·85	0·71
W34	Transfer W95	0·88	0·76	0·97	0·44	2·80*

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

The lowered productivity of mice at 32°C was due to reduced litter size at birth, resulting from increased mortality among intra-uterine young. Intra-uterine losses have been attributed to both the direct effects of elevated maternal temperatures on the embryo (Alliston *et al.* 1965) and to the indirect effects of high temperature on the uterine environment (Shah 1956). Although Sod-Moriah and Yagil (1973) demonstrated a small elevation in the body temperature of rats at 35°C, it is unlikely that the elevation in body temperature of mice at 32°C was sufficiently great to account for deaths of the developing embryos. High intra-uterine losses were most probably due to temperature-induced disturbances of maternal physiology—for example delayed lysis of corpora lutea, resulting high progesterone levels, and progesterone-induced irregularities in the timing and duration of parturition (Sod-Moriah and Bedrak 1976). Selection in the W lines, therefore, probably favoured animals in which exposure to high temperature caused the least disturbance to the endocrine changes associated with normal gestation. The decline observed in both gestation length and the interval between birth and the first feed in R95 mice selected at 34°C lends some support to this view (Pennycuik 1969*a*).

Although selection returned litter sizes of mice from the W21 and W34 lines to a level comparable with that of controls at 21°C, litter sizes showed no sign of rising *above* the levels observed in controls. This suggests that the increase in litter size which accompanied selection for numbers reared to 3 weeks of age was due almost exclusively to improved intra-uterine survivals rather than to increased ovulation rates, although it is known that mice can be selected successfully for this latter character (Bradford 1969; Eklund and Bradford 1977). These results are comparable

Table 5. Tail lengths of 6-week-old males, hair cover of adult males, weights of nests built by adult females at 21°C, and serum cholesterol levels of adult females from selection lines W34 (generation 18) and W21 (generation 15) and from corresponding control lines W95 and W70

Character	Generation examined	Control line (21°C)			Selection line (32°C)			T value
		Strain	No. of mice	Mean±s.e.	Strain	No. of mice	Mean±s.e.	
Tail length (cm)	18	W95	31	8·70±0·09	W34	20	9·40±0·11	4·49***
Hair cover (mg/cm²)	18	W95	13	3·46±0·14	W34	12	3·12±0·20	1·41
Nest weight (g)	18	W95	8	9·40±1·06	W34	10	11·80±1·11	1·53
Cholesterol (mg/100 ml)	14	W70	11	135·05±5·04	W21	15	130·66±3·05	0·79

*** $P<0·001$.

with those of Dunn (1942) and Fisher and Holt (1944) who selected mice carrying that *T* gene and mice carrying the *Sd* gene (which cause shortening of the tail by supressing the development of vertebrae in the tail region) for increased tail length. Although tail length increased in both lines, tail lengths of mutant mice never rose above those of normal mice.

Thoday and co-workers (Thompson 1975; Thoday and Thompson 1976) pointed out that very often relatively few loci are responsible for the variations observed in a character—for example sterno–pleural chaeta numbers in *Drosophila* (Spickett 1963) and tail lengths in mice carrying the *Sd* gene (Wallace 1972). Unfortunately it is not known whether the same is true of characters favouring normal uterine function in hot environments, nor is it known whether the loci responsible are acting in the hypothalamus, pituitary, satellite endocrine glands or the target tissue itself.

According to selection theory our mouse population should have been in equilibrium with respect to fitness in an environment at 21°C and additive genetic variance in fitness should have been small (e.g. Lerner 1954; Robertson 1955). By changing the environment to 32°C the population ceased to be in equilibrium for fitness and variations in fitness were exposed. It was this variation which was exploited during selection. In most selection experiments selection is for a character other than fitness and an increase in the value of the selected character is usually accompanied by a decline in fitness (e.g. Robertson 1955; Latter 1966). In the present experiment selection for fitness was found to have no effect on the expression of the unselected characters examined, even though changes in these characters are usually thought to be an advantage (in large animals at least) at high environmental temperatures—for example body weight, which declined on exposure to 32°C, remained about the same over 19 generations, and so did oxygen consumption at thermal neutrality; tails lengthened slightly at 32°C and hair cover was reduced but these changes also occurred in control mice reared at the same temperature. Failure to demonstrate changes in these characters could possibly have been due to choice of species, for small animals rely more on behavioural changes and less on structural adaptations than large animals. However, failure to demonstrate an association between improved productivity and temperature-adaptive characters suggests that it might be profitable to monitor characters associated with the selected character itself (for example, hormone levels) in searching for markers of individuals which will perform well in hot environments.

In many of the experiments in which animals were selected for improved growth on good and poor diets, progress on the poor diet usually resulted in improved growth on the good diet as well (for review see Bateman 1971). Although Barnett *et al.* (1975) were able to demonstrate an improvement in the fertility at 21°C of one of two mouse lines maintained for a number of generations at −3°C, the productivity at 21°C of mice from our 32°C selection lines was no greater than that of controls. This failure to demonstrate an improvement at 21°C was probably due to the fact that improvement in fertility at 32°C was due to improved viability of intra-uterine young, which was already high in control line mice. Bateman (1971), who examined growth rates of mice fed different proportions of maize and milk, pointed out that selection for improved growth at one end of the range may not necessarily promote improvement at the other end of the range. The results of selection for improved fertility at high and low temperatures confirm this view; at −3°C improvement was due to a decline in nestling mortality resulting from exposure to low temperatures

(Barnett 1973; Barnett *et al.* 1975), while at 32°C improvement in productivity was the result of a decline in losses among intra-uterine young. It is therefore extremely unlikely that selection at one temperature extreme would improve performance at the other. Bateman (1971) also pointed out that selection on an intermediate diet would be more likely to improve performance on diets at either extreme of the range than selection at one extreme or the other. Selection for improved fertility at 21°C would almost certainly lead to a rise in ovulation rate (Bradford 1969). Barnett *et al.* (1975) observed an increase in litter size among mice reared at −3°C and moved to 21°C; it would be interesting to know if this increase was due to an increase in the number of ovulations and if selection for a prolonged period at 32°C would also cause an increase in ovulation rate.

Although selection at 32°C had no effect on fertility at 21°C, the growth rate of selection line animals at 21°C was less than that of controls. Barnett *et al.* (1975) also observed that animals from lines maintained at −3°C grew at a different rate from controls when both were reared at 21°C. It would appear, therefore, that although an improvement in fitness in a given environment is not necessarily accompanied by changes in unselected characters, changes in these characters may be observed when the lines are moved to a new environment.

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