

## Aspects of temperature regulation in mice selected for large and small size

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### SUMMARY

We measured traits involved in physiological and behavioural thermo-regulation in the 6 replicates of a selection experiment for large and small size (6-week weight) in mice, including control lines (18 lines in all). The observed genetic correlations between body size and thermoregulatory traits are consistent with a thermoregulatory advantage of large size, including decreased weight-specific food consumption and increased nest-building, with no change in body temperature. The differences in food consumption were closely paralleled by differences in amount of brown adipose tissue, strongly suggesting that much of the decreased efficiency of the small lines is due to heat production by brown fat. These results are consistent with available observations on natural populations, that selection for temperature adaptation probably has had some influence on body size in this species.

### 1. INTRODUCTION

Temperature regulation in mammals consists of a complex array of interacting mechanisms involving aspects of behaviour and physiology as well as morphology (cf. reviews by Chaffee & Roberts, 1971; Hart, 1971). Behavioural mechanisms for decreasing heat loss may be particularly important for small mammals, and if limited food supplies correspond with periods of low temperature, a selective premium will be put on energetically efficient means of thermoregulation.

The possible adaptive association between body size and temperature regulation has been a subject of interest at least since Bergmann (1847) formulated his 'rule' that species of homeotherms from colder climates tend to be larger than species within the same genus from warmer climates. The explanation of this observation is usually put in terms of surface to volume ratio; this ratio is smaller in larger animals and they, therefore, lose relatively less heat, other things being equal.

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In this paper, we report an investigation of the relationship between body size and aspects of behavioural and physiological thermoregulation in mice which have been selectively bred for large and small size (Falconer, 1973). Since the large and small lines differ genetically with respect to size, consistent differences in thermoregulation between the lines would imply a basic biological causal relationship through pleiotropic gene action. (See Falconer (1981) for a discussion of genetic correlations and correlated response to selection.) In other words, we can ask, if we start with a single heterogeneous population, and select for differences in body size, does this result in changes in thermoregulatory ability or effectiveness?

A particular advantage of these lines is the presence of replication. Differences between replicates will be due to genetic drift, not directional selection. Thus, consistency of observations among replicates (within sizes) reduces the possibility of fortuitous associations. We measured body weight and food consumption (which together allow a measure of food-conversion efficiency), nesting behaviour, body temperature, and lipid-free weight of brown adipose tissue (an index of non-shivering thermogenesis), to provide information on morphological, behavioural, and physiological contributions to thermoregulation. The specific questions examined were the following. Do mice selected for large and small size maintain the same body temperature? Do they use the same thermoregulatory mechanisms? What are the relationships among different mechanisms of thermoregulation?

## 2. MATERIALS AND METHODS

The experimental animals were sampled from the 6 replicates (*A-F*) of large, control and small mice of Falconer's Q stocks which had been selected for body weight at 6 weeks of age (Falconer, 1973). Selection was practiced for 27 generations. After that, the lines were maintained by random mating with equal replacement from each of 8 litters per line. We employed mice from generations 62–65. Details of the selection procedures and maintenance of the stocks can be found in Falconer (1973). The temperature in the mouse house averaged 22 °C, and animals were exposed to a seasonally fluctuating photoperiod. Data collection spanned all seasons.

At 6 weeks of age, mice were weighed, individually housed, and supplied with water bottles, pre-weighed pelleted food in removeable baskets especially constructed to prevent wastage, and pre-weighed amounts of cotton wool in the food hopper of the cage lid. The cotton wool remaining in the hopper was weighed each day for 3 days, and old nests removed, so that the mice had to build a new nest each day. Additional pre-weighed cotton wool was added to the hopper if necessary. The total weight of cotton wool removed from the hopper in 3 days (total nesting score) was used as an index of nest-building behaviour. The food basket was weighed after 3 days, and food consumption calculated as the weight differential. For most analyses, food consumption was expressed per gramme body weight of mouse.

At 7 weeks of age, mice were killed by cervical dislocation, and core body temperature measured immediately with a no. 402 small animal probe inserted

3.5 cm into the colon, attached to a telethermometer (Yellow Springs Instruments). All temperatures were taken between 09.00 and 12.00 h. The interscapular brown adipose tissue pad was removed, the lipid portion extracted with a 2:1 chloroform:methanol solution, dried at 80 °C to a constant weight, and weighed. (Chaffee & Roberts (1971) reported that lipid-free dry weight is an accurate index of this organ's thermogenic capacity.) For analysis, the weight was expressed per gramme body weight of mouse. We chose the 6–7 week period for our measurements because the mice had been selected for 6-week weight and the large mice tend to become obese at older ages, a characteristic atypical of wild populations.

To the extent possible, two males and two females were sampled from each available litter. Generations were treated as separate blocks; therefore, seasonal effects are contained in blocks. Data from 1064 mice, more or less equally distributed across sizes and replicates, were subjected to analysis of variance using the least-squares program developed by Harvey (LSML76). Factors included size, sex, replicate (nested within size), family (nested within replicate), block and the appropriate interactions. Because of heterogeneity of the variances in the raw data for all variables except body temperature, log transformations were applied to body weight, food consumption and weight of brown adipose tissue, while total nesting scores were square-root transformed. These transformations minimized both the  $F_{\max}$  ratios (Sokal & Rohlf, 1969), and mean-variance correlations. However, raw scores were used for graphical representation.

Table 1. Mean squares from the least-squares analysis of variance for thermoregulatory traits in 6 replicates of a selection experiment for 6-week weight in mice

Source	D.F.	6-week body weight	Food consump. per g B.W.	Brown adipose wt. per g B.W.	Total nesting score	Body temperature
Size	2	2.8637***	0.2412	0.2223	57.17**	2.05
Replicate (within size)	15	0.0419***	0.0767***	0.0747***	5.65***	2.63***
Family (within replicate)	211	0.0606***	0.0135***	0.0066***	0.57***	0.89***
Sex	1	0.5513***	0.3596***	0.3535***	11.08***	13.30***
Block	3	0.0343***	0.0104	0.0208***	4.08***	7.08***
Pooled interaction	11	0.0041	0.0240***	0.0033	1.12**	0.33
Residual	820	0.0017	0.0048	0.0034	0.34	0.48
Transformation		log	log	log	square root	none

\*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

### 3. RESULTS

The results of the analysis of variance are summarized in Table 1. The effects of replicate and family were significant for all variables, so the mean squares for size were tested over the replicate mean squares, which were, in turn, tested over

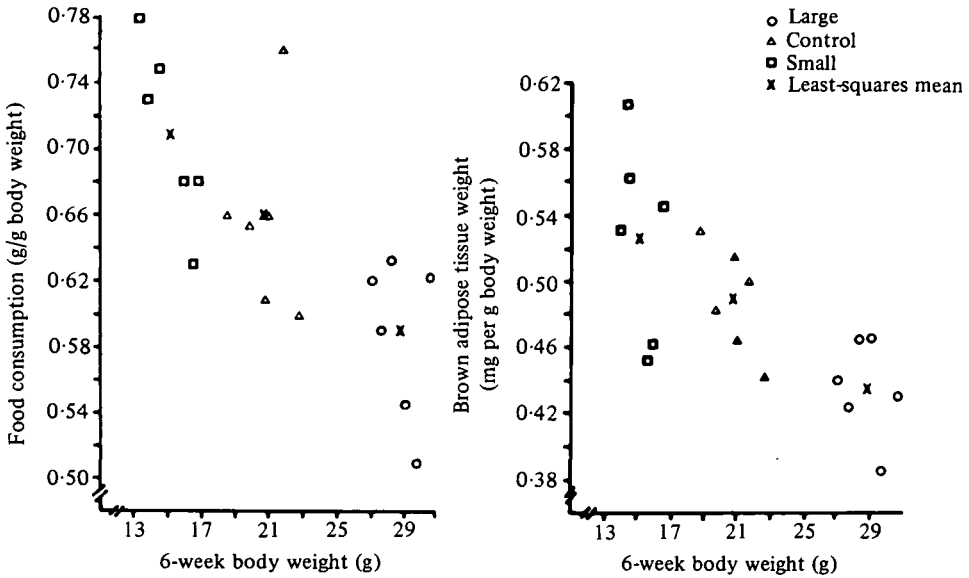


Fig. 1. Mean values of weight-specific food consumption and brown adipose tissue weight, plotted against body weight, for the 6 replicates of large, control, and small mice from a selection experiment for 6-week body weight. The overall least-squares means for the size classes are also indicated.

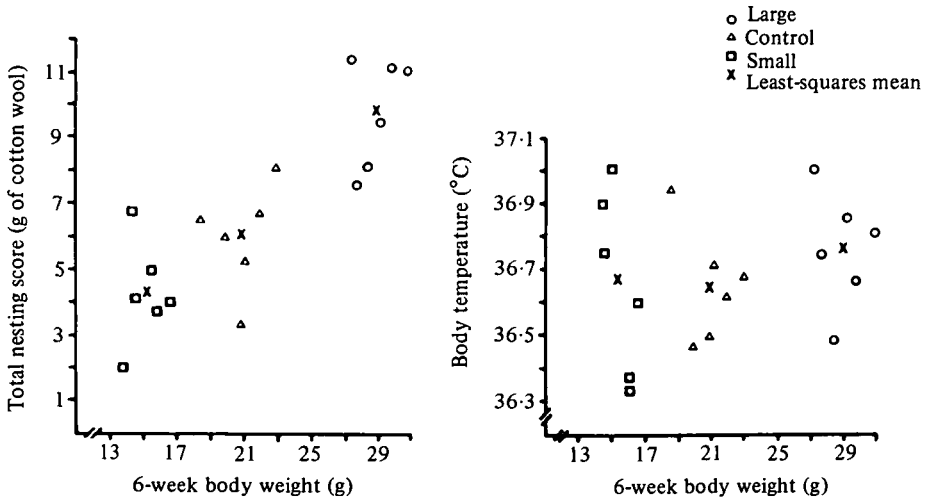


Fig. 2. Mean values of nesting scores and body temperature, plotted against body weight, for the 6 replicates of large, control, and small mice from a selection experiment for 6-week body weight. The overall least-squares means for the size classes are also indicated.

the mean squares for families. All other effects were tested over the residual mean squares.

There were large and significant sex differences for all traits, with females being lighter, consuming more food and having more brown fat per gramme, a higher body temperature, and building smaller nests than males.

Figs. 1 and 2 show the replicate and overall least-squares means for the 4 thermoregulatory traits plotted against body weight. Substantial variation among replicates prevented the effect of size from reaching formal significance in our analysis of variance for food consumption and weight of brown adipose tissue, although the large lines were significantly different from the control ( $t = 3.03$ ,  $P < 0.05$ ) and small lines ( $t = 3.61$ ,  $P < 0.02$ ) for brown adipose tissue, and large and small lines were significantly different for food consumption ( $t = 3.82$ ,  $P < 0.02$ ). In general, large mice consumed less food and had less brown fat per gramme body weight than control or small mice. Large mice built much larger nests, the difference between sizes being even greater than that for the trait under selection. Body temperature was the only trait for which there was no difference between the size classes. In contrast, random environmental effects caused blocks to differ by almost a degree, and for no other trait was the absolute sex difference larger than the difference between sizes.

#### 4. DISCUSSION

The results of this experiment can be used to examine the relationship between body mass and thermoregulation from two related perspectives. We have determined what effect selection for differences in body weight has on other traits involved in thermoregulation. Furthermore, we can now assess whether the structure of the observed genetic correlations permits an 'adaptive' modification of thermoregulation, i.e. whether large size is associated with improved effectiveness of thermoregulation.

A correlated response to selection will be directly proportional to the genetic correlation, given the presence of heritable variation in both traits. In fact, direct selection for differences in body weight has indeed altered other traits contributing to thermoregulation. Although large and small mice maintain about the same body temperature, they achieve this in different ways. On a per gramme basis, small mice eat more than large mice, and their correspondingly larger amount of brown fat suggests that they may be burning off this excess food, rather than assimilating it as body mass. It is well established that animals of many species, including mice, show a correlated response in efficiency of food conversion to selection for increased body weight (e.g. Roberts, 1979; 1981). Our results suggest that this may be due in part to differences in the amount of brown adipose tissue, which may lead to 'wasteful' food utilization through diet-induced thermogenesis (Rothwell & Stock, 1979). The situation is undoubtedly more complex, since there is also evidence for differential rates of protein turnover in large and small mice (Priestly & Robertson, 1973) which may be related to differences in appetite control (Radcliffe & Webster, 1976; Roberts, 1981). However, the ratio of food consumed to weight of brown

adipose tissue is remarkably uniform for all three sizes (between 1.35 and 1.36), implying that brown fat is a major factor in differences in food utilization.

Large mice also have a substantially greater propensity to utilize the thermoregulatory behaviour, nest-building. Since nesting increases insulation, it is one of the most efficient means of thermoregulation. Although these mice were tested at a fairly warm temperature, we expect that a similar difference would exist in the cold since the relative sizes of nests built at warm and cold temperatures are highly genetically correlated (Lynch, Connolly & Sulzbach, 1981; Sulzbach & Lynch, 1984). These nest size differences are greater than can be accounted for simply as proportional differences in body size, and there is also a difference in nest quality that is correlated with size. In a pilot study of nesting in 385 mice from two replicates of the body weight selection lines, all size groups built about the same proportion of covered nests (about 45%) but about 20% of the small mice built flat, pallet-type nests, compared to only about 5% of the large mice. The overall between-group correlation of the nest quality index (1–10) with weight of cotton wool was 0.70.

Thus, for the traits we measured, it appears that large size is genetically associated with thermoregulatory advantage. However, although these results are consistent with predictions deriving from Bergmann's 'rule', many other factors remain to be considered. While the correlated responses are in the direction of adaptation, the genetic correlations are far from one. We lack estimates of heritabilities and standard deviations of thermoregulatory traits in the specific selected population, since these would have to have been estimated in the original heterogeneous population, so we cannot assign an actual value to these correlations. However, in a separate experiment, direct selection for nesting resulted in the predicted changes in body weight, although the resulting differences were small (Lynch, 1980). Data from the base population (Lacy & Lynch, 1979) permit estimation of a realized genetic correlation of 0.25. This estimate indicates that about 75% of the additive genetic influences on this important thermoregulatory behaviour have no pleiotropic effect on body weight. The combination of relatively small genetic correlations and possible conflicting natural selection pressures on body weight means that substantial and perhaps most adaptive change in thermoregulation could occur without alteration of body size, or even in cases where body size decreases.

Furthermore, it is not clear what this genetic correlation structure means in other situations. If interest is in maximizing biomass produced, as in many animal breeding applications, then it appears that this can be an efficient process when large size is accompanied by increased thermoregulatory efficiency, as in this case. If interest is in maximizing numbers of individuals produced, as in the usual definition of Darwinian fitness, then small size has certain advantages. McNab (1971) has emphasized that large size by itself produces a relative not absolute energetic saving. Large mice still eat more than small mice on a whole-animal basis. If food supplies are limited, relatively energetically inefficient small mice would still have an advantage, including having to spend less time foraging and exposed to predators. However, at very low ambient temperatures the greater thermal conductance of smaller animals could be lethal.

For this species there is some evidence that wild populations respond to cold climate by increasing body size. Barnett *et al.* (1975) showed that wild mice which were bred for ten generations at  $-3^{\circ}\text{C}$  increased body size relative to a contemporaneous control population bred at  $21^{\circ}\text{C}$ , and that this size difference persisted when the experimental group was bred at  $21^{\circ}\text{C}$ . Berry, Peters & Van Aarde (1978) studied mice from extreme habitats and found that mice from very cold areas tended to be larger than those from more temperate regions. Plomin & Manosevitz (1974) compared mice derived from a Colorado population with those from two Texas populations and found that the Colorado mice were heaviest, although the presumed effect of drift was greater than the effect of selection. In contrast, the difference in nesting between the Colorado and Texas populations was much greater, indicating that selection had modified nesting much more extensively than body weight.

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