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# Developmental and Adult Acclimation Effects of Ambient Temperature on Temperature Regulation of Mice Selected for High and Low Levels of Nest-Building

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**Summary.** Genetic and environmental components of adaptation to cold in *Mus musculus* were assessed in a study of the effects of selective breeding for behavioral temperature regulation (indexed by high and low levels of nest-building), rearing mice from birth in the cold, and cold acclimation of adult animals, on thermoregulatory traits. Mice from the eleventh selected generation of a high-nesting line maintained higher resting metabolic rates and body temperatures, while at the same time consuming less food when compared with mice from the low-nesting line (Table 1). High-nesting mice were also more discriminating in their temperature preference when placed on a thermal gradient. Thus, common genetic loci must influence a variety of energy conservation measures important for survival in the cold, including insulative nest-building, metabolic efficiency, and optimum microhabitat selection.

Rearing mice at 5 °C from birth until 70 days of age resulted in permanent increases in nonshivering thermogenesis, weight of interscapular brown adipose tissue, and core body temperature when compared to mice raised at 22 °C (Table 1). These greater heat production capacities were accompanied by consumption of more food. Cold acclimation of adults at 5 °C for 3 weeks similarly increased measures of thermogenic capacity (nonshivering thermogenesis and interscapular brown adipose tissue) as well as food consumption, when compared to the effects of warm acclimation, but differed from the effects of cold-rearing in that while resting metabolic rates were elevated, no significant differences in body temperature were found (Table 1).

Sex differences were also noted for most of the thermoregulatory measures, with the lighter females scoring higher on thermal preference, resting metabolic rate, nonshivering thermogenesis, brown fat, and food consumption.

In general, these results suggest that a more precise partitioning of the genetic and environmental factors which influence thermoregulatory traits in mammals could eventually result in a better understanding of the differences which exist between acclimated and acclimatized animals.

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## Introduction

Small mammals encounter potentially severe problems of temperature regulation which are aggravated during winter by the reduced availability of food. Winter survival, therefore, requires either a decrease in heat loss or an increase in heat production, without incurring a prohibitively high energetic cost. Reduction of heat loss requires relatively less expenditure of energy, and can be achieved behaviorally by nest-building, huddling, postural adjustments, and microhabitat selection; or physiologically by increasing fur and subcutaneous fat, decreasing peripheral circulation, and decreasing body temperature (including the mechanisms of hibernation and torpor). Increasing heat production requires considerably more energy and can be achieved by behavioral mechanisms such as shivering and possibly gross activity (Hart, 1971), or the physiological mechanism of nonshivering thermogenesis. Thus, winter survival of free-ranging small mammals probably requires adjustment of multiple characters associated with temperature regulation.

Traits leading to the survival of individuals must be passed to offspring for evolutionary adaptation to occur. This can only happen when the modifications allowing the individual to survive have some genetic basis. It is critical, therefore, when studying the evolution of cold adaptation in small mammals, to separate genetically based adaptation from environmentally induced modification. Furthermore, en-

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vironmental influences on thermoregulatory abilities can be separated into early environmental effects acting during development, and effects of later acclimation resulting from environmental stimuli to the adult. [Although the term "acclimation" refers to the effects of a single environmental variable acting at any time in the life of an individual (Bligh and Johnson, 1973), we are drawing a distinction between developmental effects of the environment to which immature animals are exposed, and effects resulting from the 2 to 4 weeks exposure of adults typical of experimental manipulations (Hart, 1957).] Developmental effects can lead to permanent, and thus gene-mimicking differences between animals from various habitats, while adult acclimation effects are often transient.

Effects of cold acclimation on thermoregulatory responses have been extensively studied in a variety of mammals (Chaffee and Roberts, 1971; Cabanac, 1975), and results of those experiments show that cold acclimation usually augments thermoregulatory capacity. Effects of cold rearing vs. adult cold acclimation have recently been compared in laboratory mice for the thermoregulatory traits of nest-building, thermal preference, food consumption, resting metabolic rate, body temperature, and weight of brown adipose tissue (Lynch et al., 1976). That study showed that 25 days of cold exposure during early development produced lasting changes in thermoregulatory traits which, in some cases, equalled the extent of known genetic influences measured between species originating in widely different habitats.

In the present study we sought to determine whether the previously reported effects of cold rearing would be augmented by holding mice at different temperatures until development was essentially complete. In addition, we utilized lines of mice which had been subjected to artificial selection for high and low levels of nest-building, resulting in animals which were widely divergent genetically for the size of the nest they built. Use of these mice allowed initial assessment of a genetic association between nest-building and other potentially adaptive thermoregulatory traits, and thus provided us with some understanding of a possible genetic basis for the adaptive syndrome of temperature regulation in cold environments.

## Materials and Methods

### *a) Subjects*

Subjects consisted of 333 laboratory mice (*Mus musculus*) representing lines which had been subjected to 11 generations of bidirectional selection for high and low levels of thermoregulatory (individual) nesting measured as total amount of nesting material used in 4 days at room temperature (22 °C). At the 11th generation of selection, high-nesting mice used $31.6 \pm 2.4$ g of cotton, while low-nesting mice used only $4.1 \pm 0.5$ g. The selected lines were derived from an outbred stock (HS/Ibg) which was developed from an 8-way cross among inbred strains (McClearn et al., 1970).

Mice from the high and low lines were initially separated into 2 groups by rearing about half of each group in a cold room ($5 \pm 1$ °C) from birth, and half at room temperature ($22 \pm 1$ °C). To prevent high infant mortality in the cold, pregnant females were allowed 2 to 3 days to adjust to the cold prior to parturition, and were provided with 5 g of cotton with which to build a nest. Neither warm nor cold-exposed subjects were given nesting material following weaning at 30 days of age. At this time mice were housed with like-sexed littermates in polypropylene small animal cages provided with 500 $cm^3$ wood shavings. Food (Wayne Lab-Blox) and water were available *ad libitum*, and all mice were kept on a photoperiod of 16L:8D (Lights on at 6:00 AM and off at 10:00 PM).

At 70 days of age, mice from each temperature treatment were divided into 2 acclimation groups by transferring about half the animals to the opposite temperature condition. Animals were kept under these conditions for 3 weeks before testing began, and remained there for the duration of the experiment. There were approximately equal numbers of males and females in each group which yielded 16 treatment groups defined by genotype, developmental temperature, acclimation temperature, and sex.

Decreased sample sizes for some of the last-measured variables occurred due to death of some cold-acclimated mice following assessment of non-shivering thermogenesis, and mice which were not killed because they were needed for breeding.

### *b) Testing Procedures*

Tests required 3 weeks to complete and were run in the following order:

*Thermal Preference.* Temperature preference was measured in an apparatus consisting of 4 covered alleys, each 130 cm × 7 cm. Alleys were separated by opaque plexiglas and, because some animals prefer resting in corners, small partitions were placed at 5 cm intervals along the length of each alley to provide corners at all temperatures (see Lynch et al., 1976). In this study, floor temperatures were maintained in an approximately linear gradient from 24 to 42 °C by adjusting current flow through a heating tape beneath the aluminum floor of the board. Mice were allowed 3 h to adjust to the alleys, after which time the floor temperature corresponding to the position of each mouse was recorded at 10 min intervals for 1 h. All observations during which the mouse was not moving were averaged to provide an index of thermal preference, since it has been shown that mice select on the basis of substrate temperatures (Stinson and Fisher, 1953).

*Resting Metabolic Rate.* Measurements were taken in closed metabolic chambers at 32 °C. Mice were fasted for 3 h, then placed in the chambers and allowed an additional hour to adjust. Metabolic rate was indexed as the time required for an animal to consume 3 ml of oxygen. Eight or 9 successive measurements were taken for each mouse, and the 3 contiguous slowest times were averaged. Expired carbon dioxide and water vapor were adsorbed by a mixture of Ascarite and Drierite. All measurements were made between noon and 5:00 PM. Metabolic rates were expressed as ml oxygen consumed per gram body weight per h.

*Non Shivering Thermogenesis.* This was assessed immediately following establishment of resting rate by subcutaneously injecting each mouse with 0.6 mg norepinephrine (Levophed) per kg body weight, and immediately measuring oxygen consumption as above.

Nine or 10 measurements were taken and the 3 contiguous fastest times were averaged. The extent of nonshivering thermogenesis was indexed as the maximum increase in oxygen consumption per gram body weight per hour above resting levels. We noted that a control saline injection also elevated metabolism, but to a lesser extent, and the duration of the response was considerably shorter (about 10–15 min after injection). Since peak metabolism following norepinephrine injection occurred 20 min after the injection and was maintained for up to 30–45 min, we felt that this procedure indexed an increased sensitivity to norepinephrine following cold exposure (Depocas, 1960) and was not in response to the injection (see Janský and Hart, 1963; Brück, 1970). The injection of 0.6 mg norepinephrine per kg body weight used in this study results in maximal heat production in *Mus musculus* (Janský et al., 1969). The number of mice assessed here obviated the use of other indexes of nonshivering thermogenesis.

*Food Consumption.* Mice were allowed approximately a week at their respective acclimation conditions following metabolic testing before food consumption was measured at that temperature. Food in the hopper was weighed on day 0 and again 4 days later, and measurements were expressed as weight of food consumed over 4 days per gram body weight.

*Body Temperature.* Animals were killed by cervical dislocation and rectal body temperature was measured immediately with a #402 small animal probe (inserted 3.5 cm) and a telethermometer (Yellow Springs Instruments, Yellow Springs, Ohio). All body temperatures were taken between 4:00 and 5:00 PM at room temperature.

*Brown Adipose Tissue.* The interscapular brown fat pad was removed and the lipid component extracted by 3 daily changes of a 2:1 chloroform:ethanol solution. The remaining tissue was dried for 4 h in a vacuum oven at 90 °C. The resulting lipid-free dry weight of the brown fat was expressed in mg per gram body weight. J.S. Hayward (in Chaffee and Roberts, 1971) reported that lipid-free dry weight was an accurate index of the thermogenic capacity of brown fat. Similarly, Lynch (1973) found a substantial correlation between the lipid-free dry weight of interscapular brown fat and the extent of nonshivering thermogenesis.

### *c) Data Analysis*

Data for all variables were considered to represent a $2^4$ factorial arrangement of treatments and subjected to least-squares analysis of variance (Harvey, 1960). Orthogonal contrasts were used to assess significance of main effects due to genotypic differences (high vs. low nesting), developmental temperature differences (cold vs. warm), adult acclimation differences (cold vs. warm), and sex differences, as well as interactions among these factors.

## Results and Discussion

Expression of all physiological and behavioral characters is influenced by the genetic structure of the individual and by the environment to which that individual is exposed. With respect to temperature adaptation, Prosser (1967) summarized this concept in the following manner: "... two different bases of variation must be distinguished: genetically determined variation and environmentally induced variation. The former resides in differences in genetic code, and this fixes the limits within which environmentally induced variation may occur". In this experiment we have partitioned the sources of variation which result from differences in genotype (selected lines for nesting), temperature during early development, temperature as adults, and sex.

The main effects of these differences on physiological and behavioral temperature regulation in *Mus musculus* are given in Table 1. For convenience, these values denote combined means representing orthogonal comparisons between main effects (e.g., for the effect of genotype, all high-nesting mice are compared with all low-nesting mice while other treatments are distributed equally between the two main groups). All main effects listed in Table 1 are statistically significant at the 95% level of confidence. The t-values for all comparisons are listed in Table 2. Due to an adjustment of the thermal preference board between testing of mice raised in the warm and those raised in the cold, we do not feel that a valid developmental comparison can be made for thermal preference from these data, and so the relevant spaces in Tables 1 and 2 are blank. However, functioning of the board was consistent within each of these groups so that the other three possible comparisions are justified.

### *a) Effect of Genotype*

Since the subjects of this experiment were taken from genetically divergent lines of mice, genetic associations between thermoregulatory traits were to some extent assessable. In theory, any differences between a selected line and the base population from which it was selected (assuming constant environment across all generations), or between lines selected in the opposite directions are the result of changes in the allelic frequencies at genetic loci that influence the indexed trait. When a trait, in this case nest-building, is subjected to directional selection in the laboratory, measurements on a variety of potentially related traits can yield insights into the overall adaptive repertoire of the animals being studied. When it is found that additional traits have been modified by a selection experiment, such a modification is referred to as a correlated response to selection (Falconer, 1960). It may then be stated that these changes in traits which were not directly selected were also caused by the changes in allelic frequencies at loci influencing the indexed trait. For this to occur, loci must exist which exhibit pleiotropy (the action of one gene locus on several phenotypic characters), contributing to both the indexed trait and traits showing a correlated response to selection. An alternative explanation to pleiotropy is genetic linkage; however, such a possi-

**Table 1.** Grouped means of variables associated with temperature regulation in 333 laboratory mice (*Mus musculus*) representing significant effects of differences in genotype (high nesting vs. low nesting), ambient temperature during development (5 °C vs. 22 °C from 0 to 70 days of age), ambient temperature of adult acclimation (5 °C vs. 22 °C from 71 to 115 days of age), and sex

| Effects of: | Genotype | | Temperature during development | | Adult acclimation temperature | | Sex | |
|---|---|---|---|---|---|---|---|---|
| Thermal preference (°C) | | | | | | | ♀ | 32.0 |
| | | | | | | | ♂ | 30.1 |
| Resting metabolic rate ($ml\ O_2/g \cdot h$) | High | 2.28 | | | Cold | 2.30 | ♀ | 2.39 |
| | Low | 2.15 | | | Warm | 2.13 | ♂ | 2.04 |
| Non shivering thermogenesis ($ml\ O_2/g \cdot h$) | | | Cold | 6.34 | Cold | 6.21 | ♀ | 6.32 |
| | | | Warm | 5.60 | Warm | 5.72 | ♂ | 5.62 |
| Food consumption (g/g body weight) | High | 0.98 | Cold | 1.07 | Cold | 1.31 | ♀ | 1.12 |
| | Low | 1.08 | Warm | 1.00 | Warm | 0.75 | ♂ | 0.95 |
| Body temperature (°C) | High | 37.7 | Cold | 38.0 | | | | |
| | Low | 37.5 | Warm | 37.2 | | | | |
| Brown adipose tissue (mg/g body weight) | | | Cold | 0.79 | Cold | 0.85 | ♀ | 0.80 |
| | | | Warm | 0.74 | Warm | 0.68 | ♂ | 0.72 |
| Body weight (g) | | | | | | | ♀ | 24.6 |
| | | | | | | | ♂ | 28.8 |

**Table 2.** Tests of significance (t-values) of main effects of differences in genotype (high vs. low nesting), ambient temperature during development (5 °C vs. 22 °C from 0 to 70 days of age), ambient temperature of adult acclimation (5 °C vs. 22 °C from 71 to 115 days of age), and sex (female vs. male) on variables associated with temperature regulation in laboratory mice (*Mus musculus*). For orthogonal comparisons, the following conditions were assigned the + coefficient: high nesting, 22 °C and female

| Effects of: | Genotype | Temperature during development | Adult acclimation temperature | Sex |
|---|---|---|---|---|
| Thermal preference (°C) | 0.59 | | 1.93 | 8.16[c] |
| Resting metabolic rate ($ml\ O_2/g \cdot h$) | 2.92[b] | 0.56 | − 3.65[c] | 7.48[c] |
| Non shivering thermogenesis ($ml\ O_2/g \cdot h$) | 1.24 | −4.33[c] | − 2.87[b] | 4.15[c] |
| Food consumption (g/g body weight) | −4.48[c] | −3.00[b] | −23.58[c] | 7.07[c] |
| Body temperature (°C) | 1.98[a] | −8.37[c] | 0.27 | 1.65 |
| Brown adipose tissue (mg/g body weight) | 0.01 | −2.83[b] | − 9.70[c] | 4.42[c] |
| Body weight (g) | −0.41 | 1.44 | − 1.45 | −14.74[c] |

[a] Significant at $P < 0.05$
[b] Significant at $P < 0.01$
[c] Significant at $P < 0.001$

bility is less likely since recombination over many generations tends to reduce linkage disequilibrium. More importantly, most continuously varying characters, such as nesting, are thought to be influenced by allelic differences at multiple loci (Falconer, 1960). In that case, it is unlikely that all the loci involved would be closely linked on the same chromosome. Thus, selection experiments represent a potentially powerful design which can provide insight into the physiological bases of behavioral responses to envi-

ronmental change, as well as suggest the basis for interaction of different adaptive responses to produce homeostasis.

In the present study, the genetic differences brought about by selection for nest-building were found to influence food utilization, as indicated by higher metabolic rates and body temperatures accompanied by lower food consumption in the high-nesting mice. Not only does this correlated response of reduced food consumption with increased nest-building suggest that a common genetic basis must exist for behavioral mechanisms of energy conservation and physiological mechanisms leading to more efficient metabolism, but it also reveals that one or more pleiotropic loci simultaneously influence two seemingly independent aspects of thermoregulatory adaptation. The specifics of this association can now be studied. For example, has prior selection for nesting also altered the assimilation efficiencies of these animals?

Although the mean thermal preference scores of high and low-nesting mice were almost identical (31.1 °C vs. 31.0 °C, respectively), the diversity of the temperatures chosen was greatly altered by selection for nesting. The variance of thermal preference in low-nesting mice was more than twice that of high-nesting mice (6.10 vs. 2.99 for low and high-nesting mice, respectively, pooled across environmental treatment and sex), indicating that mice of the low line selected a wide range of temperatures while high-nesting mice consistently chose more intermediate temperatures.

We have two hypotheses that could explain this observed difference in variance of thermal preference scores between the high and low-nesting lines. First, alleles influencing nest-building may have a pleiotropic action on the precision of either sensory or integrative response to ambient temperature. The low-nesting mice, therefore, either would not be able to make as fine temperature discriminations, or would not act on temperature perception in as precise a manner as the high-nesting mice. Alternatively, dominance effects rather than, or as well as, additive gene action may be involved. Lynch and Hegmann (1972) have shown that nesting has a low heritability of less than 0.2, and that dominance variance is relatively high with heterosis being exhibited. Therefore, in responding to selection, the low nesting line should be more homozygous, with more fixation of alleles at those loci which influence nest-building. The presumably more homozygous low-nesters may choose either high or low temperatures, depending on what alleles they possess. The thermoneutral zone of *Mus* would be the optimum temperature, requiring no energy expenditure above the basal rate for thermoregulation, and it is within this zone that the presumably more heterozygous high nesters consistently select. This hypothesis is supported by the finding of Silcock and Parsons (1973) that while three inbred strains of *Mus* chose extreme temperatures, the heterozygous $F_1$'s chose more intermediate temperatures, even when both parent strains had extreme scores in the same direction. Loci therefore may exist which, when heterozygous, contribute to the two adaptive traits of high nesting and intermediate temperature selection.

### *b) Effect of Temperature during Development*

Since the distribution of free-ranging *Mus musculus* is extensive, differences in local weather conditions during early development probably influence expression of thermoregulatory characters as adults. In this study we found that rearing laboratory mice at 5 °C throughout their development produced lasting effects in both genotypes, independent of adults' acclimation temperature. Cold rearing resulted in higher levels of nonshivering thermogenesis, larger amounts of interscapular brown fat, elevated body temperature, and increased food consumption compared with the warm rearing condition (Table 1).

Nonshivering thermogenesis has been found to be of primary importance in the ability of rodents to withstand cold (Davis, 1963; Bartunkova et al., 1971) and brown fat is one site of nonshivering thermogenesis (Smalley and Dryer, 1963; Smith and Horowitz, 1963). Thus, the increases in nonshivering thermogenesis and lipid-free dry weight of interscapular brown fat presented here confirm the previously reported finding that mammals raised in a cold environment develop greater capabilities for heat production than do warm-reared animals (Brück and Wünnenberg, 1966; Brück et al., 1969; Lynch et al., 1976).

The permanently higher core temperatures (the 3.5 cm insertion of the rectal temperature probe used in this study yields maximum and presumably core values for body temperature) in cold-reared mice would appear to increase the temperature gradient that a mouse must maintain between itself and the environment, and therefore aggravate thermoregulatory problems in the cold. This seemingly counterproductive response of higher core temperature may be necessary to assure that peripheral tissues are kept warm enough to maintain their function in the cold. Although surface temperatures were not determined in this study, Heroux (1959) found that skin temperatures on the back, tail and feet of chronically cold-exposed adult laboratory rats were consistently higher

when compared to warm-exposed animals. In that study all rats were briefly cold-exposed before surface body temperatures were assessed. We also noted that mice reared in the cold exhibited no signs of cold injury (e.g., lesions on the ears) whereas mice reared at 22 °C and then cold-exposed often experienced such injury. Reduced cold injury in mice reared in the cold could be attributed in part to the observed increase in core body temperature, but other adjustments, such as increased vascularization of hairless surfaces and increased peripheral blood flow, could also have occurred.

Although, given the nature of the responses reported here, cold-reared mice should be more protected from severe cold stress, they pay for this added insurance with permanently higher food consumption in both warm and cold environments.

The previous study in this laboratory showed that early cold exposure had slowed morphological growth sufficiently so that at 50 days of age, cold-reared mice were substantially lighter than those raised at room temperature (Lynch et al., 1976). However, since the mice in the present study were fully adult, and all temperature groups had attained similar body weights (even though their rates of growth probably differed), it is clear that the reported differences in thermoregulatory capabilities were not mediated through differences in body size.

Since varying environmental conditions during development resulted in large differences for many of the traits we studied, it is clear that experimenters must use adequate controls when studying genetic differences between wild populations of mice from different climates. It is necessary not only to acclimate wild-caught animals to lab conditions (thereby eliminating short-term environmental effects), but also to raise one generation in the lab in order to control for differences in developmental environment between the populations. This second control has been overlooked by some investigators in the past. (For a more detailed discussion of this problem, see Lynch et al., 1976.)

### *c) Effect of Temperature during Acclimation of Adults*

Cold acclimation of adults, like cold temperatures during development, increased heat production capabilities as indicated by higher levels of non shivering thermogenesis and brown fat scores (see Table 1). Similar increases in thermogenesis (Janský et al., 1969) and in brown fat (Lynch et al., 1976) in response to cold acclimation have been previously reported for this species.

Effects of adult acclimation differed from developmental temperature effects, producing an increase in the resting metabolic rate (and in the incidence of cold injury) but no change in body temperature (Table 1). Heroux (1963) also noted increases in resting metabolism and cold injury in rats following chronic cold exposure in the laboratory, but these changes were absent in grouped animals maintained in outdoor enclosures. He attributed the increase in resting metabolism to a pathological response to chronic cold acclimation (Heroux, 1970). Our results on *Mus musculus* suggest that it is not chronic cold exposure so much as the timing of cold exposure during development which affects the level of resting metabolism in adult animals. It is possible that observed differences between acclimated and acclimatized rodents for resting rate are in part due to differences in temperature during early development. Of course, other factors, such as genetic differences between groups or seasonal change in exogeneous cues, could also contribute to differences between cold-acclimated and acclimatized animals.

### *d) Sex Differences*

The significant sex differences appearing in all traits except body temperature showed a clear trend with females scoring higher on the behavior of thermal preference, and all metabolic measures: food consumption, resting metabolic rate, non shivering thermogenesis, and brown fat (see Tables 1 and 2). Whereas the sex difference in food consumption and resting metabolic rate could simply be due to the smaller size and thus greater surface area to volume ratio of females, neither thermal preference, nonshivering thermogenesis, nor brown fat correlated with body weight in this experiment, and therefore the sex difference in those traits must be the result of some more fundamental difference between the thermoregulatory mechanisms of males and females. These sex-specific mechanisms leading to higher heat production and temperature selection of females may be adaptations for rearing offspring.

### *e) Interaction Effects*

Significant interactions of sex with ambient temperature during development ($t = -3.68$, $P < 0.001$) and sex with adult acclimation temperature ($t = -2.30$, $P < 0.05$) were found for body weight. Females responded to both periods of cold exposure by adding weight, while males tended to lose weight, leading to a decrease in the sex difference for body weight

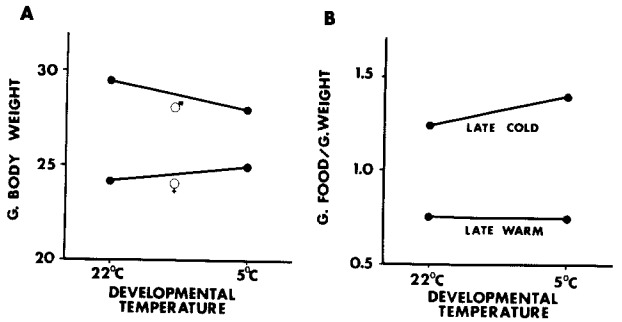


**Fig. 1.** Interaction between the effects of developmental temperature (22 or 5 °C) and a) sex on mean body weight or b) acclimation temperature of adult mice (late warm [22 °C] or late cold [5 °C]) on mean daily food consumption

in cold-exposed mice (Fig. 1). Specifically, males and females differed by 5.3 g when reared at 22 °C, but only by 3.2 g when reared at 5 °C. Similarly, there was a weight difference between the sexes of 4.9 g across all warm-acclimated adults compared to 3.6 g across cold-acclimated adults.

The opposite responses of the sexes to cold with respect to body weight suggest that a minimal level of insulative fat is necessary for cold survival, while harsh metabolic demands prevent the excessive build-up of energy stores that often occurs with males in the warm.

One additional significant effect ($t=3.33$, $P<0.001$) resulted from the interaction of temperature during development with adult acclimation temperature on food consumption (Fig. 1). Although cold rearing had little effect on food consumption of mice which were warm-acclimated as adults, it substantially increased the food intake of mice measured in the cold (0.49 g per gram body weight difference between warm and cold-acclimated warm-reared mice vs. 0.64 g per g body weight difference between warm and cold-acclimated cold-reared mice). This result further demonstrates the higher cost accompanying the increased thermoregulatory capacities of cold-reared mice.

### *f) Conclusions*

Dobzhansky (1956) viewed an adaptive syndrome as phenotypes molded by the genetic structure of a population as well as past and present environmental influences. Thus, in order to understand the adaptive syndrome associated with temperature regulation in small mammals, it is necessary to clearly distinguish the genetic and environmental components of phenotypic expression. In this study, we have demonstrated how genetically distinct lines for a thermoregulatory trait, nesting, can be employed in partitioning such genetic and environmental influences; but in order to understand adaptation of natural populations, additional data are required. As Heroux (1963) has aptly demonstrated for rats, the process of mammalian acclimation following chronic cold exposure in the laboratory differs substantially from acclimatization of free-ranging animals during winter. Yet most such comparisons fail to distinguish the specific sources of variation between acclimated and acclimatized animals. For example, the roles that genetic differences or differences in ambient temperature during early development play in explaining differences between acclimation and acclimatization are seldom evaluated. Thus, precise and thorough partitioning of genetic and environmental influences on expression of thermoregulatory phenotypes is needed before the differences between cold acclimation and cold acclimatization can be understood. At the same time such partitioning could also provide insight into the adaptive syndrome(s) associated with temperature regulation in small mammals as demonstrated here.

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## References

Bartunková, R., Janský, L., Mejsnar, J.: Nonshivering thermogenesis and cold adaptation. In: Nonshivering thermogenesis (ed. L. Janský), pp. 39–56. Prague: Academia 1971

Bligh, J., Johnson, K.G.: Glossary of terms for thermal physiology. J. appl. Physiol. **35**, 941–961 (1973)

Brück, K.: Nonshivering thermogenesis and brown adipose tissue in relation to age, and their integration in the thermoregulatory system. In: Brown adipose tissue (ed. O. Lindberg), pp. 117–154. New York: American Elsevier 1970

Brück, K., Wünnenberg, W.: Influence of ambient temperature in the process of replacement of nonshivering by shivering thermogenesis during postnatal development. Fed. Proc. **25**, 1332–1336 (1966)

Brück, K., Wünnenberg, W., Zeisberger, E.: Comparison of cold-adaptive metabolic modifications in different species, with special reference to the miniature pig. Fed. Proc. **28**, 1035–1040 (1969)

Cabanac, M.: Temperature regulation. Ann. Rev. Physiol. **37**, 415–439 (1975)

Chaffee, R.R.J., Roberts, J.C.: Temperature acclimation in birds and mammals. Ann. Rev. Physiol. **33**, 155–197 (1971)

Davis, T.R.A.: Nonshivering thermogenesis. Fed. Proc. **22**, 777–782 (1963)

Depocas, F.: The calorigenic response of cold acclimated white rats to infused norepinephrine. Canad. J. Biochem. Physiol. **38**, 107–114 (1960)

Dobzhansky, T.: What is an adaptive trait? Amer. Naturalist **90**, 337–347 (1956)

Falconer, D.S.: Introduction to quantitative genetics. New York: The Ronald Press 1960

Hart, J.S.: Climatic and temperature induced changes in the energetics of homeotherms. Rev. Canad. Biol. **16**, 133–174 (1957)

Hart, J.S.: Rodents. In: Comparative physiology of thermoregulation, Vol. II (ed. G. Causey Whittow), pp. 1–149. New York: Academic Press 1971

Harvey, W.R.: Least square's analysis of data with unequal subclass numbers. Publ. No. ARS-20-8. Washington, D.C.: United States Dept. of Agriculture 1960

Heroux, O.: Comparison between seasonal and thermal acclimation in white rats. Canad. J. Biochem. Physiol. **37**, 1247–1253 (1959)

Heroux, O.: Patterns of morphological, physiological, and endocrinological adjustments under different environmental conditions of cold. Fed. Proc. **22**, 789–792 (1963)

Heroux, O.: Pathological consequences of artificial cold acclimatization. Nature **227**, 88–89 (1970)

Janský, L., Bartunková, R., Kockova, J., Mejsnar, J., Zeisberger, E.: Interspecies differences in cold adaptation and nonshivering thermogenesis. Fed. Proc. **28**, 1053–1058 (1969)

Janský, L., Hart, J.S.: Participation of skeletal muscle and kidney during nonshivering thermogenesis in cold acclimated rats. Canad. J. Biochem. Physiol. **41**, 953–964 (1963)

Lynch, C.B., Hegmann, J.P.: Genetic differences influencing behavioral temperature regulation in small mammals. I. Nesting by *Mus musculus*. Behav. Genetics **2**, 43–53 (1972)

Lynch, G.R.: Seasonal changes in thermogenesis, organ weights, and body composition in the White-footed Mouse, *Peromyscus leucopus*. Oecologia **13**, 363–376 (1973)

Lynch, G.R., Lynch, C.B., Dube, M., Allen, C.: Early cold exposure: Effects on behavioral and physiological thermoregulation in the house mouse, *Mus musculus*. Physiol. Zool. **49**, 191–199 (1976)

McClearn, G.E., Wilson, J.R., Meredith, W.: The use of isogenic and heterogenic mouse stocks in behavioral research. In: Contributions to behavior-genetic analysis: The mouse as a prototype (ed. G. Lindzey, D.D. Theissen), pp. 3–22. New York: Appleton-Century-Crofts 1970

Prosser, C.L.: Molecular mechanisms of temperature adaptation in relation to speciation. In: Molecular mechanisms of temperature adaptation (ed. C.L. Prosser), pp. 351–376. Washington, D.C.: Amer. Assoc. Adv. Sci. 1967

Silcock, M., Parsons, P.A.: Temperature preference differences between strains of *Mus musculus*, associated variables, and ecological implications. Oecologia **12**, 147–160 (1973)

Smalley, R., Dryer, R.: Brown fat: thermogenic effect during arousal from hibernation in the bat. Science **140**, 1333–1334 (1963)

Smith, R.E., Horowitz, B.A.: Brown fat and thermogenesis. Physiol. Rev. **49**, 330–425 (1969)

Stinson, R.H., Fisher, K.C.: Temperature selection in deer mice. Canad. J. Zool. **31**, 404–416 (1953)