



# Body temperatures of house mice artificially selected for high voluntary wheel-running behavior: repeatability and effect of genetic selection

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## Abstract

We studied rectal body temperatures of house mice (*Mus domesticus*) that had been artificially selected for high voluntary wheel running.

1. At generation 17, mice from the four replicate selected lines ran, on average, 2.5-times as many revolutions/day as did mice from the four random-bred control lines.

2. During the day, repeatability of individual differences in body temperature measured 4 days apart was low; at night, repeatability was statistically significant across three time scales (1 day, 1 week, 2 weeks).

3. During the day, body temperatures of selected and control animals did not differ; at night, mice from selected lines had higher body temperatures. However, when amount of wheel running immediately prior to measurement was included as a covariate, the difference was no longer statistically significant.

Higher body temperatures, associated with increased activity, might enhance locomotor abilities through  $Q_{10}$  effects, increase metabolic rate and food requirements, affect sleep patterns, and alter expression of heat-shock proteins. © 2000 Elsevier Science Ltd. All rights reserved.

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## 1. Introduction

House mice (*Mus domesticus*) have served as common models in the study of mammalian thermoregulation. Under normothermic conditions, body temperatures of house mice range from 34 to 39°C

(Gordon, 1993). Such variation has multiple causes. We know, for example, that body temperature follows a circadian rhythm (Refinetti, 1994; Refinetti and Menaker, 1992) and that locomotor activity and body temperature are positively correlated (Bolles et al., 1968; Gordon and Yang, 1997; Honma and Hiroshige, 1978; Refinetti, 1994). Few studies, however, have focused on body temperature differences among individual animals (Hayes and Jenkins, 1997) or among populations within a single species (Garland and Adolph, 1991).

Connolly and Lynch (1981) measured body tempera-

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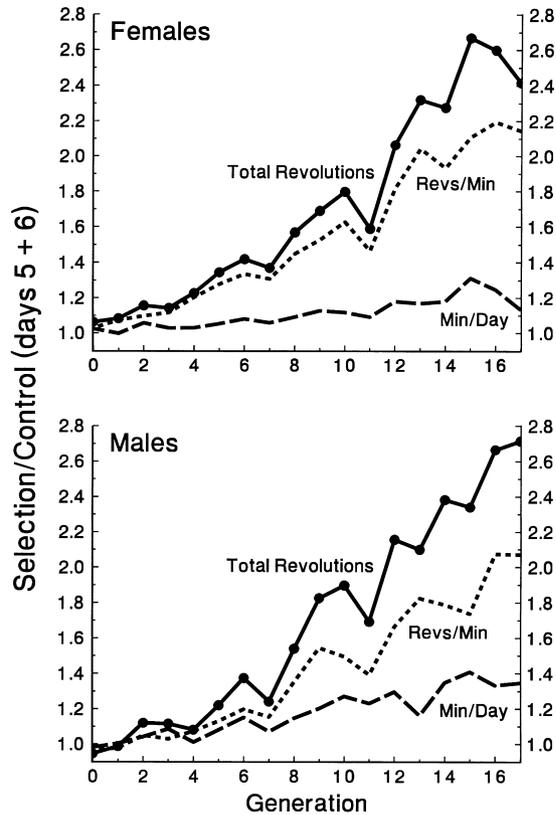


Fig. 1. Ratio (selected/control) of mean total revolutions, mean revolutions per min, and mean min per day for days 5 + 6 of a six-day test (the normal target of the artificial selection experiment: see Swallow et al., 1998a) plotted against generation ordinal number.

ture around the clock in four different inbred strains of mice to estimate broad-sense heritability (ratio of total genetic variance to phenotypic variance). Heritability of body temperature measured during the day, when mice were sleeping, was lower than at night, when they were active. Interestingly, the strains that differed most in body temperature have also been shown to differ in locomotor activity: BALB/c mice were more active in their cages than C57BL/6 (Lassalle and Le Pape, 1978) and displayed approximately 1°C higher body temperature at night (Connolly and Lynch, 1981).

Artificial selection is one tool that can be used to study the genetics and correlated evolution of different aspects of the phenotype (Garland and Carter, 1994; Gibbs, 1999; Roff, 1997). Accordingly, body temperature has also been compared among populations that diverged as a result of artificial selection. Lynch and Roberts (1984) hypothesized that mice selected for large body size would maintain higher body temperatures than those selected for small size because larger

mice have a smaller surface-to-volume ratio. In fact, no statistically significant differences were found. However, correlated effects in body temperature were found as a response to a selection for nest-building behavior (Lacy et al., 1978).

We have been conducting an artificial selection experiment for increased voluntary wheel-running behavior in *Mus domesticus* (Swallow et al., 1998a). One of the goals of the experiment is to determine whether running performance abilities, and underlying physiology, have evolved in concert with wheel running in the selected lines. We expect running performance to increase if animals from selected lines choose to run close to their maximum capacity. Presently, we do not know whether the pattern of wheel running in selected animals requires near-maximum physiological capacity (this is an area of current investigation; see also Koteja et al., 1999b). However, we do know that the selected lines have evolved higher activity levels mainly by running at higher average speeds (especially for females), rather than for more min per day (Fig. 1). In addition, the maximum revolutions/min ever exhibited over a 2-day test period is approximately 2-fold higher in the selected lines, for both sexes (unpublished results). If animals from selected lines are running near their physiological maximum speed, then body temperature may have evolved to facilitate the high running speeds. For example, selected animals might have evolved higher body temperatures to promote higher rates of muscle contraction (Bennett, 1984, 1990).

The present study has two goals. First, we estimate and discuss repeatability of the measurements of body temperature. Repeatability measures the consistency of a trait over a specified time interval and sets the upper limit to heritability. Repeatability thus provides some information about the possibility of evolutionary (genetic) change in a trait (Boake, 1989; Lessells and Boag, 1987). We know of no other study that reports the repeatability of rectal measurements of body temperature in house mice. Second, we compare body temperatures of animals from the selected and control lines to determine whether selection for high locomotor activity has resulted in increased body temperature.

Two approaches were used to distinguish immediate effects of locomotor activity on body temperature from effects of underlying thermoregulatory physiology. First, animals were measured while housed in cages either with or without access to rotating wheels. Second, for the animals housed with wheels, analysis of covariance was used to account for the effect of wheel running (or climbing in locked wheels) during the time immediately preceding measurement of body temperature.

## 2. Materials and methods

### 2.1. Animals

As described in detail in Swallow et al. (1998a), outbred, genetically variable (Carter et al., 1999; Rice and O'Brien, 1980) laboratory house mice of the Hsd:ICR strain were purchased from Harlan Sprague Dawley in 1993. After two generations of random mating, mice were randomly paired and assigned to 8 closed lines (10 pairs in each). In each subsequent generation, when the offspring of these pairs were 6–8 weeks old, they were housed individually with access to a running wheel for 6 days and a computer recorded number of wheel revolutions in one-min intervals. In the 4 “selected” lines, the highest-running male and female from each family were selected as breeders to propagate the lines to the next generation (this is termed within-family selection; Lacy et al., 1978; Roff, 1997). In the four “control” lines, a male and a female were randomly chosen from each family. Within all lines, the chosen breeders were randomly paired except that sibling matings were not allowed. The lines were propagated this way for 17 generations, at which time selected animals were running about 2.5 times as many revolutions/day as controls (Fig. 1).

In this study, we used 17th generation animals that were not among those chosen as breeders to propagate lines to the 18th generation. Because exclusion of the top runners would have caused our samples from the selected lines to be biased downwards with respect to wheel running, we also excluded the lowest-running animals in selected-line families. Of the remaining mice, 192 were randomly chosen to participate (12 of each sex per line).

### 2.2. Protocol

On 24 March 1998, the study mice (mean age  $\pm$  SD =  $70 \pm 3$  days) were placed in standard clear plastic cages ( $27 \times 17 \times 12.5$  cm) attached to Wahman-type activity wheels (1.12-m-circumference, 10-cm-wide running surface of 10-mm wire mesh bounded by clear Plexiglas walls; Lafayette Instruments, Lafayette, IN; model 86041 with modifications). Sixty four of the wheels were secured with wire ties to prevent them from rotating (“locked”) and 128 were left free to rotate (sexes and lines were equally represented within wheel-type treatments). Siblings of the same sex were never assigned to the same wheel treatment. Water and food [Harlan Teklad Laboratory Rodent Diet (W)-8604] were available ad libitum. Rooms were controlled for temperature ( $\sim 22^\circ\text{C}$ ) and photoperiod 12:12 L:D (lights on at 8 am, Central Standard Time).

For logistical reasons, cages with locked wheels were placed in one area of the room and cages with free

wheels were placed in another (i.e., wheel-types were not randomly distributed within rooms); hence, mice with free and locked wheels were not compared statistically. As well, males and females were housed in separate rooms. Because room was thus confounded with sex and because sex differences in body mass (Figs. 3 and 4; Swallow et al., 1999) and in both the amount (Fig. 5) and composition (Fig. 1) of wheel running (Swallow et al., 1998a, 1999; Koteja et al., 1999a,b) are substantial, males and females were not compared statistically.

During the day, we measured body temperatures from 11:30 am to 1:30 pm (females, April 9 and 13; males, April 10 and 14). At night, mice were measured from 10:30 pm to 1:30 am (red light provided illumination; females and males, April 20, 21, 27, 28, May 4, 5). Mice were captured by hand as quickly as possible and a thermocouple probe was inserted rectally to a depth of 2.5 cm. Mean delay time from initial handling to the moment body temperature was read on the thermometer was 59 s ( $\pm 16$  s, SD). Vaseline was used for lubrication. Two digital thermometers were used (Omega Engineering, models HH-71 and HH-23). Both were calibrated to a standard mercury thermometer and values were corrected by the mean difference between digital and mercury measurements. Body mass, time of day, behavior before capture (climbing, running, sleeping or active in cage), and delay time were also recorded. Computers recorded number of wheel revolutions in one-min intervals throughout the study.

### 2.3. Data analysis

Data were analyzed separately by sex, wheel treatment (free or locked), and circadian phase (day or night). For the day measurements, we restricted analysis to mice on free wheels because we wished to include only individuals that were sleeping prior to measurement. Thus, we excluded mice that were either observed to be active at the time they were captured or whose computer records indicated any revolutions during the 10-min period prior to measurement (justification for 10-min is discussed below).

For a repeated-measures comparison of body temperatures between selected and control lines, we used PROC MIXED in SAS (a restricted maximum likelihood procedure), modeling linetype (selected or control) as a fixed effect, line nested within linetype as a random effect, and individual nested within line nested within linetype as a random effect. The REPEATED statement was used in PROC MIXED to account for replicate measurements of body temperature within individuals. The spatial power, SP(POW), covariance structure was chosen for nighttime data to account for the fact that the repeated measurements were not

equally spaced. The compound symmetric, CS, covariance structure was used for daytime data.

To estimate repeatability of body temperature, we analyzed each day of measurements separately using PROC GLM (a general linear models procedure), with line nested within linetype entered as a random effect. Then, we calculated Pearson's correlation coefficients ( $r$ ) and intraclass correlation coefficients ( $t$ ) for the residuals of these models (Lessells and Boag, 1987). Because we recorded body temperatures at night over the course of three weeks, with two consecutive days of measurement each week, we were able to calculate correlations over three time scales: one day, one week, two weeks. Pearson's  $r$  for residuals of nighttime measurements are presented in the form of a correlation matrix.

For both MIXED and GLM procedures, we included several covariates to remove confounding influences. The delay time required for capture and measurement was always included because handling has been shown to influence body temperature (Cabanaac and Briese, 1992). Time of day and  $z$ -transformed time of day squared were always included because body temperature follows a circadian rhythm (Refinetti and Menaker, 1992); the squared term was used to account for the non-linear relationship and  $z$ -transformation was applied to avoid correlation between the predictors (orthogonal polynomial). Body mass was included because larger bodies have a smaller surface area-to-volume ratio, which may influence heat balance and consequently body temperature (Lynch and Roberts, 1984). However, selected animals were smaller than controls (see also Koteja et al., 1999b; Swallow et al., 1999), causing colinearity between linetype and body mass. Consequently, models were run both with and without body mass entered as a covari-

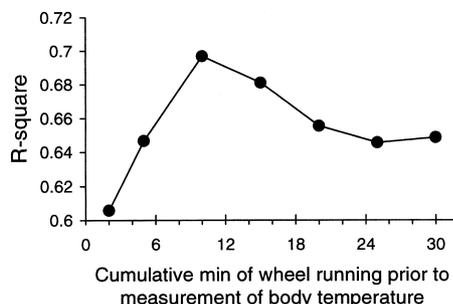


Fig. 2. Total  $R$ -squared values for statistical models predicting body temperature of females at night with access to free wheels (measured April 21, 1998) plotted against the different wheel-running covariates that were tested. In addition to square root cumulative revolutions and this variable  $z$ -transformed and squared, the models also included body mass, delay time, time of day, and  $z$ -transformed time of day squared.

ate; this allowed us to determine the extent to which repeatability and linetype differences were influenced by body mass differences among individuals or between mice from selected and control lines.

When testing for the effect of selection in the locked-wheel group, we ran PROC MIXED models with and without a dummy variable for the behavior of the animal as observed immediately prior to capture (climbing or not climbing). For the locked-wheel data, this was the only way we had to account for the immediate effect of climbing activity on body temperature. To determine whether selected animals might have climbed more than controls in locked wheels, for each animal we calculated the odds that it was climbing when captured (proportion of times it was climbing divided by 1 minus the proportion). PROC GLM (with line nested

Table 1

Repeatabilities (intraclass correlation coefficients,  $t$ ) of body temperature. Correlations are reported for residuals from ANCOVA models run separately for each measurement with and without body mass as a covariate<sup>a</sup>

		With body mass			Without body mass	
		$t$	$P$	Repeated measures	$t$	$P$
Females	Day	0.251	0.067	2	0.269	0.053
	Night-locked w/climb	0.367	< 0.001	6	0.389	< 0.001
	Night-locked w/o climb	0.476	< 0.001	6	0.479	< 0.001
	Night-free w/revs	0.301	< 0.001	6	0.297	< 0.001
	Night-free w/o revs	0.374	< 0.001	6	0.368	< 0.001
Males	Day	0.128	0.200	2	0.114	0.227
	Night-locked w/climb	0.401	< 0.001	6	0.406	< 0.001
	Night-locked w/o climb	0.568	< 0.001	6	0.559	< 0.001
	Night-free w/revs	0.165	< 0.001	6	0.161	< 0.001
	Night-free w/o revs	0.296	< 0.001	6	0.306	< 0.001

<sup>a</sup> The following covariates were always included: delay time, time of day and  $z$ -transformed time of day squared.

within linetype entered as a random effect) was used to compare the natural log of the climbing odds (equivalent to logit transformation of a proportion) between selected and control animals (Sokal and Rohlf, 1995).

When testing for the effect of selection in the free-wheel group, we ran PROC MIXED models both with and without the covariates: square root of number of wheel revolutions 10 min prior to measurement, and this variable *z*-transformed and squared. The actual min during which capture occurred was excluded. We chose 10 min after conducting preliminary analyses using wheel revolutions 2, 5, 10, 15, 20, 25, and 30 min prior to measurement. Of these, 10 min yielded the highest *R*-squared value (one example is shown in Fig. 2). In analyses without the wheel-running covariates, residuals were highly negatively skewed, so temperature data were rank transformed. We conducted analyses with and without the wheel running covariates to determine if differences in body temperature between selected and control animals were independent of the immediate positive association between activity and body temperature.

### 3. Results

#### 3.1. Repeatability

During the day, body temperature was not significantly repeatable in males and was only marginally repeatable in females (Table 1). At night, body temperature was significantly repeatable in both males and females. Including body mass in the models had little effect on the estimates of repeatability (Table 1). However, the estimates of repeatability were lower after adjusting for variation in activity prior to measurement of body temperature (i.e., climbing for locked wheels, number of revolutions during previous 10 min for free wheels).

The consistency of individual differences in body temperature at night did not appear to depend on time scale (Table 2). Overall, the repeatabilities and correlations tended to be higher in females than in males (Tables 1 and 2).

#### 3.2. Effects of genetic selection for wheel running

Among males, no statistically significant differences in body temperature were measured between selected and control lines for any comparison (Table 3). At night, however, if effects of wheel running and body mass were ignored, then males from selected lines had body temperatures that averaged 0.29°C higher than those of controls ( $P = 0.063$ ).

Among females, no significant differences in body temperature were measured during the day, whether or

not body mass was entered as a covariate (Table 3, Fig. 3). At night, however, females from selected lines housed with access to locked wheels had significantly higher body temperatures than controls (Table 3, Fig. 4). Females from selected lines climbed more than controls ( $P = 0.027$ ) and the climbing dummy variable was always a significant predictor of body temperature when it was included in models. When the climbing variable was entered, the temperature difference between selected and control lines was reduced but remained statistically significant (Table 3); if body mass was also included, then the difference was eliminated.

For mice housed with access to freely rotating wheels, females from selected lines had significantly higher rank-transformed body temperatures at night than did controls, both with and without body mass entered as a covariate (Table 3). However, when the square root of the number of wheel revolutions 10 min prior to measurement and the square of this variable *z*-transformed were included as covariates, differences were no longer significant (Table 3). Both wheel-running covariates were highly significant predictors of body temperature (Table 4), and body temperature showed an asymptotic relationship to wheel running (Fig. 5).

Replicate line was never statistically significant in any of the PROC MIXED repeated-measures analyses (e.g., Table 4). Delay time was also never significant (e.g., Table 4). In contrast, day and individual effects were always highly significant predictors of nighttime body temperature (e.g., Table 4). Body mass was a marginally significant predictor of daytime temperatures in females (estimate of partial regression coefficient = +0.035°C/g,  $P = 0.089$ ) and for nighttime temperatures in males with access to locked wheels (estimate = +0.004°C/g,  $P = 0.078$ ). Within the day or night measurement sets, time of day was a statistically significant predictor of body temperature in some analyses.

### 4. Discussion

We studied body temperatures of house mice from eight lines, four of which had been artificially selected for high voluntary wheel running and four bred randomly as controls (Swallow et al., 1998a). Repeatability of individual differences in body temperature was higher at night than during the day (Table 1). The heritability estimates of body temperature reported by Connolly and Lynch (1981) are consistent with this pattern: higher at night than during the day (note, however, that they did not correct for possible effects of differences in activity levels). If body temperature is heritable, then it has the potential to respond in a cor-

Table 2

Matrix of repeatabilities (Pearson's  $r$ ) of body temperature in mice measured at night with access to free wheels. Correlations are reported for residuals from ANCOVA models run separately for each measurement with (above diagonal) and without (below diagonal) body mass entered as a covariate<sup>a</sup>

Females ( $N = 54$ )						Males ( $N = 53$ )						
20-April	21-April	27-April	28-April	04-May	05-May	20-April	21-April	27-April	28-April	04-May	05-May	
20-April	0.485	0.478	0.073	0.478	0.283	20-April	0.042	0.007	0.098	-0.076	0.144	
21-April	0.485		0.500	0.216	0.429	0.005	21-April	0.156	0.007	0.307	0.294	0.079
27-April	0.465	0.497		0.190	0.403	0.088	27-April	0.000	0.011	0.131	0.138	0.124
28-April	0.076	0.218	0.176		0.308	0.111	28-April	0.077	0.257	0.115	0.350	0.156
04-May	0.462	0.413	0.385	0.287		0.408	04-May	-0.083	0.271	0.133	0.344	0.463
05-May	0.275	0.002	0.136	0.093	0.447		05-May	0.139	0.080	0.154	0.134	0.453

<sup>a</sup> The following covariates were included: cumulative number of wheel revolutions 10 min prior to measurement and this variable  $z$ -transformed and squared, delay time, time of day and  $z$ -transformed time of day squared.

related fashion to selection on wheel running. From this information, and from the fact that most wheel running occurs at night (Sherwin, 1998; Koteja et al., 1999a), we would predict that nighttime rather than daytime body temperatures might evolve in concert with wheel running. Consistent with this prediction,

body temperatures differed significantly between selected and control animals only at night. However, the difference can be accounted for statistically by the immediate positive association between activity (amount of wheel running in previous 10 min) and body temperature. In other words, the selected-control

Table 3

Least-square adjusted means of body temperatures ( $^{\circ}\text{C}$ ) from repeated-measures ANCOVA models. For night measurements, means are reported with and without adjusting for the activity of the animals prior to capture<sup>a</sup>

	Repeated measures	Body mass	Selected			Control			Difference		
			Mean	SE	$N$	Mean	SE	$N$	Sel-Con	$P$	
Females	Day	2	Without	34.91	0.075	24	35.07	0.085	18	-0.16	0.204
			With	34.93	0.087		35.04	0.096		-0.11	0.424
	Night-locked w/climb <sup>b</sup>	6	Without	37.53	0.061	16	37.23	0.064	14	0.29	0.016
			With	37.47	0.065		37.29	0.070		0.18	0.131
	Night-locked w/o climb	6	Without	37.53	0.071	16	37.17	0.075	14	0.36	0.012
			With	37.49	0.077		37.22	0.083		0.27	0.065
	Night-free w/revs <sup>c</sup>	6	Without	37.91	0.048	32	37.80	0.048	32	0.11	0.169
			With	37.90	0.050		37.81	0.051		0.10	0.238
Night-free w/o revs	6	Without	37.98	0.058	32	37.69	0.059	32	0.29	<sup>d</sup> 0.009	
		With	37.96	0.060		37.71	0.061		0.25	<sup>d</sup> 0.016	
Males	Day	2	Without	34.94	0.057	21	34.91	0.056	23	0.03	0.695
			With	34.95	0.058		34.89	0.056		0.06	0.497
	Night-locked w/climb	6	Without	36.67	0.146	14	36.56	0.141	16	0.11	0.599
			With	36.57	0.163		36.65	0.155		-0.08	0.755
	Night-locked w/o climb	6	Without	36.65	0.153	14	36.50	0.145	16	0.15	0.507
			With	36.55	0.170		36.58	0.159		-0.03	0.907
	Night-free w/revs	6	Without	36.98	0.042	31	37.01	0.041	31	-0.03	0.636
			With	36.96	0.043		37.02	0.042		-0.06	0.381
Night-free w/o revs	6	Without	37.15	0.082	31	36.86	0.081	31	0.29	<sup>d</sup> 0.063	
		With	37.08	0.083		36.91	0.081		0.17	<sup>d</sup> 0.266	

<sup>a</sup> The following covariates were always included: delay time, time of day, and  $z$ -transformed time of day squared.

<sup>b</sup> Climb = a dummy variable for whether or not the animals were climbing when captured.

<sup>c</sup> Revs = a covariate for cumulative wheel revolutions 10 min prior to measurement.

<sup>d</sup>  $P$ -value is for rank transformed body temperature.

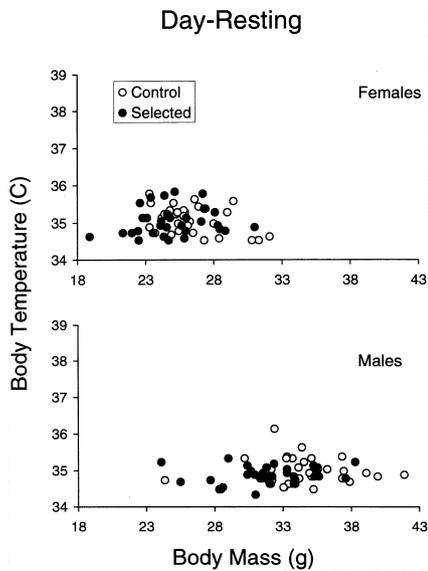


Fig. 3. Means (of both days) of daytime-resting body temperatures plotted against body mass for selected and control animals with access to free wheels. Animals that exhibited any wheel revolutions during the previous 10 min were excluded.

body temperature differences are most simply explained by underlying differences in locomotor behavior, and we see no reason to hypothesize the existence of differences in thermoregulatory physiology.

We included a locked-wheel treatment as a control for the immediate effects of activity on body temperature. Locking wheels, however, turned out not to be an effective activity control because mice from selected lines climbed significantly more in the locked wheels than did controls, even though they could not run by rotating the wheels. A recent behavioral study confirms this result (Koteja et al., 1999a). Inclusion of a dummy variable for whether or not the animals were climbing when they were captured for measurement reduced the difference in body temperature between selected and control animals in locked wheels, especially for females (Table 3). The climbing dummy variable is not as accurate a measure of activity as wheel running. First, wheel running is a continuous variable and climbing is a categorical variable. Second, the climbing dummy variable does not account for the time lag between activity and its effect on body temperature (Bolles et al., 1968). Inclusion of a more accurate measure of activity in locked-wheel analyses might have further reduced the difference in body temperature between selected and control females. We believe that mice from selected lines probably had higher body temperatures than controls in locked wheels for the same reason they had higher body temperatures in free wheels: they

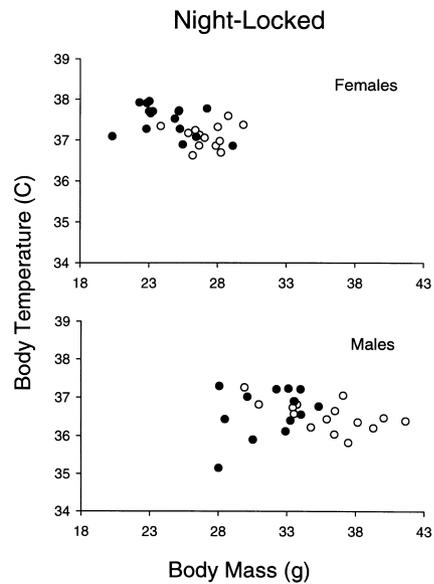


Fig. 4. Means (of all 6 days) of nighttime body temperatures plotted against body mass for selected and control animals with access to wheels that were locked to prevent rotation. Symbols as in Fig. 3.

were more active just before the measurement, and activity and body temperature are positively associated.

In rats and hamsters, regular bouts of voluntary wheel-running at night were associated with higher body temperatures during the day when the animals were inactive (Conn et al., 1990; Rowsey et al., 1993). Conn et al. (1990) and Rowsey et al. (1993) compared daytime body temperatures of animals with and without access to running wheels. Unfortunately, we could not make the analogous comparison because locked and free wheel cages were not randomly distributed in the rooms (see Materials and methods). Furthermore, climbing in locked wheels might have confounded the interpretation. Our selected animals ran more than controls at night when they had access to free wheels, and the former had higher body temperatures (e.g., Fig. 5), but during the day when mice were inactive, body temperatures of selected and control animals were similar (Fig. 3). Therefore, relative amounts of voluntary wheel running at night did not appear to affect daytime body temperatures in our mice.

Estimates of the repeatability of body temperature were lower when measurements were adjusted for activity (i.e., when the climbing dummy variable was included for mice with locked wheels and when the wheel running covariates were included for mice with free wheels). This result probably reflects the fact that intensity of locomotor activity is itself a repeatable behavior. We know this to be true for wheel running (Friedman et al., 1992; Swallow, 1998; Swallow et al.,

Table 4  
Repeated-measures ANCOVA table for 6 measurements of body temperature in females at night with access to free wheels

Effect	Estimate	Numerator DF	Denominator DF	F	P
Individual				a	< 0.001
Line				a	0.395
Linetype		1	6	1.72	0.238
Day		5	275	79.95	< 0.001
Square root revolutions	0.0449	1	275	89.24	< 0.001
Z-transformed square root revs squared	-0.1291	1	275	109.94	< 0.001
Delay time (s)	0.0010	1	275	0.62	0.432
Body mass (g)	-0.0057	1	275	0.32	0.571
Time of day (min)	-0.0003	1	275	0.41	0.525
Z-transformed time of day squared	-0.0431	1	275	3.00	0.084

<sup>a</sup> The likelihood ratio test was used to generate these *P*-values. SAS Proc Mixed does not report *F*-statistics for random effects.

1998a). Nevertheless, activity-adjusted estimates of repeatability are still significant (Table 1).

Although we found that motor activity was a positive predictor of body temperature, which is consistent with many previous studies (Gordon, 1993; Gordon and Yang, 1997; Kent et al., 1991; Refinetti, 1994), this result does not necessarily imply that wheel running caused an increase in body temperature (Gordon and Yang, 1997). For example, body temperature may have been elevated in anticipation of motor activity. Furthermore, mice may dissipate the heat generated by motor activity, maintaining high body temperatures through non-shivering thermogenesis (e.g., on rats, see

Gordon and Yang, 1997). As reviewed by Mäkinen et al. (1996), various studies of small mammals have reported partial, total, and no substitution of thermoregulatory heat by exercise-generated heat.

Depending on the degree of substitution of exercise heat for thermoregulatory heat and on the extent of  $Q_{10}$  effects, whole-body metabolism of active mice may increase over and above the increase caused simply by metabolism in skeletal muscles and other tissues (e.g., the heart) that are directly involved in support of locomotion. All else being equal, higher metabolism would increase food requirements. In generation 10 of our selection experiment, the selected mice consumed approximately 4% more food than controls (after adjusting for differences in body mass; Koteja et al., 1999b). Although this difference was presumed to reflect the difference in amount of wheel running, it might also have been affected by the difference in body temperature.

Elevated body temperatures during activity may also have functional significance with respect to locomotor abilities. Mice from our selected lines achieve a higher number of total revolutions per day on running wheels primarily because they run at higher speeds, rather than running more min per day (Fig. 1; Koteja et al., 1999a,b; Swallow et al., 1998a,b; 1999). In addition, the maximum number of revolutions run in any one-min interval is significantly higher for selected compared to control animals (unpublished data). Elevated body temperatures should facilitate the rapid rates of muscle contraction required for attaining high running speeds (Bennett, 1984, 1990). Thus, increases in body temperature may facilitate increases in wheel running. However, we have no evidence that that selected animals ran more total revolutions than controls because they had higher body temperatures. The data presented here allow us only to conclude that mice from selected lines run faster (hence more total revolutions) than controls, that wheel running and body temperature are

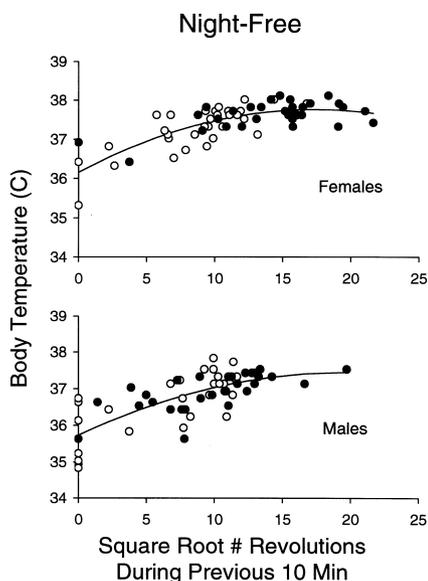


Fig. 5. Nighttime (April 21) body temperatures of mice with access to freely rotating wheels plotted against square root number of wheel revolutions during the 10 min prior to measurement. Symbols as in Fig. 3.

positively related, and that mice from selected lines experience higher body temperatures at night (ignoring activity as a covariate). Whether the increased body temperature is one of the factors that facilitates high running speeds in the mice from selected lines remains an interesting question for further investigation.

Various other effects of elevated body temperatures are also possible. For example, increased body temperature and/or wheel running (exercise) itself might affect patterns of sleep (O'Connor et al., 1998), although Koteja et al. (1999a) found no statistically significant difference in the amount of time spent sleeping in a focal-animal study of our mice sampled at generation 13. Also of interest is the possibility that temperature and/or exercise might affect the expression of heat-shock proteins (HSPs: Feder and Hofmann, 1999; Locke, 1997). Temperature and exercise can have both acute and chronic effects on HSPs in other mammals (e.g., Taylor et al., 1999), and we have previously demonstrated that eight weeks of access to running wheels increases whole-animal maximal oxygen consumption (Swallow et al., 1998b) as well as isotonic endurance and oxidative capacity (succinate dehydrogenase activity) of the medial gastrocnemius muscle (Zhan et al., 1999) in both our selected and control lines sampled at generation 10. The ways in which activity levels, body temperature, and the stress-protein response coadapt is a promising area for future work (see also Feder and Hofmann, 1999).

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