

Effects of Size, Sex, and Voluntary Running Speeds on Costs of Locomotion in Lines of Laboratory Mice Selectively Bred for High Wheel-Running Activity

Enrico L. Rezende*
 Scott A. Kelly
 Fernando R. Gomes
 Mark A. Chappell
 Theodore Garland Jr.

Department of Biology, University of California, Riverside,
 California 92521

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ABSTRACT

Selective breeding for over 35 generations has led to four replicate (S) lines of laboratory house mice (*Mus domesticus*) that run voluntarily on wheels about 170% more than four randomly bred control (C) lines. We tested whether S lines have evolved higher running performance by increasing running economy (i.e., decreasing energy spent per unit of distance) as a correlated response to selection, using a recently developed method that allows for nearly continuous measurements of oxygen consumption ($\dot{V}O_2$) and running speed in freely behaving animals. We estimated slope (incremental cost of transport [COT]) and intercept for regressions of power (the dependent variable, $\dot{V}O_2/\text{min}$) on speed for 49 males and 47 females, as well as their maximum $\dot{V}O_2$ and speeds during wheel running, under conditions mimicking those that these lines face during the selection protocol. For comparison, we also measured COT and maximum aerobic capacity ($\dot{V}O_{2\text{max}}$) during forced exercise on a motorized treadmill. As in previous studies, the increased wheel running of S lines was mainly attributable to increased average speed, with males also showing a tendency for increased time spent running. On a whole-animal basis, combined analysis of males and females indicated that COT during voluntary wheel running was significantly lower in the S lines (one-tailed $P = 0.015$). However, mice from S lines are significantly smaller and attain higher maximum speeds on the wheels; with either body mass or maximum speed (or both) entered as a covariate,

the statistical significance of the difference in COT is lost (one-tailed $P \geq 0.2$). Thus, both body size and behavior are key components of the reduction in COT. Several statistically significant sex differences were observed, including lower COT and higher resting metabolic rate in females. In addition, maximum voluntary running speeds were negatively correlated with COT in females but not in males. Moreover, males (but not females) from the S lines exhibited significantly higher treadmill $\dot{V}O_{2\text{max}}$ as compared to those from C lines. The sex-specific responses to selection may in part be consequences of sex differences in body mass and running style. Our results highlight how differences in size and running speed can account for lower COT in S lines and suggest that lower COT may have coadapted in response to selection for higher running distances in these lines.

Introduction

Locomotion is an important aspect of behavior from a variety of perspectives. Maximal locomotor abilities may be important for escape from predators, for prey capture, and even during some aspects of courtship or mating behavior (review in Irschick and Garland 2001; Miles 2004; Perry et al. 2004). Energetic costs of locomotion can be major components of total daily energy budgets in some mammals (Garland 1983; Gorman et al. 1998; Corp et al. 1999; Girard 2001) and especially in some lizards (Christian et al. 1997; Drent et al. 1999). More generally, Dickinson et al. (2000, p. 100) have claimed that "locomotion...is the behavior that most dictates the morphology and physiology of animals. Evolutionary pressures for efficient, rapid, adjustable, or just plain reliable movement often push the envelope of organism design."

In the wild, locomotor behavior varies tremendously among species, especially if one makes extreme comparisons, such as between sessile organisms (e.g., various marine invertebrates) and migratory birds. Even within a smaller phylogenetic grouping, however, quantitative variation can be orders of magnitude. Among species of "lizards" (nonsnake squamates), for example, home range area varies by more than four orders of magnitude at a given body size (Perry and Garland 2002). Among mammals, home range areas vary by up to three orders of magnitude for a given body size (Harestad and Bunnell 1979; Kelt and Van Vuren 2001; Ferguson and Lariviere 2002) and even 130-

* Corresponding author. Present address: Integrative Ecology Group, Estacion Biologica Donana, CSIC, Apartado 1056, E-41080 Seville, Spain; e-mail: enrico.rezende@ebd.csic.es.

fold within a single rodent species (*Apodemus sylvaticus*; Corp et al. 1999). Daily movement distances are also quite variable in mammals, with interspecific values covering almost two orders of magnitude for a given body size (Garland 1983; Goszczynski 1986; Carbone et al. 2005) and one order of magnitude within a single species (e.g., Corp et al. 1999; Girard 2001).

Locomotor performance (e.g., running speed or endurance) also differs considerably within and among species, and several morphological specializations have been associated with “cursorial” locomotion. For example, cursorial animals generally have relatively long and light limb elements, which allow for increased stride length with reduced energetic cost of cycling the limbs (e.g., Myers and Steudel 1985; Steudel 1990), thus being advantageous for increased running speeds and/or endurance (with respect to human evolution, for instance, Bramble and Lieberman [2004] have argued that selection for endurance-running ability played an important role). Although the relation between locomotor performance and fitness is not necessarily straightforward (e.g., Miles 2004), one would intuitively expect that animals with higher daily movement distances and home range areas should evolve lower costs of locomotion (i.e., by increasing locomotor efficiency). More efficient runners would spend less energy to travel a given distance or could travel faster for the same energy cost (Alexander 1989, p. 1201).

However, comparative studies of the ways in which locomotor behavior (including motivation), whole-animal performance capacities, life-history traits, and subordinate morphological and physiological traits evolve in a correlated fashion are in their infancy (e.g., Garland 1999; Ferguson and Lariviere 2002). As an alternative to comparative studies, experimental evolution can be used to approach such questions about the evolution of complex phenotypes (e.g., Koch and Britton 2005; Rhodes et al. 2005; Swallow and Garland 2005). Selective-breeding experiments are currently employed to study evolution in action; researchers can study changes in the phenotypic and genotypic architecture across generations under more controlled and reproducible environments (and selective regimes) and address how traits at different levels of organization can evolve in a coherent fashion (e.g., Garland and Carter 1994; Bennett 2003; Garland 2003 and references therein).

We are studying the correlated evolution of behavior and physiology in four replicate lines of laboratory house mice that have been selectively bred for increased voluntary wheel-running activity, measured as total running distance during the last 2 d of a 6-d trial (Swallow et al. 1998a; Garland 2003; see “Material and Methods”). After 16 generations, mice from the selected lines (S) ran about 170% more revolutions per day than their control-line (C) counterparts, and this differential has persisted through generation 41 (Rhodes et al. 2000, 2003; Girard et al. 2001; Koteja and Garland 2001; T. Garland, unpublished data). This difference in wheel running between the S and C lines is similar to the range of variation that was found

among 13 species of wild rodents (see Fig. 4 in Garland 2003), suggesting that it is reasonable to expect correlated evolutionary changes in other traits.

For several reasons, we hypothesized that running economy would increase in the S lines. First, on simple intuitive grounds, one might expect animals that routinely cover long distances to be efficient runners (e.g., John-Alder et al. 1986; Carrano 1999), although this is not necessarily the case for mammals (Taylor et al. 1982; Bramble and Lieberman 2004). Second, it has turned out that running distance of the S lines has increased mainly by increased speed rather than amount of time spent running (Swallow et al. 1998a; Garland 2003). Running faster is generally expected to be a more economical way to increase total distance covered (Taylor et al. 1982; Koteja et al. 1999), but it also means that S mice might run at speeds that tax their physiological capacities, possibly approaching or even exceeding their maximal aerobic (sustainable) speeds (see top panel of Fig. 1). One way to reduce this possibility would be to lower the incremental cost of transport (COT; Fig. 1), that is, the cost to move a unit of distance (typically expressed on a per-unit-body-mass basis). Third, S lines run more intermittently on wheels (Girard et al. 2001; movie at <http://www.biology.ucr.edu/people/faculty/Garland/Girard01.mov>), and intermittent locomotion has sometimes been suggested to increase locomotor efficiency. Fourth, S mice performed fewer turns in open-field behavioral trials (Bronikowski et al. 2001); if they also perform fewer reversals while wheel running, then this could lead to a lower cost per revolution. Fifth, reduced hindlimb muscle mass, especially in two of the S lines (Houle-Leroy et al. 2000, 2003; Garland et al. 2002), might reduce the energetic cost of cycling the limbs (Kent and Miller 1997; Carrano 1999; Liem et al. 2001; Bramble and Lieberman 2004). Finally, mice from the S lines exhibit reduced directional and fluctuating asymmetry in their hindlimb bone lengths (Garland and Freeman 2005).

The main goal of this study was to test whether the evolution of high wheel running activity has been accompanied by a correlated increase in running efficiency (decrease in COT). Effects of body size and sex were also analyzed because (i) COT is known to vary allometrically (nonlinearly) in relation to body size (Taylor et al. 1982; Kram and Taylor 1990); (ii) the sexes differ considerably in body mass; (iii) running behavior has evolved differently in males and females (S-line males have also evolved an increase in amount of time running per day; Koteja and Garland 2001; T. Garland, unpublished data; see “Discussion”); and (iv) selection for high wheel running activity has resulted in smaller body size at maturity in both sexes (Swallow et al. 1999). We also compared estimates of COT during voluntary wheel running with values obtained during forced exercise on a motorized treadmill, which has been the main protocol employed to estimate COT (e.g., Hoyt and Taylor 1981; Taylor et al. 1982; Hoyt and Kenagy 1988; Kram and Taylor 1990).

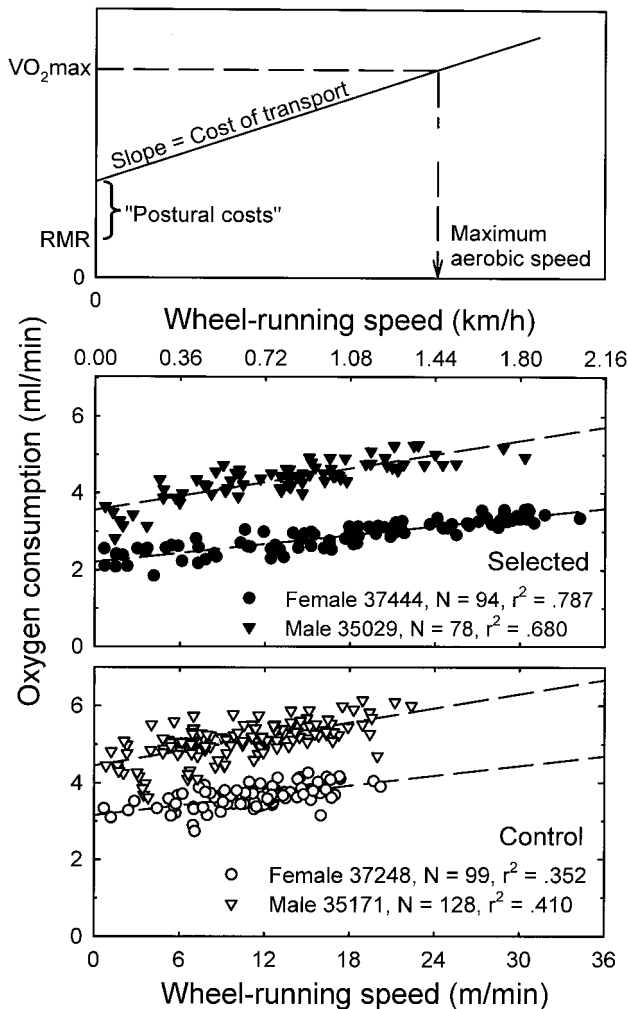


Figure 1. *Top*, Diagram summarizing how incremental cost of transport was calculated for individual mice by regressing $\dot{V}O_2$ against running speed based on 1-min averages obtained with stepped sampling (see text). Resting metabolic rate (RMR) was estimated as the lowest metabolic rate recorded during any consecutive 5-min interval over the 24-h period. Postural costs were estimated as the difference between RMR and the y -intercept of the $\dot{V}O_2$ on speed least squares linear regression. Maximum $\dot{V}O_2$ during any 1-min interval was also recorded for the wheel trials and during separate forced-exercise trials on a motorized treadmill ($\dot{V}O_{2,max}$). $\dot{V}O_{2,max}$ and the COT regression are used to compute maximum aerobic speed. *Middle*, Representative male and female from selected lines (42.8 and 23.0 g, respectively). Dashed lines represent the following linear regression: $\dot{V}O_2 = (0.060 \pm 0.005) \times \text{speed} + (3.55 \pm 0.07)$ for the male and $(0.039 \pm 0.002) \times \text{speed} + (2.20 \pm 0.04)$ for the female (mean \pm SE). *Bottom*, Representative male and female from control lines (45.0 and 31.1 g, respectively); results from linear regression were $\dot{V}O_2 = (0.062 \pm 0.007) \times \text{speed} + (4.45 \pm 0.07)$ for the male and $(0.043 \pm 0.005) \times \text{speed} + (3.17 \pm 0.06)$ for the female.

Material and Methods

Animals

Wheel measurements were performed on males and females from generations 32 and 34 postselection, respectively, from an ongoing artificial-selection experiment for high wheel-running activity (Swallow et al. 1998a; Garland 2003). The original progenitors were outbred, genetically variable laboratory house mice (*Mus domesticus*) of the Hsd:ICR strain. After two generations of random mating, mice were randomly paired and assigned to eight closed lines with 10 families per line. In each subsequent generation, when the offspring of these pairs were 6–8 wk old, they were housed individually with access to running wheels (circumference = 1.12 m) for 6 d. Daily wheel-running activity was monitored with a computer-automated system.

In the four S lines, the highest-running males and females from each family (highest number of revolutions during days 5 and 6) were chosen as breeders to propagate the lines to the next generation. In C lines, breeders were chosen at random from each family. Breeders within all lines were randomly paired, except that sibling matings were not allowed. Measurements on wheels and selective breeding were interrupted for four generations (31 to 34), and breeders were picked at random as in C lines.

Aerobic metabolism ($\dot{V}O_2$) during activity was initially measured for 49 males during day 6 of wheel running, mimicking conditions faced by these animals during selection. Although $\dot{V}O_2$ and activity measurements were identical for males and females, the experimental protocol differed slightly for logistical reasons. Males were retired breeders that averaged 18 wk of age (i.e., older than the average age of selection) and 38 g in body mass (Table 1). After being weaned at 21 d of age, they were kept four to a cage with water and food ad lib. (Harlan Teklad Laboratory Rodent Diet 8604). After breeding, males were maintained in individual cages until they were given access to "acclimatory" running wheels (same as in routine wheel testing for the selection protocol) for 5 d and were then placed in wheels enclosed in metabolic chambers (see below) at the beginning of day 6 (at approximately 11:30 a.m.).

Females were measured at a younger age (10 wk) than males and were smaller on average (Table 1). After weaning, 47 females (six individuals per line, each from a different family; one S female was not included in the analyses because of measurement problems) were randomly assigned and housed four to a cage (two S and two C mice in each) and maintained with water and food ad lib. Mice were left 4 d in acclimatory wheels and 2 d in the metabolic chambers, allowing for a 24-h period without disturbance (i.e., displacement from one wheel to the other) before the start of metabolic recording, which was not the case for males. In addition, maximum aerobic capacity ($\dot{V}O_{2,max}$) during forced exercise on a motorized treadmill was measured twice (see below). In females, measurements were

Table 1: Sample size, body mass, and age for laboratory mice measured during voluntary exercise on wheels, and fitness for linear and quadratic regressions employed to estimate cost of transport (COT)

	Males		Females	
	Selected	Control	Selected	Control
Number of individuals	26	23	23	24
Body mass (g) ^{*,**}	34.5 ± .6 (30.2–42.8)	40.8 ± .7 (35.0–46.5)	23.5 ± .5 (19.4–27.2)	27.1 ± .6 (22.4–34.1)
Age (d) [*]	120.5 ± 2.5 (97–146)	129.2 ± 3.1 (103–147)	72.0 ± 3.0 (47–94)	71.5 ± 2.8 (48–95)
Total samples [*]	92.6 ± 5.8 (45–150)	69.7 ± 6.7 (24–138)	97.4 ± 4.7 (44–146)	98.5 ± 5.0 (50–141)
Outliers	.73 ± .18 (0–3)	.78 ± .31 (0–6)	1.00 ± .44 (0–9)	1.25 ± .33 (0–6)
Samples used [*]	91.9 ± 5.8 (45–150)	68.9 ± 6.5 (24–137)	96.4 ± 4.8 (44–146)	97.2 ± 5.0 (50–141)
r^2 (linear) ^{*,**}	.686 ± .029 (.30–.87)	.545 ± .032 (.22–.80)	.602 ± .036 (.19–.82)	.398 ± .033 (.14–.79)
Adjusted r^2 (linear) ^{*,**}	.681 ± .029 (.29–.87)	.536 ± .033 (.21–.80)	.597 ± .036 (.18–.82)	.390 ± .033 (.13–.79)
r^2 (quadratic) ^{*,**}	.701 ± .028 (.31–.87)	.556 ± .032 (.22–.80)	.614 ± .036 (.19–.85)	.413 ± .033 (.14–.79)
Adjusted r^2 (quadratic) ^{*,**}	.693 ± .029 (.29–.86)	.537 ± .035 (.18–.79)	.606 ± .036 (.18–.84)	.399 ± .034 (.12–.79)

Note. Descriptive statistics on stepped samples employed in linear regressions are also summarized: total number of samples after filtering values below 0.5 rpm (~0.57 m/min); number of outliers outside 3 SD from overall regression, removed after visual inspection; number of samples used to estimate COT; and mean fit of individual linear and quadratic regressions (r^2 and adjusted r^2). Values represent day 6 of wheel access (day 1 in chamber for males, day 2 for females; see “Material and Methods”). Values are simple means ± SE calculated separately for each group, with range of values shown in parentheses. Effects of linetype and sex were tested by two-way nested ANOVA in SAS PROC MIXED without sex × linetype interaction ($P > 0.05$ in all cases) or any covariates.

* $P_{\text{sex}} < 0.01$.

** $P_{\text{selection}} < 0.01$.

taken immediately after wheel trials; for males, there was a variable window (4–33 d) between measurements on wheels and treadmill (they were measured on a daily basis on wheels and in batches of about 12 individuals on treadmill). All animal procedures were in compliance with the University of California, Riverside, Institutional Animal Care and Use Committee (IACUC 0212042) and U.S. laws.

Metabolism and Running Speed during Forced and Voluntary Exercise

We estimated COT in S and C lines during voluntary activity employing the system used for deer mice (*Peromyscus maniculatus*) by Chappell et al. (2004). In short, we enclosed running wheels identical to those employed during the selective-breeding protocol in a Plexiglas metabolic chamber connected to computer-interfaced gas analyzers (photos at http://www.biology.ucr.edu/people/faculty/Garland/Wheel_Metab_Alone_1.jpg and http://www.biology.ucr.edu/people/faculty/Garland/Wheel_Metab_Two_2.jpg). The metabolic chamber surrounded the wheel connected to an individual cage, where animals had water and food ad lib. and free access to the wheel (Fig. 1 in Chappell et al. 2004).

We recorded $\dot{V}O_2$ with open-system respirometry, as well as running speeds and ambient temperature, every 1.5 s for 23.5 h (from 12:00 noon to 11:30 a.m.) with a Macintosh computer equipped with National Instruments A-D converters (Austin, TX) and LabHelper software (Warthog, <http://www.warthog.ucr.edu>). Airflow was maintained at 2,500 mL/min \pm 1%, with mass flow controllers (Applied Materials, Sunnyvale, CA; Tylan, Billerica, MA; Porter Instruments, Hatfield, PA), and reference readings (lasting 2.5 min each) were obtained every 45 min with an automated system. By monitoring the time needed for wheel speed to decay from \sim 80 rpm to zero, we estimated rotational resistance before every measurement in females but only every few days in males. Animals were measured on a photoperiod of 12L : 12D (from 7:00 a.m. to 7:00 p.m.).

Maximum aerobic capacity during forced exercise ($\dot{V}O_{2\max}$) was estimated twice on consecutive days by running mice in an enclosed motorized treadmill as described previously (Rezende et al. 2005). Mice ran at increasing speeds, starting at 0.15–0.20 m/s and raised in step increments of about 0.1 m/s every 45 s until they could no longer maintain position on the treadmill and $\dot{V}O_2$ no longer increased. Trials lasted from 6 to 17 min, and reference readings of incurrent gas were obtained at the start and end of each trial. All treadmill measurements were performed with a 25° inclination, which yields the highest values of $\dot{V}O_{2\max}$ in mice, according to Kemi et al. (2002). For 11 males, $\dot{V}O_{2\max}$ was not obtained because of technical difficulties. Trial quality was also assessed using a subjective scale (five categories from “poor” to “excellent”; Swallow et al. 1998b), and two males were excluded because their trial quality was “poor” (i.e., uncooperative, did not run) in both trials.

Data for treadmill $\dot{V}O_{2\max}$ for the females have been reported previously (Rezende et al. 2005) and are presented again here to facilitate comparisons and because they are used to calculate maximal aerobic speeds.

Smoothing, baseline, and lag corrections for both wheel and treadmill trials were performed using the LabAnalyst software. We employed the “instantaneous correction” of $\dot{V}O_2$ (Bartholomew et al. 1981) to provide accurate resolution of short-term metabolic changes during activity. Throughout the 24-h record on the wheel, mean $\dot{V}O_2$ and rotational speeds (i.e., running speeds) during 1-min periods separated by intervals of 3 min were saved in ASCII files containing case range and time from when mean values were calculated. With these sampling periods, we were able to obtain $\dot{V}O_2$ values statistically independent of each other (i.e., no autocorrelation), as detailed in Chappell et al. (2004). To maximize the range of speeds for regressions, sampling started from the highest running speed (1-min average) and its respective $\dot{V}O_2$. Similarly, $\dot{V}O_{2\max}$ on the treadmill was calculated as the highest mean for a 1-min period (see also Swallow et al. 1998b; Rezende et al. 2005). During wheel trials, we also estimated resting metabolic rate (RMR) as the lowest metabolic rate recorded in a 5-min interval throughout the 24-h period. The first hour of recording for males and females during day 1 was discarded because O_2 in the chamber did not attain a steady-state condition.

Locomotion Costs on the Treadmill

To determine whether both methods yield similar results, we estimated COT on the motorized treadmill using females from generation 38. Mice were not trained to run on the treadmill before the trials, and we used $\dot{V}O_2$ values only from those individuals that ran cooperatively enough to attain steady-state values of $\dot{V}O_2$ for more than 5 min at constant speeds of 12, 21, and 30 m/min. Because animals were neither trained nor preselected for cooperativity, only 22 of 64 females met the criteria for inclusion, and so not all of the eight lines were represented in the final data set ($N = 15$ S and seven C mice). These females had mass and age ranges (25.8 ± 0.8 and 28.9 ± 1.1 g for S and C lines, respectively; 88.4 ± 0.3 d of age [\pm SE]) similar to those of the females employed in the wheel trials (Table 1). The measurement protocol was similar to the one employed to estimate $\dot{V}O_{2\max}$, and $\dot{V}O_2$ for each speed was calculated as the lowest 1-min average (results were similar for 2- and 5-min averages, so we report results with 1-min means for consistency with the wheel data).

Costs of Locomotion on Wheels and Statistical Analyses

The relationship between metabolic rate and running speed is approximately linear over a broad range of speeds for most quadrupedal mammals, and COT can be calculated as the slope of the regression of power (the dependent variable) on speed

Table 2: Effects of selection history, sex, body mass, and maximum running speed

	$P_{\text{selection}}$	P_{lines}	P_{sex}	$P_{\text{sex} \times \text{linetype}}$	$P_{\text{body mass}}$	$P_{\text{max speed}}$
Slope (mL O ₂ /m), full model	.446	1	.044	.226	.122	.003
Slope (mL O ₂ /m) ^a	.394	1	.059	.488	.071	
Slope (mL O ₂ /m)	.976	1	<.0001	.125		.002
Slope (mL O ₂ /m) ^b	.030	1	<.0001	.280		
Intercept (mL O ₂ /min), full model	.039	.966	.009	.307	<.0001	.271
Intercept (mL O ₂ /min) ^a	.010	.551	.010	.388	<.0001	
Intercept (mL O ₂ /min)	.641	.569	.008	.772		.715
Intercept (mL O ₂ /min) ^b	.749	.900	.008	.722		
Maximum $\dot{V}O_2$ on wheel (mL O ₂ /min), full model	.034	.452	.414	.276	<.0001	<.001
Maximum $\dot{V}O_2$ on wheel (mL O ₂ /min) ^a	.001	1	.627	.701	<.0001	
Maximum $\dot{V}O_2$ on wheel (mL O ₂ /min)	.924	1	<.0001	.796		.009
Maximum speed on wheel (m/min) ^b	<.001	1	.229	.189		
$\dot{V}O_{2\text{max}}$ on treadmill (mL O ₂ /min) ^c	.011	.127	.727	.007	<.0001	
RMR (mL O ₂ /min)	.141	.867	.0017	.496	<.0001	
Total distance (m) ^b	.003	.046	.035	.921		
Time spent running (min) ^b	.254	<.0001	.057	.181		
MAS _{$\dot{V}O_{2\text{max}}$} (m/min) ^{b,c,d}	.150	.011	.024	.188		
MAS _{$\dot{V}O_{2\text{wheel}}$} (m/min) ^{b,d}	<.001	1	.029	.179		
Postural costs (mL O ₂ /min) ^e	.011	.570	.109	.460	<.0001	

Note. Comparisons between selected and control lines pooling sexes in the nested model including sex as a fixed factor (tested over sex \times line [linetype], $df = 1,6$), performed with SAS PROC MIXED. Age was not included in the model because it was highly correlated with mass when both sexes were pooled (males were considerably older and larger than females). Values shown are two-tailed P not corrected for multiple comparisons. Boldface indicates statistically significant effects ($P < 0.05$). $N = 96$ for all traits, except for treadmill trials where 13 males had missing data.

^a Maximum running speed not included in the model.

^b Mass and maximum running speed not included in the model.

^c Trial for which 13 males had missing data.

^d Calculated as $(\dot{V}O_2 - \text{intercept})/\text{slope}$.

^e Calculated as $\text{intercept} - \text{RMR}$.

relation (Fig. 1; Taylor et al. 1970, 1982; Hoyt and Taylor 1981). We plotted $\dot{V}O_2$ against speed to estimate COT during voluntary wheel running for each individual. The relationship between $\dot{V}O_2$ and speed was analyzed as follows. Data with absolute wheel speeds less than 0.5 rpm (5.7 m/min) averaged over the 1-min block were discarded to reduce potential problems of electrical noise and activity outside of running wheels. Then, two different steps were performed to remove outliers for each individual (mainly because of the high number of files to be analyzed; i.e., 94 files for females and 49 for males). We performed visual inspections of the original records (e.g., Fig. 2 in Chappell et al. 2004) followed by preliminary regressions of $\dot{V}O_2$ versus speed plots (Fig. 1). Those data points falling outside 3 SD from the regression were removed. If after visual inspection, however, it was unclear whether a data point could potentially affect the regression in spite of not falling outside the range of 3 SD, we employed the test described in Cook and Weisberg (1999) to determine whether the point should be removed. The method consists of computing a 0–1 dummy variable, assigning 1 to the data point that is a putative influ-

ential point, adding this variable to the model, computing the P value, and then multiplying the P value by the total number of data points in the data set. When this corrected P value is <0.05 , the point is removed.

Using least squares linear regressions, we estimated the slope (i.e., incremental COT) and intercept for each individual (Fig. 1). From the slopes and intercepts estimated for each individual, we calculated the hypothetical maximum aerobic speeds (MAS) that mice could attain if they ran on the wheels at their maximum $\dot{V}O_2$ (measured during forced and voluntary exercise; Fig. 1). For simplicity, we will refer to these parameters as MAS _{$\dot{V}O_{2\text{max}}$} and MAS _{$\dot{V}O_{2\text{wheel}}$} (Tables 2 and 3). So-called postural costs of locomotion were calculated as the difference between RMR and the zero-speed intercept of the speed versus cost regression (Taylor et al. 1970, 1982).

The power versus speed relationship may not be linear, however, if (1) mice occasionally run above their MAS _{$\dot{V}O_{2\text{max}}$} or (2) costs of locomotion on wheels are actually nonlinear in general. As a simple test for nonlinearity, we employed quadratic regressions and tested significance of the (negative) quadratic

Table 3: Values obtained for males and females at day 6 of wheel access

	Selected	Control	$P_{\text{selection}}$	P_{lines}	$P_{\text{body mass}}$	$P_{\text{max speed}}$
Males:						
Slope (mL O ₂ /m)	.0642 ± .0039	.0641 ± .0042	.982	1	.853	.264
Slope (mL O ₂ /m) ^a	.0612 ± .0029	.0675 ± .0030	.191	1		
Intercept (mL O ₂ /min)	3.118 ± .113	2.873 ± .120	.254	.428	<.0001	.009
Intercept (mL O ₂ /min) ^a	3.019 ± .088	2.976 ± .094	.756	1		
Maximum \dot{V}_{O_2} on wheel (mL O ₂ /min)	5.091 ± .144	4.870 ± .156	.420	.674	<.001	<.001
Maximum \dot{V}_{O_2} on wheel (mL O ₂ /min) ^b	5.333 ± .137	4.592 ± .149	.020	1	.008	
Maximum running speed (m/min) ^a	30.9 ± 1.1	22.2 ± 1.2	.002	.185		
$\dot{V}_{\text{O}_2\text{max}}$ treadmill (mL O ₂ /min)	7.144 ± .191 ^c	6.070 ± .208 ^c	.019	1	.165	
RMR (mL O ₂ /min)	.923 ± .042	.817 ± .045	.190	1	.002	
Total distance (m) ^a	7,512 ± 1,050	3,924 ± 1,056	.053	<.0001		
Time spent running (min) ^a	357.9 ± 52.1	252.6 ± 52.4	.205	<.0001		
MAS $\dot{V}_{\text{O}_2\text{max}}$ (m/min) ^{a,d}	63.3 ± 3.5 ^c	45.2 ± 3.6 ^c	.012	.649		
MAS $\dot{V}_{\text{O}_2\text{wheel}}$ (m/min) ^{a,d}	35.1 ± 1.1	28.8 ± 1.2	.009	1		
Postural costs (mL O ₂ /min) ^e	2.304 ± .070	1.924 ± .076	.019	1	.001	
Females:						
Slope (mL O ₂ /m)	.0422 ± .0022	.0360 ± .0022	.145	1	.302	<.001
Slope (mL O ₂ /m) ^a	.0366 ± .0024	.0412 ± .0023	.235	.437		
Intercept (mL O ₂ /min)	2.825 ± .092	2.497 ± .090	.072	.724	<.001	.918
Intercept (mL O ₂ /min) ^a	2.657 ± .074	2.656 ± .073	.993	1		
Maximum \dot{V}_{O_2} on wheel (mL O ₂ /min)	4.286 ± .092	3.749 ± .090	.013	1	<.0001	.010
Maximum \dot{V}_{O_2} on wheel (mL O ₂ /min) ^b	4.390 ± .090	3.649 ± .088	.002	1	.0004	
Maximum running speed (m/min) ^a	35.0 ± 1.6	21.9 ± 1.6	.001	.911		
$\dot{V}_{\text{O}_2\text{max}}$ on treadmill (mL O ₂ /min)	5.455 ± .173	5.095 ± .171	.219	.093	<.001	
RMR (mL O ₂ /min)	.945 ± .031	.870 ± .030	.172	.785	<.001	
Total distance (m) ^a	9,639 ± 631	5,832 ± 551	.005	1		
Time spent running (min) ^a	388.3 ± 26.4	384.7 ± 26.1	.927	.252		
MAS $\dot{V}_{\text{O}_2\text{max}}$ (m/min) ^{a,d}	72.7 ± 7.4	68.5 ± 7.3	.697	.048		
MAS $\dot{V}_{\text{O}_2\text{wheel}}$ (m/min) ^{a,d}	44.3 ± 2.3	30.0 ± 2.2	.004	1		
Postural costs (mL O ₂ /min) ^e	1.891 ± .081	1.619 ± .080	.071	.300	.006	

Note. $N = 26$ and 23 for S and C males and 23 and 24 for S and C females, respectively, with the exceptions noted. Values in table correspond to day 1 in the metabolic chamber for males and day 2 for females (see “Material and Methods”). Adjusted means were calculated from SAS PROC MIXED for a male of 37.4 ± 1.0 g, 125 d of age, and maximum running speed 26.9 m/min (1.6 km/h) and for a female of 25.3 ± 0.2 g, 72 d of age, and maximum speed 28.3 m/min (1.7 km/h), in models in which mass and speed were covariates. Adjusted SE are also shown, and boldface indicates statistically significant effects ($P < 0.05$). P values are for two-tailed tests not corrected for multiple comparisons. For males, raw (unadjusted) means (\pm SE) for treadmill body mass were 35.0 ± 0.7 and 41.3 ± 0.7 g for S and C males, whereas $\dot{V}_{\text{O}_2\text{max}}$ on the treadmill averaged 7.034 ± 0.164 and 6.261 ± 0.148 mL O₂/min (0.2015 ± 0.0052 and 0.1520 ± 0.0037 mL O₂/min g on a mass-specific basis), respectively. For females, raw mean body mass (\pm SE) was 22.8 ± 0.4 and 26.8 ± 0.6 g for S and C lines, and mean $\dot{V}_{\text{O}_2\text{max}}$ on the treadmill was 5.201 ± 0.115 and 5.341 ± 0.121 mL O₂/min (0.229 ± 0.006 and 0.200 ± 0.004 mL O₂/min g on a mass-specific basis), respectively.

^a Mass and maximum running speed not included in the model.

^b Maximum running speed not included in the model.

^c $N = 18$.

^d Calculated as $(\dot{V}_{\text{O}_2} - \text{intercept})/\text{slope}$.

^e Calculated as $\text{intercept} - \text{RMR}$.

component (MAS was not calculated employing quadratic regressions, however). Adjusted r^2 was calculated as a measure of goodness of fit to perform comparisons between linetypes (Table 1) and models (linear vs. quadratic; Zar 1999, p. 423; note that all comparisons of COT use the slopes from simple linear regressions, not quadratic regressions).

Comparisons between linetypes were performed with nested

ANCOVA separately for males and females, employing SPSS for Windows 11.5 and SAS PROC MIXED 8.02 (SAS Institute 1996). We employed a one-way nested ANCOVA with type III sums of squares to estimate linetype effects (S vs. C) within each sex. Linetype was the grouping variable, and replicate lines ($N = 8$ in total) were nested within linetype as a random factor. In addition, body mass and age were included as covariates in

some models. Likelihoods of the models estimated with and without replicate lines as a factor in the model were used to test the statistical significance of line effects (the difference in $-2 \ln$ likelihoods follows a χ^2 distribution with 1 df). When sexes were pooled, we employed a two-way nested ANCOVA with sex and linetype as grouping variables (sex effects and the sex \times linetype interaction were always tested over the sex \times line (linetype) interaction, with $df = 1,6$). In these models, age was not included as a covariate because the sexes differed considerably in age.

Mass-adjusted least squares means were calculated to estimate differences between S and C lines, and correlations among residuals from nested ANCOVAs were assessed with Pearson product-moment correlations. Repeatability between days for all traits was also estimated with Pearson correlations, employing raw values or residuals from the nested design, whereas differences between days 1 and 2 were assessed with paired t -tests. Statistical significance is reported with both $\alpha = 0.05$ and α corrected after sequential Bonferroni correction for multiple comparisons (Rice 1989). Unless indicated otherwise, results are shown as mean \pm SE. For simplicity, we report two-tailed P values, although in some cases we have directional predictions (e.g., S lines run faster than C), and so some effects are discussed according to one-tailed P values.

Results

Repeatability and Linear and Quadratic Regressions on Wheels

The stepped sampling routines (Chappell et al. 2004) provided on average 81.8 and 97.9 data points for males and females, respectively. Few individuals had more than two outliers removed (four males and nine females; Table 1), and in only three cases (one male and two females) did we remove more than six outliers because of problems observed within an entire range of measurement, as suggested by inspections of the raw data. Because results remained qualitatively identical when analyses were performed after removing these 13 individuals that had at least two outliers, here we report values obtained from analyses with all individuals included after outliers were removed.

In females, incremental COTs were significantly repeatable between days after accounting for linetype, body mass, and age ($N = 47$, $r = 0.256$, one-tailed $P = 0.041$). All remaining traits—intercept, maximum $\dot{V}O_2$ and speed attained on wheels, RMR, total running distance, and time spent running—were highly repeatable between days 1 and 2 when either raw values or residuals were correlated, controlling for linetype, line, mass, and age ($r > 0.471$, $P < 0.001$ in all cases).

In females, slope estimates from day 1 (0.043 ± 0.002 mL O_2 /m) were on average higher than those from day 2 (0.039 ± 0.001 mL O_2 /m; $t_{46} = 2.05$, $P = 0.046$), with the opposite being true for regression intercepts (2.48 ± 0.05 and 2.66 ± 0.05 mL O_2 /min for days 1 and 2, respectively; $t_{46} =$

-3.99 , $P < 0.001$). In addition, females spent significantly more time running during day 1 regardless of linetype (7.3 ± 0.3 and 6.4 ± 0.2 h for days 1 and 2; $t_{46} = 3.25$, $P = 0.002$). In contrast, there were no differences in maximum $\dot{V}O_2$, RMR, running speed, or total distance between days 1 and 2 ($P > 0.05$ in all cases).

The linear component of quadratic regressions of speed versus $\dot{V}O_2$ was always statistically significant ($P < 0.05$). The quadratic component was always negative, as would be expected, and it was statistically significant in 22 of 95 individuals (10 S and two C males, five S and five C females; mean coefficient = -0.066 ± 0.006). Adjusted r^2 values were slightly but significantly higher with the quadratic model. For all 95 mice pooled, adjusted $r^2 = 0.554 \pm 0.020$ and 0.562 ± 0.020 for linear and quadratic regressions, respectively (paired $t_{94} = -3.9$, two-tailed $P < 0.001$). For the 48 males, the respective values were 0.616 ± 0.024 and 0.622 ± 0.025 ($t_{47} = -2.2$, $P = 0.016$); for the 47 females, the respective values were 0.492 ± 0.029 and 0.500 ± 0.029 ($t_{46} = -3.5$, $P < 0.001$). Quadratic coefficients, nevertheless, were not repeatable between days in females ($r = 0.148$, one-tailed $P = 0.160$; repeatability was not estimated for males; see above).

Selection History, Mass, and Sex

As expected from previous studies, males were heavier than females, and C mice were heavier than S mice (Table 1). Also as expected, wheel-running distances were higher in S lines and in females as compared with males, and S lines reached substantially higher running speeds than C in both sexes (Tables 2, 3). Consistent with previous studies, female S and C mice spent similar amounts of time running (388 vs. 385 min/d, respectively; Table 3). Males from S lines spent 42% more time running as compared with C males (358 vs. 253 min/d), but the difference was not statistically significant (two-tailed $P = 0.205$).

In addition to COT (slope of the $\dot{V}O_2$ on speed linear regression), body mass was significantly correlated with all measures of $\dot{V}O_2$ except for treadmill $\dot{V}O_{2\max}$ in males (Tables 2, 3). The correlations were always positive, except for MAS (see below). When males and females were analyzed together, sex effects on COT were statistically significant, with males having costs 1.3% higher than females after body mass and running speed (Table 2) were accounted for. When we omit the sex \times linetype interaction term and recalculate the partial regression coefficients, a general equation that describes incremental COT is

$$\text{COT} = (0.0324 \pm 0.0274) + \text{mass} \times (0.00086 \pm 0.00044) + \text{max speed} \times (-0.0006 \pm 0.0002), \quad (1)$$

where coefficients are shown \pm SE, incremental COT is expressed in milliliters O_2 per meter, body mass is in grams, and

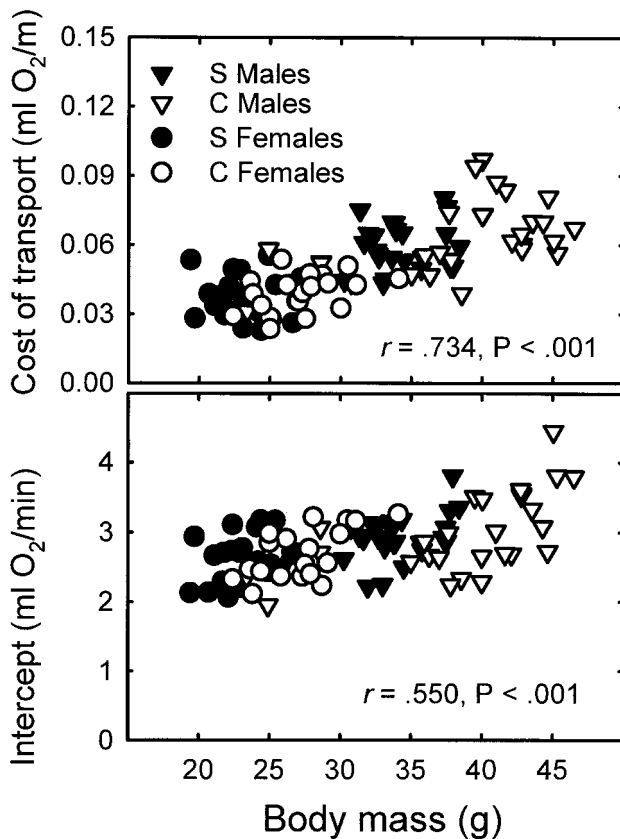


Figure 2. Slopes (i.e., costs of transport; see Fig. 1 and text) and intercepts obtained from linear regressions of oxygen consumption versus speed relationships, plotted against body mass for males and females from S and C lines ($N = 92$). Pearson product-moment correlations were computed using raw data for all animals pooled, and P values represent two-tailed tests. As would be expected, larger individuals generally have absolutely greater COT and intercepts.

maximum speed is in meters per minute. This equation refers to a female from a C line. For a male, the value 0.0136 (± 0.0060) would be added, and for an S-line mouse, the term 0.0033 (± 0.0040) would be added. Note that the above equation does not correspond exactly to any of the models shown in Table 2 because it does not include the sex \times linetype interaction term.

If maximum running speed is excluded from the model (similar to the second row of Table 2, but after removing the sex \times linetype interaction; see Fig. 2 and "Discussion"), then the equation becomes

$$\text{COT} = (0.0136 \pm 0.0224) + \text{mass} \times (0.00094 \pm 0.00046). \quad (2)$$

For a male, the value 0.0138 (± 0.0062) would be added, whereas for an S individual, 0.0029 (± 0.0035) would be subtracted.

As shown in Table 2, COT was significantly lower in S lines only when body mass and speed were not included in the model (Table 2; Fig. 3). There were no significant effects of either selection history or body mass when males and females were analyzed separately (Table 3), possibly because of reduced statistical power with smaller sample size and narrower ranges of body mass. Age effects were controlled for in the models with sexes analyzed separately (Table 3) and were never significant ($P > 0.10$ in all cases), whereas age was not controlled for when sexes were pooled (Table 2; see "Material and Methods").

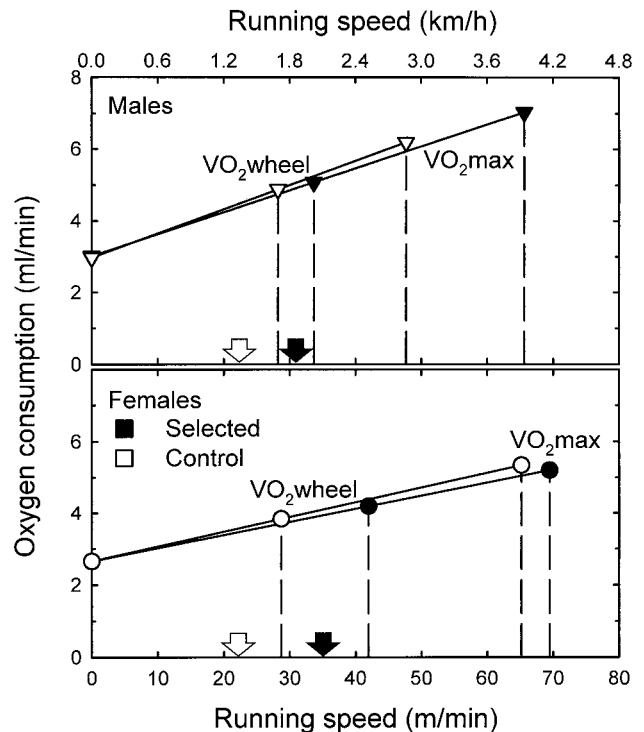


Figure 3. Schematic representation of the relationship between incremental cost of transport (COT), intercept, and maximum $\dot{V}O_2$ attained during voluntary wheel running ($\dot{V}O_{2\text{wheel}}$) and during forced exercise on a motorized treadmill ($\dot{V}O_{2\text{max}}$). Symbols represent adjusted means for 1-min period $\dot{V}O_2$ from nested ANCOVA performed on each sex separately without controlling for body mass. Dashed lines show estimated maximum aerobic speed (MAS; obtained from maximum $\dot{V}O_2$ on wheels and treadmill) and our estimates of COT). Arrows show mean maximum running speeds actually recorded in each group. *Top*, $\dot{V}O_2 = 0.0612 \times \text{speed} + 3.019$ for S males and $0.0675 \times \text{speed} + 2.976$ for C. Measured $\dot{V}O_{2\text{wheel}} = 5.08$ and 4.88 mL O_2 /min, and $\dot{V}O_{2\text{max}} = 7.03$ and 6.19 mL O_2 /min, for S and C, respectively. Calculated $MAS_{\dot{V}O_{2\text{wheel}}} = 33.6$ and 28.2 m/min, $MAS_{\dot{V}O_{2\text{max}}} = 65.6$ and 47.7 m/min, and maximum measured running speed was 30.9 and 22.2 m/min, for S and C, respectively. *Bottom*, $\dot{V}O_2 = 0.0366 \times \text{speed} + 2.657$ for S females and $0.0412 \times \text{speed} + 2.656$ for C. $\dot{V}O_{2\text{wheel}} = 4.19$ and 3.83 mL O_2 /min, and $\dot{V}O_{2\text{max}} = 5.20$ and 5.34 mL O_2 /min for S and C, respectively. $MAS_{\dot{V}O_{2\text{wheel}}} = 42.0$ and 28.7 m/min, $MAS_{\dot{V}O_{2\text{max}}} = 69.4$ and 65.2 m/min, and maximum voluntary running speed was 35.0 and 21.9 m/min, for S and C, respectively.

Based on mean values for each of the eight lines, body mass and COT were significantly positively correlated in males ($N = 8$, $r = 0.799$, one-tailed $P = 0.009$) but not as strongly correlated in females ($r = 0.548$, $P = 0.081$). When means for both sexes were pooled and sex was entered as a 0–1 dummy variable, COT and mass remained highly correlated (partial $F_{1,15} = 48.6$, $P < 0.001$).

With body mass and maximum running speed controlled for, intercepts of the $\dot{V}O_2$ versus speed regressions were significantly higher in females and in S lines as compared with C lines (Table 2; Fig. 2). This linetype tendency was also evident in males and females separately, although it was never statistically significant in these analyses (Table 3).

Corresponding to their higher maximum voluntary running speeds on the wheels, mice from S lines also exhibited higher maximum voluntary $\dot{V}O_2$ (Table 2). In addition, females from S lines ran voluntarily closer to their treadmill $\dot{V}O_{2,max}$ than did C females (82.4% vs. 71.7%, respectively; one-tailed $P_{selection} = 0.032$), whereas no effects of linetype or sex on the ratio $\dot{V}O_{2,wheel}/\dot{V}O_{2,max}$ was detected when both sexes were pooled, after mass was controlled for ($P_{selection} = 0.251$, $P_{sex} = 0.524$, $P_{sex \times linetype} = 0.069$). Differences between S and C mice were no longer significant after differences in maximum voluntary running speeds were accounted for ($P_{selection} > 0.36$ in all cases).

Although males ran voluntarily at a similar proportion of their $\dot{V}O_{2,max}$ irrespective of linetype, note that S males attained a $\dot{V}O_{2,max}$ on the treadmill about 18% higher than did C males (Table 3; two-tailed $P = 0.019$). Thus, treadmill $\dot{V}O_{2,max}$ was significantly higher in S lines for males but not for females, and the sex \times linetype interaction was highly significant (Table 2; $P = 0.006$). Both $MAS_{\dot{V}O_{2,max}}$ and $MAS_{\dot{V}O_{2,wheel}}$ (calculated from maximum $\dot{V}O_2$ obtained on the treadmill and the wheel, respectively) were significantly higher in females when body mass was not included (Table 2), although there were no differences between sexes when mass was included in the model ($P_{sex} = 0.989$ and $P_{body\ mass} = 0.178$). $MAS_{\dot{V}O_{2,max}}$ and $MAS_{\dot{V}O_{2,wheel}}$ were significantly higher in S mice in the pooled analysis when mass was not included, and the same was the case in males (Tables 2, 3). $MAS_{\dot{V}O_{2,max}}$ did not differ between S and C females, however.

Although RMR was not affected by linetype, S lines had higher “postural costs” (Tables 2, 3). In the pooled analyses, females had higher RMRs than males, but the sexes did not differ in postural costs (Table 2).

Correlations among Traits

After mass and age effects were accounted for, COTs were negatively correlated with intercepts in females and with both sexes pooled, whereas no significant relationship was detected for males (Table 4). In addition, COTs were highly (negatively) correlated with maximum running speed only in females

($P < 0.001$), and correlations between COT and maximum $\dot{V}O_2$ attained on wheels were significant in both sexes ($P < 0.05$) but with opposite signs.

Correlations between metabolic variables estimated during wheel measurements—intercept, RMR, postural costs, and maximum voluntary $\dot{V}O_2$ —were always significant and positive even after selection history, body mass, and age effects were removed ($P < 0.05$; Table 4). In addition, maximum $\dot{V}O_2$ attained during wheel running was highly correlated with maximum wheel-running speeds in both males and females. $\dot{V}O_{2,max}$ during forced exercise, however, was never significantly correlated with any wheel variable. As would be expected, both estimates of MAS were significantly correlated with all traits from which they were calculated (Table 4).

Total wheel-running distance was positively correlated with maximum $\dot{V}O_2$ and maximum running speed attained on wheels in both males and females, although the relationship between speed and distance was stronger in females (Table 4). Running distance was also negatively correlated with COT, but only in females (Fig. 4). When maximum running speed was included in the model, however, COT was not correlated with distance ($P_{COT} = 0.286$ and $P_{speed} < 0.001$).

Given that the range of the independent variable (i.e., running speed) is generally correlated with both the slope and r^2 in linear regressions, we tested whether there was a significant correlation between maximum running speeds, goodness of fit, and COT. Indeed, COT was negatively correlated with maximum running speeds ($N = 97$, $r = 0.223$, two-tailed $P < 0.028$), whereas adjusted r^2 was positively related with running speeds ($r = 0.672$, $P < 0.001$), and both variables remained significant in a multiple regression ($P < 0.001$ for both variables). Results (not shown) remained similar for residuals from nested ANCOVAs.

Costs of Transport in Forced versus Voluntary Exercise

In females, COTs (i.e., slopes) did not differ significantly between protocols (forced vs. voluntary exercise) when tested over the protocol \times line(linetype) interaction ($F_{1,3} = 0.83$, $P_{protocol} = 0.429$, $P_{selection} = 0.447$; Fig. 5), according to a nested two-way ANCOVA controlling for mass and linetype effects (analyses were identical to those for sex effects but included “forced vs. voluntary” rather than “sex” as a 0–1 dummy variable; $df = 3$ because we did not obtain data for mice from two C lines and one S line). Adjusted means (\pm SE) were 0.0340 ± 0.0048 mL O_2/m for forced exercise and 0.0392 ± 0.0030 mL O_2/m for voluntary exercise. Results remained unchanged when lines were not included as a random factor ($F_{1,65} = 1.11$, $P = 0.296$), suggesting that the lack of differences between slopes is real and not attributable entirely to low statistical power.

Extrapolated intercepts (i.e., metabolic rate at running speed = 0) were not significantly different between protocols

Table 4: Pearson product-moment correlations (r) between residuals from nested ANCOVAs

	Intercept	RMR	Postural Costs	$\dot{V}O_2$ wheel	$\dot{V}O_2$ max	Max Speed Wheel	MAS $\dot{V}O_2$ max	MAS $\dot{V}O_2$ wheel	Total Distance
Males + females ($N = 92$):									
Slope	-.181*	.127	-.314**	.127	.010	-.260**	-.581***	-.571***	-.164
Intercept		.663***	.914***	.742***	.056	.077	-.028	.132	.336**
RMR			.311**	.509***	.172	.001	-.085	-.128	.171
Postural costs				.665***	-.010	.106	-.017	.245**	.340***
$\dot{V}O_2$ wheel					-.005	.349***	-.170	.384***	.427***
$\dot{V}O_2$ max ^a						-.068	.614***	-.098	-.039
Max speed wheel							.246*	.743***	.570***
MAS $\dot{V}O_2$ max ^a								.404***	.102
MAS $\dot{V}O_2$ wheel									.472***
Males ($N = 45$):									
Slope	-.092	.138	-.216	.297*	.283	.046	-.463**	-.448***	.136
Intercept		.710***	.895***	.728***	-.003	.341*	-.214	.095	.468***
RMR			.330*	.540***	.246	.266*	-.088	-.123	.431**
Postural costs				.640***	-.121	.278*	-.174	.205	.351**
$\dot{V}O_2$ wheel					.048	.549***	-.410**	.407**	.482***
$\dot{V}O_2$ max ^a						.097	.594***	-.044	-.013
Max speed wheel							-.026	.401**	.310*
MAS $\dot{V}O_2$ max ^a								.064	-.326*
MAS $\dot{V}O_2$ wheel									.040
Females ($N = 47$):									
Slope	-.347**	.076	-.446**	-.245*	.044	-.500***	-.621***	-.767***	-.439***
Intercept		.579***	.929***	.791***	-.039	-.016	-.109	.227	.236
RMR			.258*	.430**	-.053	-.143	-.255*	-.121	-.097
Postural costs				.756***	-.018	.030	-.015	.323*	.311*
$\dot{V}O_2$ wheel					-.127	.363**	-.148	.505***	.501***
$\dot{V}O_2$ max ^a						-.085	.620***	-.066	-.210
Max speed wheel							.324*	.829***	.667***
MAS $\dot{V}O_2$ max ^a								.500***	.157
MAS $\dot{V}O_2$ wheel									.657***

Note. Residuals were calculated from SAS PROC MIXED, with linetype, line, and sex as factors and body mass and age as covariates.

^a Males with measurement problems in $\dot{V}O_2$ max ($N = 13$) were not included.

* $P < 0.05$ according to a Pearson's one-tailed correlation (not corrected for multiple comparisons).

** $P < 0.01$ according to a Pearson's one-tailed correlation (not corrected for multiple comparisons).

*** $P < 0.001$ according to a Pearson's one-tailed correlation (not corrected for multiple comparisons).

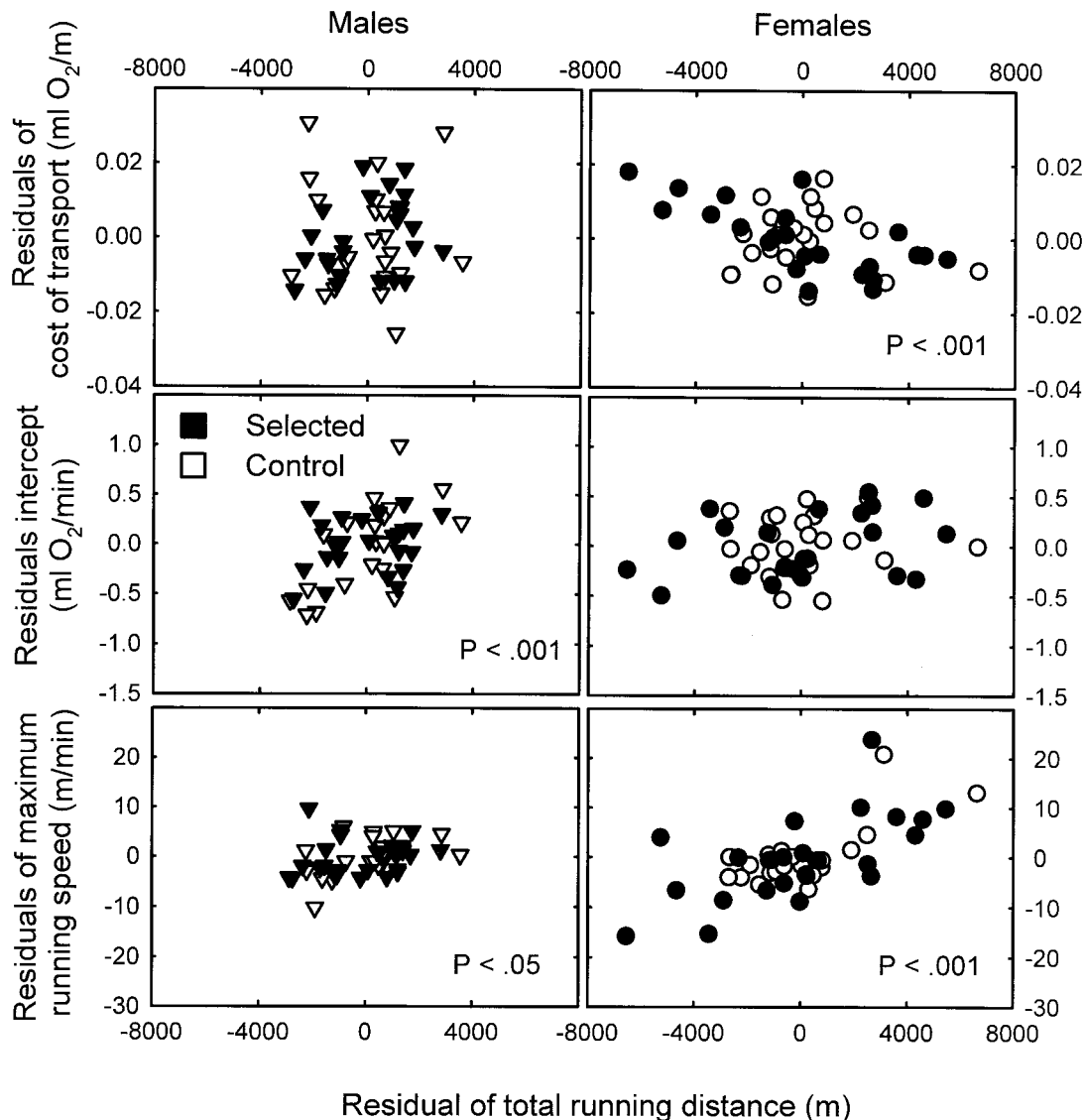


Figure 4. Residuals from nested ANCOVA of COT, intercept, and maximum running speed plotted against residuals of total wheel-running distance throughout the 24-h trial. Residuals were calculated separately for males (*left*) and females (*right*), including mass and age as covariates. *P* values obtained from Pearson correlations are shown when the relationship was statistically significant (two-tailed tests not corrected for multiple comparisons). Results for the nested model are listed in Table 4.

($F_{1,3} = 3.23$, $P = 0.170$; mean adjusted values of 2.93 ± 0.11 and 2.69 ± 0.07 mL O₂/min for forced and voluntary exercise, respectively) but increased significantly with body mass ($F_{1,55} = 8.90$, $P = 0.004$) and tended to be higher in S females ($F_{1,3} = 5.89$, $P = 0.093$; Fig. 5). After replicate lines were removed as a factor in the model, the effect of linetype was significant ($P = 0.018$), and the effect of protocol bordered on significance ($F_{1,64} = 0.71$, $P = 0.077$), which may indicate low power to detect differences between intercepts with our nested design.

Discussion

Costs of Transport during Voluntary and Forced Exercise

Although considerable information about treadmill-derived locomotor energetics exists for small rodents (e.g., MacMillen and Hinds 1992), including house mice (Taylor et al. 1970; Kemi et al. 2002), comparisons between energetic costs of forced and voluntary exercise should be performed with caution (Chappell et al. 2004; see also Girard 2001 regarding costs of locomotion in the field). Nonetheless, despite the

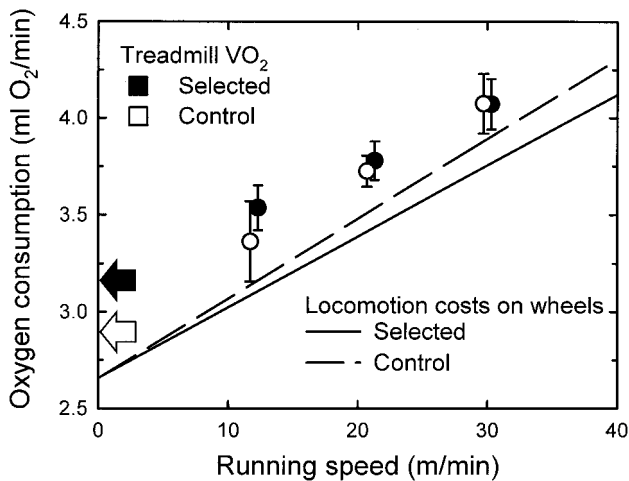


Figure 5. Values for $\dot{V}O_2$ consumption (symbols are means \pm SE) obtained at three different speeds (12, 21, and 30 m/min) in treadmill trials for 22 females (15 S and seven C) from generation 38, compared with cost of transport estimates obtained from wheel measurements (solid and dashed lines for S and C, respectively; equations shown in Fig. 3). Based on these values, incremental costs of locomotion on the treadmill were $\dot{V}O_2 = (0.0298 \pm 0.0086) \times \text{speed} + (3.1692 \pm 0.1864)$ for S lines and $(0.0395 \pm 0.0126) \times \text{speed} + (2.8905 \pm 0.2728)$ for C lines (regression lines for treadmill values not shown for clarity; arrows indicate intercepts of the regressions).

considerable individual variation in $\dot{V}O_2$ within each speed on the treadmill (Fig. 5), as well as variation in slopes and intercepts, mean incremental COT values were remarkably similar to those obtained in the wheels. Mean slopes on the wheels tended to be higher than slopes obtained during forced exercise (22.8% higher in S and 4.2% higher in C lines), but they were not statistically different. In contrast, y -intercepts estimated from wheel trials were 16.2% and 8.1% lower in S and C lines, respectively (Fig. 5), and although we could not detect significant differences between intercepts obtained with forced and voluntary running, this result may be a consequence of low statistical power. Although several additional factors might be involved (see Girard 2001; Chappell et al. 2004), we believe that stress or anxiety during forced exercise might explain the trend for higher intercepts and lower slopes in the treadmill trials. Although this pattern was true in both S and C mice, discrepancies between estimates were more pronounced in S lines; despite the lower COT in S lines during voluntary exercise, S females tended to have higher $\dot{V}O_2$ than C females running at similar speeds on the treadmill, especially at the lowest speed employed (Fig. 5). The behavioral profile, neurobiology, and high basal corticosterone levels of S lines suggest that the selective breeding has affected the hypothalamic-pituitary-adrenal axis (see Girard and Garland 2002; Rhodes et al. 2003, 2005), which could account for the difference between S and C mice. Nevertheless, we must emphasize that our mice were not trained before treadmill trials.

In this context, training may considerably affect cooperativity and running quality during trials (e.g., Thompson et al. 1980), running economy (Wisløff et al. 2001; Kemi et al. 2002), and anxiety levels associated with being in a novel environment (Wunder and Morrison 1974; Girard 2001).

Selection, Sex, and Body Mass

In our samples, mice from S lines were significantly smaller than those from C lines (Table 1), and this difference must be considered when interpreting the evolutionary change, or lack thereof, in transport costs. Within the range for each sex, body mass was not a statistically significant predictor of COT (Table 3). Similarly, COT was not significantly correlated with body mass in *Peromyscus* weighing between 15.6 and 32.5 g (Chappell et al. 2004)—an approximately twofold range of mass, as within each sex in our sample. When the sexes were pooled, the P value for body mass was reduced (Table 2; Fig. 2), which would be expected, given the increase in both sample size and range of body mass. Although age was not controlled for statistically when both sexes were pooled (see “Material and Methods”), it seems unlikely that costs would change dramatically with age within the range of this study, apart from the direct effects of body mass (but see Chappell et al. 2003).

Despite the lack of statistical significance, the body mass range in this study can affect COT considerably; the estimated partial regression coefficient (Eq. [2]) predicts a mean increment of 62.1% in whole-animal COT as mass increases from 20 to 45 g. Accordingly, differences in body size might be enough to account for the lower COT reported for deer mice (*Peromyscus maniculatus*) by Chappell et al. (2004). Their mean slope of 0.582 (speed in km/h, costs in mL O_2 /min [their Table 6]; or 0.0349 ± 0.0048 mL O_2 /m) obtained for 16 deer mice (15.6–32.5 g) at room temperature (22°–25°C) was 12.5% lower than would be predicted for a 22.2-g mouse in our sample (i.e., sex and linetype effects were averaged; COT = 0.0400 mL O_2 /m). Furthermore, mass effects on COT were similar to those obtained by Taylor et al. (1982) in an interspecific analysis of a variety of birds and mammals, despite the contrasting difference in mass range (Table 1) between both studies (7 g–260 kg in Taylor et al. 1982). Their parameter estimates ($10.7 \times \text{mass}^{0.684}$; costs in kJ/km, mass in kg) fall within the range of variation ($\pm 95\%$ confidence interval) of our estimates (COT = $5.21 \times \text{mass}^{0.529 \pm 0.266}$), calculated with log-transformed data and a conversion factor of 20.1 J/mL O_2 . (Despite the twofold difference in intercepts, back-transformation of SE from log scale resulted in intercepts ranging between 1.91 and 14.16.)

As mentioned above, selection for higher wheel-running performance resulted in a decrease in body size at maturity in our lines (Swallow et al. 1999), as was also observed in rats selected for high treadmill endurance (Koch and Britton 2001). How does the reduction in mass in S lines affect COT? In our sample, S males were on average 6.3 g (15.4%) smaller than C males

(Table 1), which would lead to a 7.8% decrease in COT, whereas in females the 3.6-g (13.3%) difference would result in a 5.3% COT reduction in S lines (Fig. 3). From adjusted means, COT averaged 13.6% lower in S mice when size differences were not accounted for ($P_{\text{selection}} = 0.030$; Table 2) but only 6.0% lower ($P = 0.394$) after mass was controlled for. Our results show that an S female of 23.5 g would spend in average 559.8 mL O_2 to run 10 km per night, a distance over which a C female (27.1 g) would have spent 590.9 mL O_2 , resulting in a total economy of 31.1 mL O_2 —enough O_2 to sustain an individual's metabolic rate at rest (i.e., RMR) for over 30 min.

Thus, the reduction in body size of S lines leads to reduced energetic COT on a whole-animal basis, but whether this should be viewed simply as a by-product of the reduction in size or as a possible adaptation in and of itself is unclear. During the routine selective-breeding protocol, mice always have ad lib. food. Nevertheless, over a 6-d exposure to wheels some mice lose body mass (T. Garland, unpublished data; see also Koteja et al. 1999 regarding shorter trials and Syme et al. 2005 regarding normal vs. mini-muscle mice), indicating that energy intake is not keeping pace with energy expenditure. This mismatch could have various causes, including alterations in the reward circuitry of the brain that have apparently evolved as a mechanism to “motivate” higher wheel running in the S lines (see Rhodes et al. 2005), but it may also have effects on other behaviors, such as feeding, predatory aggression (Gammie et al. 2003), and thermoregulatory nesting (Carter et al. 2000).

Our results are consistent with previous studies showing that higher running distances have evolved in S lines mainly by increasing average running speed rather than time spent running (Swallow et al. 1998a; Koteja et al. 1999; Girard et al. 2001; Koteja and Garland 2001). This pattern is more pronounced in S females, which, in the present study, attained maximum speeds on average 60% faster than C females, contrasting with the 39% increase in speed in S males compared to C males (Table 3). On the basis of the computer-recorded data, obtained in 1-min bins, S females from generation 31 postselection ran at mean speeds 2.6 times higher than C females, whereas in males the difference was only a factor of 2.2 (T. Garland, unpublished data; see also Rhodes et al. 2000). Conversely, during that generation S males were spending 38% more time running, whereas S females increased running time by only 14%, which is also supported by our results—a mean increase of 42% in time spent running in S males versus <1% in females (Table 3).

Could COTs account for these differences between males and females in their response to selection? Assuming mean slopes of 0.0641 and 0.0391 mL O_2 /m for males and females, respectively (Table 3), running speeds would increase on average 1.65 times in females if selection resulted in S mice willing to run voluntarily at consistently higher $\dot{V}O_2$ levels than C mice, regardless of sex. Accordingly, maximum $\dot{V}O_2$ during wheel running differed between linetypes but not sexes regardless of running speed (Table 2). During voluntary wheel running, S males

and females attained $\dot{V}O_2$ on average 0.741 mL O_2 /min higher than their C counterparts (Table 3). On the other hand, selection resulted in mean increases in maximum running speeds of 8.7 and 13.1 m/min in S males and females, respectively, over C values. Hence, because of the smaller size and lower transport costs in females, running speeds should evolve proportionally more in females if we have been selecting animals willing to run voluntarily at increasingly higher aerobic levels, as our results indicate (Table 3).

Whether sex affects COT in any manner independently of size remains unclear. Although transport costs differed significantly between the sexes after mass and maximum speed were controlled for (Table 2), age may be a confounding factor because males were considerably older than females, and comparisons between sexes did not account for age differences (see “Material and Methods”). Nevertheless, males and females have adopted different strategies to increase total running distances in response to selection (e.g., S females running relatively faster than males when compared to C), and it is possible that correlated responses in $\dot{V}O_{2\text{max}}$ may be sex specific as well. Differences in $\dot{V}O_{2\text{max}}$ between S and C lines were statistically significant for males (Table 3), as was also reported by Swallow et al. (1998b), at generation 10 but not for females (Table 3; see also Rezende et al. 2005).

Maximum Running Speeds and Transport Costs

In general, running at higher speeds should decrease net COT (i.e., COT at a given speed; Koteja et al. 1999), and metabolic economy is maximized during locomotion at speeds close to MAS (Taylor et al. 1970, 1982; Kenagy and Hoyt 1989). Thus, one might predict that S lines would have evolved to run faster, and even near their MAS (or their lactate threshold velocity, which is not significantly different from MAS in different strains of laboratory mice; Billat et al. 2005). Although S lines do run considerably faster than C lines (see “Results”), they still rarely approach their estimated MAS (Fig. 3). Similar results were reported by Chappell et al. (2004) in *P. maniculatus*, which ran preferentially at speeds below their MAS. Interestingly, there was a temperature-related shift in preferred running speeds in *P. maniculatus*, which ran at faster average speeds at lower temperatures. During wheel running at room temperature, S lines attain higher body temperatures (T_b) than C lines (Rhodes et al. 2000), and one can only speculate whether T_b regulation is somehow associated with chosen running speeds on wheels (e.g., perhaps S lines avoid running faster to keep from overheating). It is also possible that Q_{10} effects associated with higher T_b could increase metabolic rates of the S lines at the higher speeds, preventing them from running faster.

Although running at higher speeds should decrease net COT (incremental COT \times running speed + intercept; Fig. 1), we also observed significant reductions in incremental COT (i.e., slopes) as maximum running speeds increased in the pooled

analysis (Table 2) and among females (Tables 3, 4), contradicting our expectations based on the nature of least squares regressions (everything else being equal, slopes should be lower as the range of running speeds decreases). This “extra” economy may be because S females now run more intermittently, with shorter and more frequent bouts, as compared with C lines, which “exhibit pauses that are long enough to allow significant deceleration of the wheel” (Girard et al. 2001). Accordingly, in our study wheel speed variation within the highest 1-min recorded speed—that is, acceleration and deceleration of the wheel within that minute, estimated as the standard deviation of 40 samples of instantaneous speed recorded every 1.5 s (SD_{speed})—was negatively correlated with maximum running speed in males ($r = -0.457$, $P < 0.001$) and in females ($r = -0.255$, $P = 0.042$) but was not correlated with COT ($P > 0.05$).

Interestingly, the decrease in slopes with higher running speeds and intermittency in S females would be analogous to the reduction in costs by changing gaits as speed increases; both behaviors can minimize energy waste (i.e., energy that would be spent accelerating either the wheel forward or the center of gravity upward), increasing overall running efficiency (e.g., Kram and Taylor 1990). In this context, several factors could explain why transport costs were not statistically affected by running speeds in males (Tables 3, 4). For instance, the range of maximum running speeds on wheels was broader in females (Fig. 4; Table 3), and S females ran faster than S males. Furthermore, females appear to run at high speeds more steadily than males regardless of selection history; SD was around 20% lower in females (SD_{speed} was ± 3.08 m/min in females vs. ± 3.75 m/min in males) but did not differ between linetypes ($P_{\text{selection}} = 0.159$, $P_{\text{sex}} = 0.045$, $P_{\text{speed}} = 0.161$). Mass differences could account for these differences and might be playing an important role, since coasting behavior should decelerate wheels faster in males when they stop running simply because they are considerably heavier.

Differences in size (and hence in COT) between the sexes could also explain why in males, wheel-running activity, estimated as mean revolutions per day in trials lasting 3 d (Koteja et al. 1999) or 8 wk (Swallow et al. 1999; final 7 d of running analyzed), was negatively correlated with final body mass, but there was no such correlation in females. In lines of rats selected for high and low treadmill endurance, Koch and Britton (2001) reported that males and females from the low-selected line were, on average, 16% and 20% heavier, respectively, than their counterparts from the high-selected line, and they reported that body mass accounted for a statistically significant fraction of the response to selection for both the low and high lines ($P < 0.001$ in both cases). Within our sample, it is possible that size-related effects on net COT (see above) are increasingly important as mass increases. With net COT (mL O₂/min) = $0.0641 \times \text{speed} + 3.00$ for males (pooling S and C data) and $0.0391 \times \text{speed} + 2.656$ for females (Fig. 3), males would spend 4.60 mL O₂/min to run at 25 m/min—in other words, 5.2 times

an average RMR of 0.87 mL O₂/min—whereas females would spend only 3.63 mL O₂/min, or 4.0 times RMR (0.91 mL O₂/min), to sustain the same speed (females have higher absolute and mass-specific RMRs than males, however). If costs are higher at larger sizes, then two nonexclusive explanations could account for the discrepancy between results in males and females. First, smaller males might run more time (or faster) simply because increased running economy, as mass decreases, becomes more pronounced within the range of sizes of males but does not in females (Table 1). Second, wheel running might have more pronounced “training effects” in males because of their larger size and overall higher net COT, and hence good runners lose proportionally more mass if they are male. This was not the case in our data set, and there were no differences between sexes in mass loss after 6 d of wheel access. (Females lost 2.7%, vs. 0.1% in males [$P_{\text{sex}} = 0.094$].) In addition, females ran on average more than males, and sex differences are not significant after wheel running at day 6 is controlled for [$P_{\text{sex}} = 0.170$].) However, sex differences in mass regulation may exist, and how wheel running and body mass interact requires further study (e.g., Koteja et al. 1999; Swallow et al. 1999).

Concluding Remarks

We have shown that S lines may have evolved lower COT, although this effect is confounded with differences in body size and running behavior between linetypes (S vs. C) as well as between sexes. Although our results demonstrate that selection on increased daily movement distances could lead to reduced COT as a correlated response, we emphasize that analyses of correlated behavioral and physiological responses to selection are environment dependent, and extrapolation of these results to natural populations should be performed with caution. By running faster (and more intermittently), S lines (particularly females) may reduce their incremental COT on wheels, but we do not know if this is a pattern that occurs during evolution in the wild. However, it is worth noting that increasing running distances mainly by increasing speed rather than time spent running is in agreement with field studies and theoretical models that suggest the former to be the more energetically economical strategy.

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