

Running Behavior and Its Energy Cost in Mice Selectively Bred for High Voluntary Locomotor Activity

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ABSTRACT

Locomotion is central to behavior and intrinsic to many fitness-critical activities (e.g., migration, foraging), and it competes with other life-history components for energy. However, detailed analyses of how changes in locomotor activity and running behavior affect energy budgets are scarce. We quantified these effects in four replicate lines of house mice that have been selectively bred for high voluntary wheel running (S lines) and in their four nonselected control lines (C lines). We monitored wheel speeds and oxygen consumption for 24–48 h to determine daily energy expenditure (DEE), resting metabolic rate (RMR), locomotor costs, and running behavior (bout characteristics). Daily running distances increased roughly 50%–90% in S lines in response to selection. After we controlled for body mass effects, selection resulted in a 23% increase in DEE in males and a 6% increase in females. Total activity costs (DEE – RMR) accounted for 50%–60% of DEE in both S and C lines and were 29% higher in S males and 5% higher in S females compared with their C counterparts. Energetic costs of increased daily running distances differed between sexes because S females evolved higher running distances by running faster with little change in time spent running, while S males also spent 40% more time running than C males. This increase in time spent running impinged on high energy costs because the majority of running costs stemmed from “postural costs” (the difference between RMR and the zero-speed intercept of the speed vs. metabolic rate relationship). No statistical differences in these traits were detected between S and C females, suggesting that large changes in locomotor behavior do not

necessarily effect overall energy budgets. Running behavior also differed between sexes: within S lines, males ran with more but shorter bouts than females. Our results indicate that selection effects on energy budgets can differ dramatically between sexes and that energetic constraints in S males might partly explain the apparent selection limit for wheel running observed for over 15 generations.

Introduction

Locomotion is fundamental to most behaviors. It is intrinsic in movements related to territorial defense, foraging, predator escape, mating interactions, migration, and others, and it is the presumed ancestral source of many evolved displays, such as the ritualized locomotor patterns used as signals in courtship, aggression, and other social interactions (e.g., Irschick and Garland 2001; Alexander 2003; Perry et al. 2004; Husak et al. 2006; Oufiero and Garland 2007). In these contexts, both the limits to performance and the energy costs of locomotion are of considerable interest for behavioral and ecological physiology. With respect to performance, interspecific comparisons have shown that locomotor abilities can be correlated with aspects of behavioral ecology (Garland et al. 1988; Garland 1999). With respect to energetics, energy gathering, assimilation, and/or partitioning are assumed to be central to Darwinian fitness in a broad range of studies, and in many analytical approaches (e.g., optimal foraging models), energy is used as a proxy for fitness when testing hypotheses.

Numerous comparative studies show that locomotion can be energetically demanding, and in many mammals, the highest attainable whole-animal metabolic rates are achieved during running or flying (e.g., Taylor et al. 1982; Weibel et al. 2004). Thus, the energy costs of locomotion could potentially constitute a substantial portion of an animal's daily energy expenditure (DEE). Because animals are generally presumed to be limited in their abilities to acquire and expend energy (e.g., see Koteja et al. 2001; Vaanholt et al. 2007a), biologists routinely assume that trade-offs exist between expenditures on locomotion, other maintenance requirements, and such fitness-critical life-history traits as growth and reproduction (Karasov and Martinez del Rio 2007). However, the importance of locomotor costs to the overall energy budget—and hence their potential importance to behavioral evolution—remains a matter of debate, especially for small mammals. Some studies have suggested that these costs can have significant effects on DEE under natural conditions (Karasov 1992; Gorman et al. 1998; Corp et al. 1999; Girard 2001), but others indicate that loco-

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motor costs often comprise a minor fraction of DEE (Garland 1983; Altmann 1987; Baudinette 1991).

Whether energy use in locomotion is high or low has important implications, both for the fitness consequences of behaviors that require movement and for the mechanistic, sub-organismal traits that underlie locomotor behavior. Along with potential trade-offs within the energy budget, high costs of locomotion and activity, when coupled with ecological or social requirements for substantial movement, are expected to generate selection favoring increased locomotor efficiency. For example, we would expect morphological, physiological, and behavioral traits that reduce locomotor costs to evolve concomitantly with increased home range area or daily movement distances (DMDs), which show enormous variability among species of mammals (Harestad and Bunnell 1979; Garland 1983; Garland et al. 1993; Corp et al. 1999; McLoughlin and Ferguson 2000; Kelt and Van Vuren 2001; Carbone et al. 2005). Indeed, some anatomical correlates of interspecific variation in locomotor movements and costs involving limb structure and dimensions have been identified (e.g., Kelly et al. 2006; Sockol et al. 2007). Also, behavior can have a substantial effect on locomotor costs through an animal's choice of speeds, gait, or movement intermittency (Hoyt and Taylor 1981; Kenagy and Hoyt 1989; references in Girard et al. 2001).

Given the presumed importance of locomotion and energetics in ecological and evolutionary physiology, direct, controlled tests of how variation in locomotor behavior affects energy budgets or how selection on locomotor behavior affects running economy are surprisingly rare. Numerous comparative studies provide useful insights into metabolic rates during locomotion and the correlated evolution of behavioral and physiological traits (Baudinette 1991; Garland 1999; Irschick and Garland 2001; Rezende et al. 2004; Healy et al. 2005; Kelly et al. 2006), but without information on running time or distance or behavioral or physiological trade-offs between locomotion and other energetic demands, they provide limited insight as to the significance of locomotion in energy budgets. Moreover, interspecific comparative analyses are complicated by statistical issues related to "phylogenetic signal" and often by confounding of independent variables (e.g., Clobert et al. 1998; review in Garland et al. 2005). For example, among mammals, the species with the largest home ranges and DMDs tend to be carnivores in the order Carnivora (Harestad and Bunnell 1979; Garland 1983; Garland et al. 1993; Corp et al. 1999; Kelt and Van Vuren 2001; Carbone et al. 2005), so it is difficult to determine whether locomotor behavior and energetics have evolved in concert with diet, activity levels, or other traits that differ, on average, between Carnivora and other mammals. Another potential problem with most estimates of locomotor energetics is that costs are typically measured during forced exercise at constant speeds over long durations (e.g., Taylor et al. 1982), a situation that contrasts with the often highly intermittent running and walking behavior of freely moving animals (Kenagy and Hoyt 1989; Girard et al. 2001; Vásquez et al. 2002).

Here, we use an experimental evolution approach (Garland 2003; Swallow and Garland 2005; Garland and Kelly 2006; Gar-

land and Rose 2009; Rhodes and Kawecki 2009) to examine how locomotor activity and energy budgets evolve in a small mammal. This study is unique for two reasons. First, it employs a respirometry system that allows measurements of behavior and energy costs of voluntary exercise with high temporal resolution over long periods (up to several days). We developed an enclosed running wheel coupled with a standard housing cage that permitted essentially continuous measurements of oxygen consumption ($\dot{V}O_2$, a standard measure of metabolic rate) and running performance. A study of deer mice (*Peromyscus maniculatus*; Chappell et al. 2004) validated the method and showed that individual variation in running behavior was substantial, that mice rarely used speeds that approached maximum sustainable speeds, and that mice ran at a wide range of speeds (contrary to hypotheses that high speeds should be preferred to maximize transport efficiency; see also Chappell et al. 2007 on gerbils).

Second, this study involves mice from a long-term selective breeding experiment (Swallow et al. 1998; Garland 2003; Rezende et al. 2006b). This project has produced four replicate lines of mice that have undergone multigenerational selection for high voluntary wheel running (S lines) and four nonselected control lines (C lines). After more than 40 generations, the S lines, compared with the C lines, run more than twice as far per day on wheels during days 5 and 6 of a 6-d period of wheel access and show a similar elevation of home cage activity when housed without wheels (Rhodes et al. 2001; Malisch et al. 2008, 2009; Vaanholt et al. 2008). The motivation and/or reward systems of the S lines have diverged in terms of wheel-running behavior (Belke and Garland 2007), gene expression in the hippocampus (Bronikowski et al. 2004), and brain neurochemistry and pharmacological responses related to wheel running (review in Rhodes et al. 2005; Keeney et al. 2008). Beyond locomotor activity, the S lines show differences from the C lines in predatory aggression (Gammie et al. 2003), thermoregulatory nest building (Carter et al. 2000), open-field tests (Bronikowski et al. 2001), and other behaviors when observed in regular housing cages (Koteja et al. 1999a). Compared with mice from the C lines, mice from the S lines are smaller in body size, have less body fat, and show differences in relative organ sizes and hind limb bone dimensions (Swallow et al. 1999, 2005; Houle-Leroy et al. 2003; Garland and Freeman 2005; Kelly et al. 2006; Middleton et al. 2008), and at least some of these alterations seem clearly related to running abilities (e.g., Garland and Freeman 2005). The S lines show elevated maximal oxygen consumption during forced treadmill exercise (Rezende et al. 2006a, 2006b), higher treadmill endurance (Meek et al. 2009b), and increased insulin-stimulated glucose uptake by isolated extensor digitorum longus muscles (Dumke et al. 2001) but few differences in resting glycogen levels or in depletion of glycogen stores during nightly running (Gomes et al. 2009), aside from those related to individuals expressing the mighty minimuscle phenotype (Houle-Leroy et al. 2003; Hartmann et al. 2008). Mice from the S lines show differences in circulating corticosterone, leptin, and adiponectin levels (Girard and Garland 2002; Girard et al. 2007; Malisch et al. 2007, 2008; Vaanholt et

al. 2007b, 2008) compared with those of the C line mice, but whether these affect locomotor abilities or energetics is unclear. As of generation 21–22, the S mice showed no evidence of reduced reproductive performance (Girard et al. 2002; see also Pontzer and Kamilar 2009). Mice from S lines, compared with those from C lines, show higher body temperatures at night when they are active (Rhodes et al. 2000) and maintain body mass better when cold challenged (Koteja et al. 2001) but apparently do not differ in maximum cold-induced food consumption (Koteja et al. 2001) or in maximum food consumption when forced to work (run on a wheel) to obtain food (Vaanholt et al. 2007a). Overall, the S and C lines can offer robust insights into how locomotor propensities coadapt with other traits, including energy budgets, and the ways in which physical activity can affect energy expenditure in freely locomoting animals.

This study had three primary goals. The first was to measure with unprecedented accuracy the fraction of the energy budget composed of locomotor activity to determine whether it is potentially significant, that is, more than the roughly 1% of DEE predicted by several authors (Garland 1983; Altmann 1987; Baudinette 1991). This is essential for gauging the likelihood that locomotor costs have a substantial effect on DEE and components of Darwinian fitness (e.g., litter size) and hence lead to adaptations that reduce locomotor costs. The second was to analyze how the evolution of locomotor activity can affect energy expenditure and vice versa. Specifically, we tested whether the evolutionary increase in daily movement distance in S lines resulted in a proportionally increased DEE and addressed whether energetic constraints might be involved in the selection limit in running distances observed after 16 generations of selection (Garland 2003). The third was to test whether differences in running behavior between S and C lines (running speeds, number and duration of running bouts, etc.) affect locomotor efficiency and energy expenditure.

Material and Methods

Animals and Selection Procedure

As described by Rezende et al. (2006b), we studied mice from generations 32 (males) and 34 (females) of the ongoing selection experiment. As detailed by Swallow et al. (1998), the original progenitors were outbred, genetically variable laboratory house mice (*Mus domesticus*) of the Hsd:ICR strain (Harlan Sprague Dawley). After two generations of random mating, mice were randomly paired and assigned to eight closed lines (10–15 pairs in each; about 10 families per line were selected). In each subsequent generation, ~6–8-wk-old offspring were housed individually, with access to a running wheel for 6 d. Wheel running was monitored by an automated system and quantified as the total number of revolutions on days 5 and 6 of the 6-d test. In four selected lines (S), the highest-running male and female from each family were selected as breeders for

the next generation. In four control lines (C), a male and a female were randomly chosen from each family. Within all lines, sibling matings were not allowed. All aspects of animal housing and experimental procedures were approved by the University of California, Riverside, Institutional Animal Care and Use Committee and are in compliance with U.S. laws.

Metabolic Rate Measurements

To determine energy costs of voluntary running, we used the protocol reported by Chappell et al. (2004) and Rezende et al. (2006b). Briefly, we enclosed a commercially available rodent wheel (circumference 1.12 m; the same model used in the selection procedure) and a standard plastic mouse cage within an airtight Lucite chamber (Fig. 1 in Chappell et al. 2004). The mouse cage contained bedding (wood shavings) as well as a food hopper and water tube that were available ad lib. Mice entered and exited the wheel at will through an access port cut into the side of the mouse cage and could run at speeds, directions, and durations of their choice. The enclosures had paired incurrent and excurrent ports for airflow and an internal fan that rapidly recirculated air to facilitate mixing.

Two wheel enclosures were housed in a large environmental cabinet that controlled ambient temperature (T_a ; 20°–27°C) and photoperiod (12L : 12D; dark period 1900–0700 hours). Temperature and light cycles during measurements were similar to conditions in our animal room. We regulated airflow to the enclosures (2,500 mL/min \pm 1%) with upstream mass flow controllers (Applied Materials, Sunnyvale, CA; Tylan, Billerica; or Porter Instruments, Hatfield, PA). About 100 mL/min of excurrent air was subsampled and dried (magnesium perchlorate) for gas analysis by a Sable Systems (Las Vegas, NV) Oxzilla dual-channel O₂ analyzer and Sable Systems CA-2A CO₂ analyzers. Oxygen and CO₂ concentration, flow rates, wheel speed, and T_a were recorded every 1.5 s on Macintosh computers equipped with National Instruments A-D converters and LabHelper software (Warthog Systems, <http://www.warthog.ucr.edu>). Wheel speed was measured with tachometers (not by counting wheel revolutions; Eikelboom 2001; Koteja and Garland 2001). Computer-controlled valves took 2.5-min gas reference readings every 45 min. Procedures for baseline adjustment, smoothing, respirometry calculations, and instantaneous conversions to estimate oxygen consumption rates ($\dot{V}O_2$) and CO₂ production rates ($\dot{V}CO_2$) concomitantly with wheel running are detailed elsewhere (Chappell et al. 2004; Rezende et al. 2006b).

Experimental Protocol

To mimic conditions during selection trials and allow acclimation to the wheel system, mice that were used for exercise cost trials had access to a standard running wheel and mouse cage setup for 4–5 d before measurements. Mice were weighed to the nearest 0.1 g before entering the acclimation wheel (day

0). Following the acclimation period, they were placed in a wheel respirometry chamber between 1130 and 1200 hours (i.e., the middle of the inactive phase of the daily cycle) for recording of behavior and metabolism. The home cage was transferred to the wheel chamber with each mouse, so animals were exposed to familiar bedding and odors during measurements. However, it was not feasible to completely clean the wheel chambers between trials.

We first tested a set of male retired breeders from generation 32 postselection. These mice were wheel acclimated for 5 d and measured on day 6; we report data for 49 individuals that provided reliable measurements according to visual inspection of the original records. Subsequently, we tested 48 females from generation 34 postselection (six individuals from each of four S and four C lines, each individual from a different family). After weaning at 21 d of age, females were randomly mixed four per cage (except to meet the condition of two individuals from the C line and two from the S line per cage). Measurements began at about 8 wk of age. In a slight change from the procedure for males, the females had access to acclimation wheels for 4 d. At day 5, they were placed inside a metabolic chamber, and metabolism and behavior were recorded during days 5 and 6; we used the data from day 6 in analyses comparing males and females (see Rezende et al. 2006b).

Because only two animals could be measured simultaneously, measurements were randomly scheduled across lines, except that we roughly attempted to control for age effects (e.g., mice that were born first were also measured first), and we always attempted to measure one S and one C mouse concomitantly. The latter criterion was not always met for males because only one metabolic chamber was available during some trials. For females, we tested rotational resistance before and after each measurement by spinning wheels to a high speed (~80 rpm) with an electric drill fitted with a rubber friction disk and then monitoring the time needed for speed to decay to 0. No changes in resistance occurred during the trials.

The following variables were extracted for each trial (total recording period of 23.5–23.75 h, between 1200 and roughly 1130 hours on the following day): $\dot{V}O_2$ vm, maximum 1-min average $\dot{V}O_2$ during trial (mL/min); RMR, resting metabolic rate (measured as the lowest continuous 5-min running average of $\dot{V}O_2$ during periods of inactivity; mL/min); DEE, average daily energy expenditure (total $\dot{V}O_2$ /total trial duration; mL/min); D_{run} and T_{run} , total distance run (m) and time spent running (calculated using speeds above 0.5 rpm [0.56 m/min] to remove effects of electrical noise; min); V_{mean} , mean running speed (D_{run}/T_{run} ; m/min, log transformed to improve normality); $V_{max,1}$, maximum 1-min average wheel speed (m/min); V_{peak} , maximum instantaneous wheel speed over 1.5 s (m/min, log transformed to improve normality); N_{bouts} , numbers of running bouts (log transformed), defined as a period of wheel rotation lasting 3 s or more at speeds above 0.5 rpm in either direction of rotation (Chappell et al. 2004); and BD_{mean} and BD_{max} , mean and maximum duration of running bouts, respectively (expressed in s, log transformed in both cases).

Comparisons between measurements in day 5 versus day 6

in females show that these variables are highly repeatable (one-tailed $P \leq 0.003$ in all cases), so the observed variation should accurately reflect interindividual differences in physiology and behavior (detailed results are listed in Rezende 2005, p. 215). We also calculated the cost of transport (COT) from the relationship between wheel speed and $\dot{V}O_2$ for each mouse. Absolute COT at a given speed is $\dot{V}O_2$ /speed. The slope of the speed versus $\dot{V}O_2$ regression is the incremental COT (the energy costs strictly associated with displacement, in units of energy distance⁻¹; Taylor et al. 1982; Garland 1983). The zero-speed intercept of this regression is typically elevated above RMR, and the difference (intercept – RMR) is assumed to be a “postural cost” of locomotion (Taylor et al. 1982; Fig. 1; see also Dlugosz et al. 2009). Analyses of incremental COT for these mice are shown by Rezende et al. (2006b).

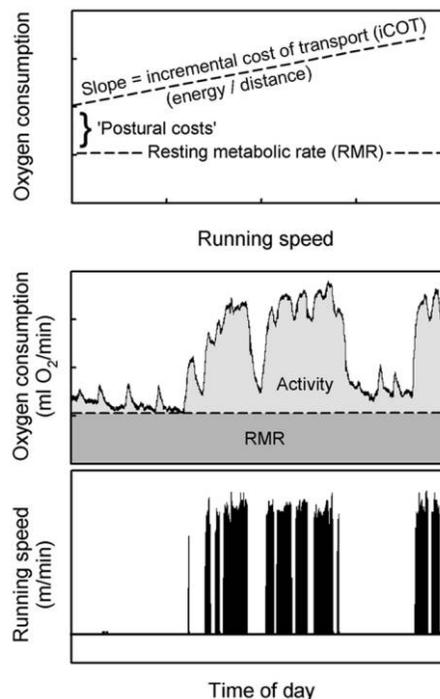


Figure 1. Interactions between running speed and metabolic rate for a typical small mammal, including the mice in this study (top). Resting metabolic rate (RMR) is the minimal rate of energy expenditure of an inactive animal and is assumed not to change during running. Absolute cost of transport at a given running speed is metabolic rate/speed. The slope of the regression of metabolic rate on speed is the incremental costs of transport (iCOT, in units of energy per unit distance). The intercept of the regression is typically elevated above RMR, and this difference is called the “postural cost” of locomotion. Continuous records of oxygen consumption (middle) and wheel speeds (bottom) allowed us to partition energy expenditure into different components, as shown diagrammatically for daily energy expenditure (DEE), where $DEE = RMR + \text{activity costs}$ (gray, middle). Activity costs can be subsequently partitioned into postural costs, daily locomotor costs (DCOT), and extra costs, as detailed in “Material and Methods.”

Energy Budgets

To calculate the components of energy budgets for each individual, DEE was broken down into RMR and activity costs (net activity costs in mL O₂/min = DEE – RMR in mL/min, and relative activity costs = net costs/DEE, expressed as %). Note that costs of activity, as defined here, refer to the total oxygen consumption above RMR averaged throughout the entire trial and not costs associated only with wheel running (Fig. 1). Costs of locomotion (total daily costs of transport [DCOT] + postural costs) are a subset of total activity costs (Fig. 1). We calculated DCOT as incremental COT × D_{run} . Similarly, we estimated the daily contribution of postural costs as (intercept – RMR) × T_{run} . For comparisons with rates of energy use (e.g., DEE, RMR), we divided DCOT and postural costs by trial duration (ca. 23.5 h). Activity costs include additional energy expenditures not directly related to wheel locomotion (e.g., thermoregulation, digestion, activity outside of the running wheel). We estimated the contributions of these “extra” factors by subtracting locomotion costs from activity costs (i.e., extra = net activity costs – [DCOT + postural costs]).

Statistical Analyses

Because males and females were measured under similar but not identical conditions (Rezende 2005, pp. 240–241) and the sexes have increased total running distance in somewhat different manners in response to selection (Swallow et al. 1998; Garland 2003), analyses were initially performed separately for each sex. However, results were consistent between sexes, and so we also report analyses of pooled data (both sexes) with sex in the model as an additional factor. Age was removed from these analyses because it was highly correlated with mass when sexes were pooled—that is, males were considerably older and larger than females (Table 2). Effects of selection (line type; S vs. C) were estimated using a one-way (males and females separately) or two-way (pooled sexes) nested ANCOVA with Type III tests of fixed effects using mixed models in SPSS for Windows and SAS PROC MIXED (ver. 8; SAS Institute, Cary, NC). Line-type effects with 1 and 6 df were tested over the replicate lines’ random effect ($N = 8$) nested within line type. Testing the effect of line type relative to the variation among lines is necessary in principle because it matches the experimental design (Garland and Rose 2009) and in practice because various traits show significant differences among the replicate lines (e.g., Koteja et al. 1999b; Swallow et al. 2001; Gammie et al. 2003; Bronikowski et al. 2006; Rezende et al. 2006b; Malisch et al. 2007; Kane et al. 2008; results of this study). Body mass and age were initially included in the model as covariates. In some cases where absolute rather than mass-corrected values are of interest (e.g., running speeds and distances), analyses were also performed without body mass in the model. Some traits were log transformed to improve normality of residuals or to allow estimation of allometric scaling exponents. Adjusted least squares means and associated standard errors were cal-

culated to estimate the magnitude of differences between S and C lines.

Although we report two-tailed P values in the tables for simplicity (unless otherwise noted), we tested directional hypotheses whenever appropriate to increase statistical power. For example, we expect that selection has led to higher DEE and total running distances in S mice because these lines voluntarily run more than C lines.

To analyze how behavior, running performance, and metabolic rates correlate at the level of individual variation, we employed Pearson product-moment correlations between residuals of each variable as computed from the nested ANCOVAs described above. We controlled for Type I errors in these multiple simultaneous tests by use of the false discovery rate method (Storey and Tibshirani 2003), testing P values with the QVALUE library (<http://faculty.washington.edu/jstorey/qvalue>) in the R statistical package (R Foundation for Statistical Computing). Results (see Table 3) were considered statistically significant if the false discovery rate associated with each test that resulted in $P < 0.05$ was smaller than 5% (i.e., $Q < 0.05$).

Results

We base our calculations of metabolism and energy use on rates of oxygen consumption and used the \dot{V}_{CO_2} data to test for selection-induced changes in respiratory exchange ratio ($\text{RER} = \dot{V}_{\text{CO}_2}/\dot{V}_{\text{O}_2}$). We found no statistical differences between S and C mice in average daily RER ($F_{1,6} = 3.49$, $P = 0.11$), RER at rest (i.e., estimated during the 5-min period of RMR; $F_{1,6} = 0.22$, $P = 0.65$), or RER during maximum vol-

Table 1: Effects of selective breeding for high voluntary wheel running (S vs. C lines), variation among replicate lines, and sex differences while controlling for effects of body mass

	$P_{\text{selection}}$	P_{lines}	P_{sex}^a	$P_{\text{body mass}}$	$P_{\text{sex} \times \text{selection}}^a$
Body mass	.0010	.1398	<.0001		.0838
$\dot{V}_{\text{O}_2\text{vm}}$.0015	1	.6270	<.0001	.7014
DEE	.0475	.0066	.0502	<.0001	.2579
RMR	.1411	.8674	.0017	<.0001	.4964
DEE – RMR	.0634	.0002	.6938	.0068	.2578
V_{max}^1	.0012	.4976	.9619	.4480	.1573
V_{peak}^b	.0010	.7057	.9309	.9163	.0474
V_{mean}^b	.0011	1	.6069	.9881	.4883
D_{run}	.0104	.0477	.4405	.4551	.8310
T_{run}	.3916	<.0001	.3876	.4798	.2038
N_{bouts}^b	.0212	.2712	.0334	.3006	.0487
$\text{BD}_{\text{mean}}^b$.0017	1	.0149	.7588	.3896
BD_{max}^b	.0063	.6672	.1527	.5355	.3143

Note. Comparisons between S and C lines in the nested model included sex as a fixed factor (SAS PROC MIXED). Age was not included in the model because it was highly correlated with mass when sexes were pooled (i.e., males were considerably older and heavier than females). All P values are for two-tailed tests, and values significant at $P < 0.05$ are in bold. See text for descriptions of variables and abbreviations.

^a Tested over the sex × line interaction ($df = 1, 6$).

^b Analyses performed with log-transformed data (see text).

Table 2: Adjusted least squares means and standard errors for metabolic and locomotor traits based on separate analyses of males and females

	Selected	Control	S/C	$P_{\text{selection}}$	P_{lines}	$P_{\text{body mass}}$
Males ($N = 49$):						
$\dot{V}_{O_2\text{vm}}$ (mL O ₂ /min)	5.333 ± .137	4.592 ± .149	1.16	.020	1	.008
DEE (mL O ₂ /min)	2.563 ± .152	2.085 ± .155	1.23	.088	.047	.019
RMR (mL O ₂ /min)	.923 ± .042	.817 ± .045	1.13	.190	1	.002
DEE – RMR (mL O ₂ /min)	1.640 ± .131	1.269 ± .132	1.29	.110	.006	.154
$V_{\text{max}1}$ (m/min) ^a	30.87 ± 1.13	22.25 ± 1.16	1.39	.002	.185	
$V_{\text{max}1}$ (m/min)	30.25 ± 1.19	22.93 ± 1.24	1.32	.009	.339	.278
V_{peak} (m/min) ^{a,b}	35.81 [33.46, 38.33]	30.23 [28.24, 32.37]	1.20	.004	.154	
V_{peak} (m/min) ^b	35.96 [33.78, 38.35]	30.39 [28.23, 32.04]	1.18	.014	.140	.676
V_{mean} (m/min) ^{a,b}	18.25 [16.75, 19.88]	13.12 [11.95, 14.40]	1.39	<.001	1	
V_{mean} (m/min) ^b	18.51 [17.24, 19.87]	12.91 [11.97, 13.93]	1.43	.003	1	.507
D_{run} (m) ^a	7,511.9 ± 1049.4	3,923.4 ± 1055.9	1.91	.053	<.0001	
D_{run} (m)	7,357.8 ± 1086.2	4,081.7 ± 1095.2	1.80	.088	.0001	.628
T_{run} (min) ^a	357.9 ± 52.2	252.6 ± 52.4	1.42	.205	<.0001	
T_{run} (min)	356.9 ± 54.3	253.9 ± 54.8	1.41	.247	<.0001	.934
N_{bouts}^b	463 [326,655]	565 [399,802]	.82	.424	.057	.637
BD _{mean} (s) ^b	45.3 [38.5, 53.3]	27.7 [23.2, 33.1]	1.63	.012	1	.719
BD _{max} (s) ^b	427.3 [314.0, 581.3]	259.5 [190.5, 353.7]	1.65	.061	.417	.784
Females ($N = 47$):						
$\dot{V}_{O_2\text{vm}}$ (mL O ₂ /min)	4.351 ± .093	3.687 ± .090	1.18	.004	1	.003
DEE (mL O ₂ /min)	2.174 ± .060	2.052 ± .059	1.06	.239	.703	.012
RMR (mL O ₂ /min)	.939 ± .032	.876 ± .031	1.07	.252	.586	<.001
DEE – RMR (mL O ₂ /min)	1.238 ± .050	1.174 ± .049	1.05	.432	.492	.446
$V_{\text{max}1}$ (m/min) ^a	35.04 ± 1.65	21.90 ± 1.62	1.60	.001	.911	
$V_{\text{max}1}$ (m/min)	34.56 ± 1.96	22.36 ± 1.91	1.54	.008	.922	.640
V_{peak} (m/min) ^{a,b}	39.46 [36.15, 43.08]	27.53 [25.27, 30.00]	1.43	.001	1	
V_{peak} (m/min) ^b	39.16 [35.17, 43.59]	27.74 [24.99, 30.79]	1.43	.005	1	.934
V_{mean} (m/min) ^{a,b}	20.96 [18.22, 24.11]	13.14 [11.46, 15.07]	1.59	.003	1	
V_{mean} (m/min) ^b	20.97 [17.67, 24.89]	13.13 [11.12, 15.51]	1.59	.010	1	.840
D_{run} (m) ^a	9,639.2 ± 630.9	5,832.5 ± 617.6	1.65	.005	1	
D_{run} (m)	9,099.0 ± 741.2	6,350.2 ± 721.3	1.43	.057	1	.184
T_{run} (min) ^a	388.3 ± 26.4	384.7 ± 26.1	1.01	.927	.252	
T_{run} (min)	367.8 ± 27.6	404.1 ± 27.0	.91	.423	.358	.107
N_{bouts}^b	228 [170,307]	471 [353,629]	.48	.022	1	.818
BD _{mean} (s) ^b	95.3 [72.9, 124.4]	48.8 [37.6, 63.26]	1.95	.019	1	.844
BD _{max} (s) ^b	722.3 [563.7, 925.5]	420.7 [330.6, 535.4]	1.72	.033	1	.202

Note. Significance of the effects of selection history (S vs. C), line, and body mass are from nested ANCOVA, including age as a covariate. Adjusted means ± SEs were calculated for a male of 37.4 g (125 d of age) and a female of 25.3 g of 71 d of age. P values are for two-tailed tests, and significant values are in bold. See text for descriptions of variables and abbreviations.

^a Body mass not included in the model.

^b Values are back transformed from log means obtained in the mixed model; back-transformed 95% confidence intervals are shown in brackets.

untary \dot{V}_{O_2} ($F_{1,6} = 3.35$, $P = 0.12$). However, in all cases females had significantly lower RER than males, and the sex × line type interaction was never significant. Although statistical comparisons were not presented, Figure 1 of Kane et al. (2008) also shows that females had lower RERs than males under basal conditions (fasted 3 h) at 23 mo of age. However, a study of one S and one C line by Vaanholt et al. (2008) did not show sex differences for RER over 24 h on either a high-carbohydrate or a high-fat diet (see their Tables 2 and 3; again, statistical comparisons were not presented).

Body Size, Resting Metabolic Rate, and Daily Energy Expenditure

Body mass was significantly smaller in S lines regardless of sex, and males were significantly larger than females (Table 1). Mean mass was 23.5 ± 0.5 g for S females versus 27.1 ± 0.6 g for C females; S males averaged 34.5 ± 0.6 g, whereas C males weighed 40.8 ± 0.7 g. Accordingly, we controlled for mass and sex when comparing among behavioral and metabolic traits.

Both body mass and sex (females higher) strongly affected

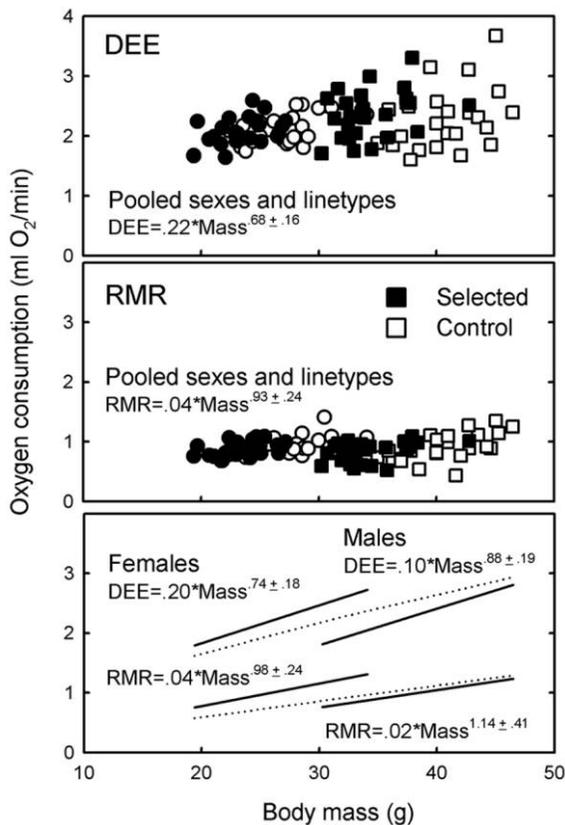


Figure 2. Relationship between body mass, resting metabolic rate (RMR), and daily energy expenditure (DEE) for 49 males (squares) and 47 females (circles). Symbols are raw values for DEE (top; the average energy expenditure for the entire 23.5-h period) and RMR (middle; the lowest 5-min average in the entire trial). After log transformation, slopes (\pm SE) and intercepts were calculated from one- or two-way nested ANCOVAs controlling for age and running activity by including D_{run} as a covariate in DEE analyses. Regressions for DEE and RMR with both sexes and line types included (dotted lines) were calculated for a 96-d-old individual running 5,975.4 m (equations in top, middle). For sexes analyzed separately (but pooling line types), regressions (solid lines) were calculated for a mean age of 125 d and D_{run} of 5,116.5 m for males and 72 d and 6,870.8 m for females (bottom). Allometries appear to be straight lines, despite the linear axes, because the curvature is nearly imperceptible in this range of body mass. Equations for the complete models are shown in “Results.”

RMR, but line type (i.e., selection on voluntary running) had no effect in either sex (Tables 1, 2; Fig. 2). If we recalculate the partial regression coefficients from a model similar to the one in Table 1, a general equation that describes RMR in an S line male is

$$\log_{10} \text{RMR} = (-1.43 \pm 0.30) + (0.875 \pm 0.198) \log_{10} \text{mass},$$

where coefficients are shown \pm SE, body mass is in grams, and RMR is in milliliters of oxygen per minute. For females, 0.174 (± 0.038) would be added to the constant, and for a C line mouse, the constant would be decreased by 0.025 (± 0.021). Accordingly, after correcting for mass differences, the RMR of

females was about 49% higher than that of males, and the RMR of S mice was roughly 6% higher than that of C mice (not significant). A previous study of basal (i.e., fasted) metabolic rate in ICR mice from the same source that was used to establish the selection experiment discovered no statistical effect of sex (Dohm et al. 2001), so the difference found here for RMR may reflect the fact that males were much older than females (see also Kane et al. 2008).

Body mass also significantly affected DEE, and after correcting for running distance, line type did not. However, in contrast to RMR, DEE was not affected by sex (Table 1; Fig. 2). For DEE in an S line male, a generalized equation that corrects for running distance is

$$\log_{10} \text{DEE} = (-0.69 \pm 0.21) + (0.62 \pm 0.13) \log_{10} \text{mass} \\ + (0.015 \pm 0.002) D_{\text{run}},$$

where DEE is in milliliters of oxygen per minute and D_{run} is in kilometers. For a female, the value 0.0419 (± 0.0257) would be added to the constant, and for a C line mouse, the term 0.0008 (± 0.0158) would be added. Hence, at a given mass and running distance, female DEE is roughly 10% higher than that of males, and the DEE of C line mice is about 2% higher than that of S line mice, although neither difference is statistically significant.

These equations do not correspond exactly to the models in Table 1: to estimate partial regression coefficients, we log transformed data to compute allometric relationships, the sex \times line type interaction term was not included (it was never significant when included; $P > 0.1675$), and D_{run} was added as a covariate for DEE. Comparisons of DEE and RMR among S and C in Table 1 were performed with untransformed data, testing for a sex \times line type interaction, and testing for DEE did not control for differences in D_{run} (which differed significantly between S and C lines; Table 1).

Activity and Locomotor Costs

As previously reported for this sample of mice (Rezende et al. 2006b), individuals from S lines ran much more than C mice (Table 2); D_{run} in S and C lines was about 7.51 km versus 3.92 km in males (S 92% higher than C) and 9.64 km versus 5.83 km in females (S 65% higher than C). The change in running behavior was associated with changes in energy metabolism. Differences between S and C lines in DEE and overall activity costs (DEE – RMR) were statistically significant (one-tailed $P < 0.05$; Table 1) but proportionally considerably smaller than for D_{run} . In males, mass-adjusted DEE in S lines was 22.8% higher than in C lines (2.56 vs. 2.08 mL O₂/min), but in females it was only 5.8% higher (2.17 vs. 2.05 mL O₂/min). After controlling for the effects of body mass and selection history, all three metabolic indexes (minimum, mean, and maximum power output; RMR, DEE, $\dot{V}_{O_2\text{vm}}$, respectively) were significantly positively correlated with each other in both males and females (Table 3). S mice of both sexes had higher $\dot{V}_{O_2\text{vm}}$ than

Table 3: Pearson product-moment correlations between residual metabolic and locomotor traits

	DEE	RMR	$V_{\max 1}$	V_{peak}^a	V_{mean}^a	D_{run}	T_{run}	$N_{\text{bouts}}^{a,b}$	$BD_{\text{mean}}^{a,b}$	$BD_{\text{max}}^{a,b}$
Males ($N = 49$):										
$\dot{V}O_{2\text{vm}}$.647	.521	.530	.394	.587	.483	.368	-.089	.529	.567
DEE		.710	.223	.156	.330	.769	.830	.374	.413	.533
RMR			.223	.127	.171	.419	.467	.253	.171	.282
$V_{\max 1}$.688	.812	.340	.098	-.296	.621	.613
V_{peak}^a					.512	.215	.063	-.113	.320	.353
V_{mean}^a						.543	.236	-.391	.887	.749
D_{run}							.879	.211	.595	.609
T_{run}								.575	.330	.444
N_{bouts}^a									-.466	-.191
BD_{mean}^a										.837
Females ($N = 47$):										
$\dot{V}O_{2\text{vm}}$.769	.449	.335	.352	.460	.526	.205	-.209	.378	.145
DEE		.646	-.108	-.109	.087	.395	.535	.253	.035	-.210
RMR			-.141	-.161	-.125	-.023	.169	.191	-.129	-.275
$V_{\max 1}$.967	.900	.659	-.182	-.745	.730	.676
V_{peak}^a					.874	.621	-.174	-.719	.697	.652
V_{mean}^a						.801	-.079	-.817	.879	.722
D_{run}							.462	.371	.739	.519
T_{run}								.522	-.038	-.121
$N_{\text{bouts}} (N = 46)^{a,b}$									-.859	-.674
$BD_{\text{mean}} (N = 46)^{a,b}$.735

Note. Correlations were performed between residuals from one-way nested ANCOVA with line type as the grouping factor and lines nested within line types (see “Statistical Analyses”). Age and body mass were included as covariates. Statistically significant correlations after correcting for multiple comparisons (see “Statistical Analyses”) are shown in bold. See text for descriptions of variables and abbreviations.

^a Analyses performed with log-transformed data.

^b Influential point was removed (female 37255).

C mice (Table 2), consistent with the higher running speeds in S lines (Tables 1, 2). Additionally, $\dot{V}O_{2\text{vm}}$ was positively correlated to D_{run} and (in males only) to T_{run} . In summary, selection has resulted in a substantial increase in daily wheel-running distance in both sexes, but the effect on total DEE is substantial only in males.

After accounting for differences in body mass, activity costs (DEE – RMR) in females were not significantly different in S and C lines, but in males, activity costs were 29.2% greater in S lines (one-tailed $P = 0.055$; Table 2). Expressed as a percentage of DEE, activity costs were positively correlated with body mass ($F_{1,79} = 4.05$, two-tailed $P = 0.0475$) but were significantly lower in females (54.9 vs. 64.3%; $F_{1,6} = 13.21$, $P = 0.0109$; Fig. 3). No significant sex \times line type interactions were detected.

As previously reported (Rezende et al. 2006b), S and C mice do not differ in incremental COT (i.e., the slope of the relationship between speed and $\dot{V}O_2$) after accounting for differences in body mass. In our sample, the net DCOT was independent of body size, regardless of whether D_{run} was included in the model (Table 4). As for D_{run} and (in males) T_{run} , both selection history and line significantly affected DCOT, with S mice having considerably higher DCOT than C mice (mass-adjusted DCOT about 40.5% higher in S than in C in females

and 77% higher in S than in C in males; Table 4). As a fraction of DEE, DCOT was also higher in S mice than in C mice, although the difference was small (about 6.3%–8.7% of DEE in C lines vs. 8.7%–12.7% in S lines; Table 4; Fig. 3). There were also significant differences in DCOT among lines, both as a mass-adjusted value and as percent of DEE (Table 4).

In addition to DCOT, the other component of energy use in locomotion is postural costs, which are dependent on T_{run} but independent of the speed of running. Postural costs in our mice were independent of selection history and sex but were positively correlated to body mass and varied among lines (Table 4); T_{run} did not differ significantly among S and C females (Table 2), and T_{run} in S males was about the same as for S and C females (Tables 2, 4). Hence, C males, which ran for considerably less time than S males or females of either line type, tended to have lower postural costs than other groups for both mass-adjusted values or percent of DEE (Table 4). Differences in mass-adjusted postural costs approached significance in pairwise comparisons between C males and both S males and S females (two-tailed $P = 0.092$ and $P = 0.070$, respectively). Postural costs exceeded DCOT (both mass adjusted and percent of DEE) by roughly a factor of three in both sexes and line types, with the smallest difference in C males (Table 4; Fig. 3). These results show that postural costs encompass the largest

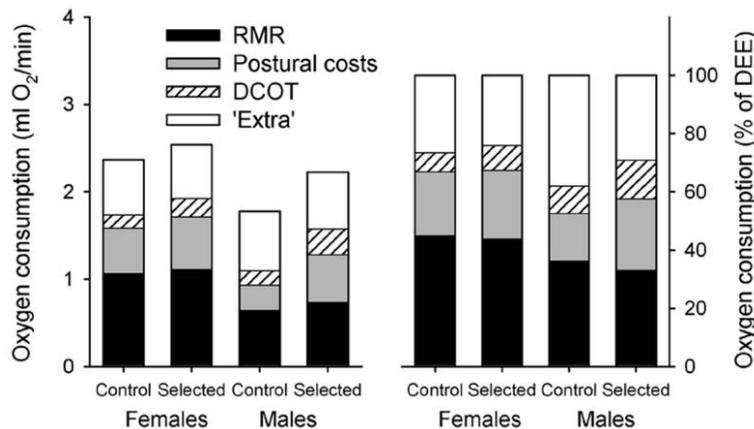


Figure 3. Contribution of resting metabolic rate (RMR), postural costs, and daily costs of transport (DCOT) to daily energy expenditure (DEE). Values are shown as average $\dot{V}O_2$ for the entire 23.5-h trial, adjusted for variation in body mass (*left*) and relative to DEE (= 100%; *right*). The total energy cost of running is the sum of postural costs and DCOT. “Extra” is the amount of DEE not included in RMR, postural costs, or DCOT, and it presumably results from thermoregulatory costs, energy spent on digestion, and activity out of the running wheel (see “Material and Methods”). Bars represent adjusted means for a 31.4-g individual calculated with SAS PROC MIXED from a two-way nested ANCOVA controlling for age and for sex \times line type interaction (but not for D_{run} ; i.e., model 2 in Table 3).

fraction of energy expenditure involved in wheel running in S and C lines whereas net costs of displacement are less important.

Although S mice ran almost twice as far per day as C animals, we did not find a consistent line-type effect on total running costs (DCOT + postural costs) in either sex (Table 5). Line type was not a significant predictor of total running costs in most models, although it approached significance in one analysis for females.

Running Behavior

Numerous aspects of running behavior differed between S and C line types, but some of these differences were dissimilar in males and females, and there was also considerable variance among the replicate lines. In S lines, females increased D_{run} mainly by increasing running speed with little change in running time, while in S males, both speed and T_{run} tended to increase (Table 2; Fig. 4; Rhodes et al. 2000; Rezende et al. 2006b). As expected, energy use was affected by running behavior: correlations between DEE and D_{run} or T_{run} were positive and highly significant in both sexes. After accounting for body mass differences, RMR was strongly positively correlated with T_{run} and D_{run} in males ($P < 0.001$ in both cases) but not in females (Table 3). Net activity costs were highly correlated with D_{run} in both sexes, and T_{run} (rather than speed) was the main factor accounting for this association after controlling for other covariates (Table 5). Similarly, relative activity costs (% of DEE) were significantly correlated with T_{run} in both sexes but not with running speed (Table 5).

Selective breeding increased the maximum (V_{peak} , V_{max1}) and the mean voluntary wheel-running (V_{mean}) speeds of both sexes (Fig. 5), with corresponding increases in power use during running (Fig. 6). All three speed indexes were positively cor-

related with maximum power output ($\dot{V}O_{2vm}$) but not with DEE or RMR. Selection also affected the number of running bouts (N_{bouts}) and their duration (both BD_{mean} and BD_{max} ; Table 2); sex also affected N_{bouts} and BD_{mean} . In both sexes, S mice had fewer running bouts (significantly in females; Table 2) but increased bout duration compared with C mice; D_{run} was also significantly correlated with N_{bouts} among females but not males. Interestingly, males but not females showed a strong positive association between T_{run} and BD_{mean} and BD_{max} . Both BD_{mean} and BD_{max} were negatively correlated with N_{bouts} in both sexes, although this association was weaker in males (not significant for BD_{max} ; Table 3). DEE was positively correlated with N_{bouts} in males but not in females. The same was true for correlations between DEE and bout duration (both BD_{mean} and BD_{max}), which were positive and significant only for males. In summary, energy costs seem to be primarily determined by the amount of time spent running rather than running speeds, and differences between males and females may stem from the different strategies adopted by each sex to run longer distances.

Discussion

The intent of this study was to examine behavioral and energetic consequences of selection-induced variation in voluntary running behavior. These issues are of broad importance for understanding the effects of behavioral evolution on energy budgets and vice versa. Physiologically, running is one of the most demanding and energetically expensive of all behaviors; therefore, it is intuitive to expect that behaviors involving extensive locomotion might comprise substantial portions of total DEE. Whether selection favoring increased running activity in any ecological or social context can result in correlated responses to improve running economy will depend on the effect of locomotion on total time and energy budgets. For instance, sep-

Table 4: Contribution of resting metabolic rate (RMR), postural costs, and daily costs of transport (DCOT) to daily energy expenditure (DEE), expressed in absolute values and relative to the total

	RMR (mL O ₂ /min)	Postural (mL O ₂ /min)	DCOT (mL O ₂ /min)	“Extra” (mL O ₂ /min)	RMR (% DEE)	Postural (% DEE)	DCOT (% DEE)	“Extra” (% DEE)
Model 1:								
Females C	1.079 ± .040	.560 ± .050	.181 ± .018	.616 ± .028	44.9 ± 1.4	22.8 ± 1.3	7.4 ± .7	24.9 ± 1.4
Females S	1.098 ± .056	.480 ± .062	.149 ± .024	.644 ± .039	47.3 ± 1.9	19.2 ± 1.7	6.5 ± .9	27.2 ± 1.9
Males C	.663 ± .061	.402 ± .065	.229 ± .026	.637 ± .043	35.0 ± 2.0	20.7 ± 1.9	10.7 ± 1.0	33.3 ± 2.1
Males S	.731 ± .035	.502 ± .046	.271 ± .016	.651 ± .024	34.7 ± 1.2	22.2 ± 1.2	11.8 ± .6	31.3 ± 1.2
<i>P</i> :								
Line type	.3830	.8578	.8731	.5355	.5311	.5280	.8840	.9365
Line	.8005	.0396	.3422	1	.5248	.3027	.1617	.8466
Body mass	<.0001	.0068	.3337	.0069	.0513	.8456	.2761	.1246
Sex	.0019	.4038	.0356	.7944	.0036	.8401	.0106	.0486
Sex × line type	.4586	.0868	.0481	.7610	.2385	.0448	.1669	.0902
<i>D</i> _{run}	.1327	<.0001	<.0001	.0033	<.0001	<.0001	<.0001	<.0001
Model 2:								
Females C	1.068 ± .040	.520 ± .081	.153 ± .034	.631 ± .028	45.8 ± 1.7	21.5 ± 2.3	6.3 ± 1.1	26.6 ± 2.0
Females S	1.117 ± .055	.599 ± .092	.215 ± .041	.617 ± .040	45.1 ± 2.2	23.2 ± 2.7	8.7 ± 1.3	23.6 ± 2.6
Males C	.646 ± .060	.291 ± .096	.168 ± .044	.661 ± .044	37.1 ± 2.4	17.0 ± 2.8	8.7 ± 1.5	36.6 ± 2.8
Males S	.740 ± .034	.544 ± .078	.297 ± .032	.639 ± .024	33.8 ± 1.6	23.6 ± 2.2	12.7 ± 1.0	29.9 ± 1.9
<i>P</i> :								
Line type	.1411	.0906	.0447	.5582	.3507	.1368	.0395	.0732
Line	.8674	<.0001	.0034	1	.0597	.0001	.0412	.0949
Body mass	<.0001	.0415	.8039	.0042	.0475	.9788	.2212	.4214
Sex	.0017	.2395	.3609	.6504	.0109	.5469	.1084	.0503
Sex × line type	.4964	.3011	.2573	.8619	.3783	.2883	.3715	.2872

Note. “Extra” refers to the fraction of DEE not accounted for by the previous estimates (see “Material and Methods”). Values are adjusted means ± SE obtained from SAS PROC MIXED independently for each trait, controlling or not for differences in total running distance (Model 1 and Model 2, respectively). Significance of each factor is listed for each trait, and values statistically significant (two-tailed $P < 0.05$) are in bold. Percentages were always approximately normally distributed, so no transformation was performed before analyses. See text for descriptions of variables and abbreviations.

arate selection experiments in rats and mice support the hypothesis of a positive genetic correlation between voluntary activity and maximum aerobic performance during forced exercise (Swallow et al. 1998; Rezende et al. 2005, 2006a; Waters et al. 2008), but it remains unclear to what extent increased wheel-running distances add additional costs to DEE. In this context, the results of our study are unique because we were able to directly estimate the associations between running behavior, locomotor performance, and energy budgets as well as how they evolve in response to selection for high activity levels.

Costs of Locomotion and Running Behavior

Although locomotion costs are frequently assumed to be a substantial portion of total energy use, broadscale comparative analyses by Garland (1983), Altmann (1987), and Baudinette (1991) predicted transport costs of roughly 1% of DEEs in mammals smaller than 100 g rising to perhaps 10%–15% of DEE in large, mobile carnivores. These surprisingly low values derive from calculations based on incremental costs of transport (iCOT; Fig. 1) and estimates of DMD in natural habitats (i.e., locomotor cost = iCOT × DMD); Garland (1983) termed this the “ecological cost of transport” (ECT). The ECT is useful

in an ecological context because its units (energy mass⁻¹ distance⁻¹) are independent of speed and, accordingly, its estimation does not require detailed information on locomotor behavior. An ECT amounting to ~1% of DEE seems unlikely to have much effect on resource allocation and hence aspects of Darwinian fitness, suggesting little potential for selection to improve running economy and little selection against increased mobility (at least from the standpoint of energy costs). However, our results indicate considerably higher locomotor costs. If running costs are calculated as DCOT (DCOT = iCOT × DMD; Table 4), then our mice expended from 6.3% to 12.7% of their DEE on locomotor behavior, with S mice having slightly higher DCOT than C mice (Fig. 3). These estimates are similar to previous reports of DCOT in mice from earlier generations of our selection experiment (4.4%–7.5% of DEE based on food consumption and wheel-running data; Kotaja et al. 1999b) and also resemble results from deer mice (*Peromyscus maniculatus*) tested in the same wheel respirometers (6.3% of DEE; Chappell et al. 2004) and from free-living golden-mantled ground squirrels (*Spermophilus saturatus*; 13%; Kenagy and Hoyt 1989).

What accounts for the contrast between the very low predicted ECT and the much higher measured DCOT? To some

Table 5: Relationship of activity and running costs with line type (S vs. C lines), total running distances, total running time, and average running speed in males and females

Model	df	Total Activity Costs (DEE – RMR)				Running Costs (DCOT + Postural Costs)			
		Net (mL O ₂ /min)		Relative (% DEE)		Net (mL O ₂ /min)		Relative (% DEE)	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Males:									
Line type	1, 6	3.51 ^b	.1102 ^b	.60	.4681	3.00 ^b	.1341 ^b	2.49	.1657
Line type	1, 6	.12	.7383	.04	.8469	.00	.9924	.24	.6394
<i>D</i> _{run}	1, 37	84.53	<.0001	6.94	.0122	345.5	<.0001	248.6	<.0001
<i>N</i> _{bouts}	1, 37	4.60	.0386	.34	.5616	21.3	<.0001	11.9	.0014
Line type	1, 6	.69	.4394	.02	.8935	.58	.4767	.46	.5217
<i>T</i> _{run}	1, 36	53.77	<.0001	3.85	.0575	261.8	<.0001	173.0	<.0001
<i>V</i> _{mean} ^a	1, 36	.10	.7571	.02	.8946	2.73	.1074	8.53	.0060
<i>N</i> _{bouts}	1, 36	1.05	.3127	.10	.7593	2.07	.1585	.45	.5057
Females:									
Line type	1, 6	1.70	.2403	.02	.8907	1.92	.2155	1.00	.3553
Line type	1, 6	.42	.5402	.57	.4806	1.88	.2195	.65	.4496
<i>D</i> _{run}	1, 35	21.81	<.0001	13.42	.0008	100.6	<.0001	124.8	<.0001
<i>N</i> _{bouts}	1, 35	5.31	.0272	1.50	.2292	35.3	<.0001	47.0	<.0001
Line type	1, 6	.86	.3884	.43	.5378	5.47	.0579	2.19	.1891
<i>T</i> _{run}	1, 34	23.38	<.0001	8.25	.0070	167.7	<.0001	228.1	<.0001
<i>V</i> _{mean} ^a	1, 34	.33	.5671	.77	.3858	6.18	.0180	17.85	.0002
<i>N</i> _{bouts}	1, 34	.37	.5496	.03	.8606	.02	.8970	2.09	.1574

Note. One-way ANCOVAs with replicate lines nested within line types (SAS PROC MIXED) were performed separately for males and females. Body mass and age were included as covariates in all models. Number of bouts was included in some models to control for variation in running behavior. All statistically significant effects had a positive sign. *P* values are from two-tailed tests. See text for descriptions of variables and abbreviations.

^a Log-transformed to improve normality.

^b Significant line differences ($P < 0.01$).

degree, methodological differences may be responsible. Estimates of running costs derived from treadmill tests might be biased relative to costs of running in the field for several reasons (see Altmann 1987; Karasov 1992; Corp et al. 1999; Rezende et al. 2006b). Similarly, running in wheels may have somewhat different costs from running over typical terrain, although wheel-derived slopes (*i*COT) are not substantially different from those obtained via treadmill testing (Chappell et al. 2004; Rezende et al. 2006b). Another possible problem is that running time in wheels is often tabulated in 1-min bins, which has been criticized for not accurately reflecting running behavior (Eikelboom 2001; Girard et al. 2001; Koteja and Garland 2001). However, the most likely cause of the ECT-DCOT discrepancy is estimates of movement distances. Some studies have argued that ECT calculations were biased due to underestimation of DMD in the field (Altmann 1987; see also Kenagy and Hoyt 1989; Baudinette 1991; Karasov 1992; Corp et al. 1999). For example, daily wheel running in 10–55-g murid rodents ranges from 3 to 16 km (Dewsbury 1980; Garland 2003; Table 2), but estimated DMD from field studies of small (<100 g) rodents is less than 1.0 km (Garland 1983; Carbone et al. 2005). It is certainly possible that lab-reared animals with ad lib. food, no risk of predation, and so on, run more than would be the case for free-living wild animals (see Sherwin 1998), but it seems unlikely that this alone could account for the severalfold dif-

ference between field and laboratory estimates of DMD, ECT, and DCOT. Clearly, accurate information on daily movements in natural habitats is a prerequisite for robust estimation of ECT for free-living animals, as was emphasized by Garland (1983).

A critical consideration for calculating the energetic consequences of running behavior is the so-called postural costs of locomotion: the elevation above resting metabolism of the intercept of the speed versus metabolic rate regression (see also Dlugosz et al. 2009). Postural costs are independent of speed and appear to be an unavoidable expense in studies of both forced (e.g., Taylor et al. 1982) and voluntary (Chappell et al. 2004; Rezende et al. 2006b) running, although they may be lower in the latter (Chappell et al. 2004, 2007). In our mice, postural costs were several times larger than DCOT in both S and C lines (Table 4; Fig. 3). Consequently, the total cost of running (DCOT + postural cost) comprised 26%–28% of the energy budget in C lines and 32%–36% of the energy budget in S lines. The greater running distance in S lines compared with C lines did increase running expenditures and DEE. However, those increases were proportionally quite small compared with the almost twofold difference in running distance between line types and generally did not attain statistical significance (Table 5). Similarly, at generation 10, Koteja et al. (1999b) found

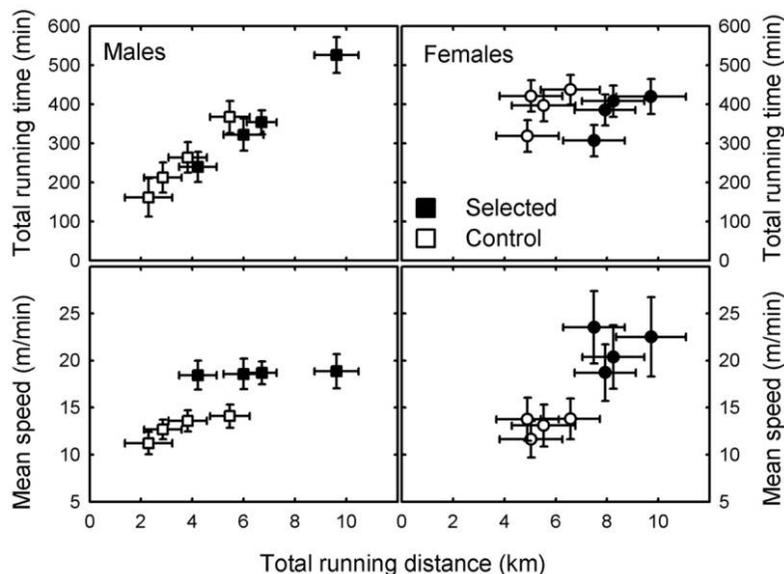


Figure 4. Relationship between mean values of total distance and time run and mean running speed (D_{run} , T_{run} , and V_{mean} , respectively) for each of the four selected and four control lines in males (left) and females (right). Adjusted means \pm SEs were calculated separately for each sex, with SAS PROC MIXED in a model with line type excluded and lines as the fixed effect and controlling for differences in body mass and age.

only a 4% difference in mass-adjusted daily food intake between S and C lines.

Given the high total cost of locomotion, has intense selection on wheel running affected the economy of running in S line mice? In absolute terms, yes: whole-animal costs of running are lower in S mice than C mice of both sexes (Fig. 3; Rezende et al. 2006b). However, this is caused by the considerably smaller size of S mice (Table 1). Postural costs and iCOT do not differ between S and C line types after accounting for differences in body mass (Rezende et al. 2006b). Therefore, although they have become smaller (among other morphological changes; Houle-Leroy et al. 2003; Garland and Freeman 2005; Swallow et al. 2005; Kelly et al. 2006; Middleton et al. 2008), the S lines apparently do not possess altered relations between speed, body mass, and metabolic power requirements.

Effects of Selection on Running Behavior

Given that most energy expenditure during running is due to time-dependent but speed-independent postural costs and relatively little to DCOT, a logical expectation is that selection for increased running distance should lead to increased running speed instead of increased running time. Working with earlier generations of the same S and C lines of mice, Koteja et al. (1999b; see also Swallow et al. 2001) suggested that substantial increases in DMD can be accomplished with proportionally much smaller increases in energy expenditure by running at faster speeds. Also, the absolute COT ($\dot{V}O_2/\text{speed}$, the energy cost of moving a given mass a given distance) is lowest at high speeds (Taylor et al. 1982), again suggesting that selection for

increased running distance should favor higher speeds instead of increases in running duration (Kenagy and Hoyt 1989). The running behavior of S line mice partially fulfilled those predictions: essentially all of the increase in daily wheel-running distance in S females was accomplished by speed increases with no change in running time (Table 2; Fig. 5). Males from S lines also showed substantial increases in running speed (Table 2; Fig. 5), but in contrast to predictions, they also tended to increase running time. The effect of selection on running time in males was not statistically significant in our tested mice, but higher running times for S males have been described in other recent generations of the selection experiment (e.g., Koteja and Garland 2001; Swallow et al. 2005; Kelly et al. 2006; T. Garland, unpublished data).

The prediction that most running should occur at high speed in order to minimize absolute COT (e.g., Kenagy and Hoyt 1989) was not met by either sex or line type. Instead, mice in all groups used a wide range of running speeds, with the frequency distributions of speeds used by S lines shifted upward relative to C lines (Fig. 5). Use of a broad range of speeds was also observed in voluntary running by deer mice (Chappell et al. 2004), gerbils (Chappell et al. 2007), and several wild-caught small mammals (M. A. Chappell, unpublished data). A possible explanation is that high postural costs relative to DCOT greatly reduce the potential energy savings of high-speed running and hence weaken selection for running primarily at high speeds.

Although mass-specific running economy apparently has not responded to selection, some details of running behavior have diverged in S and C lines. In addition to the substantial speed increase seen in S lines, the intermittency of running bouts is

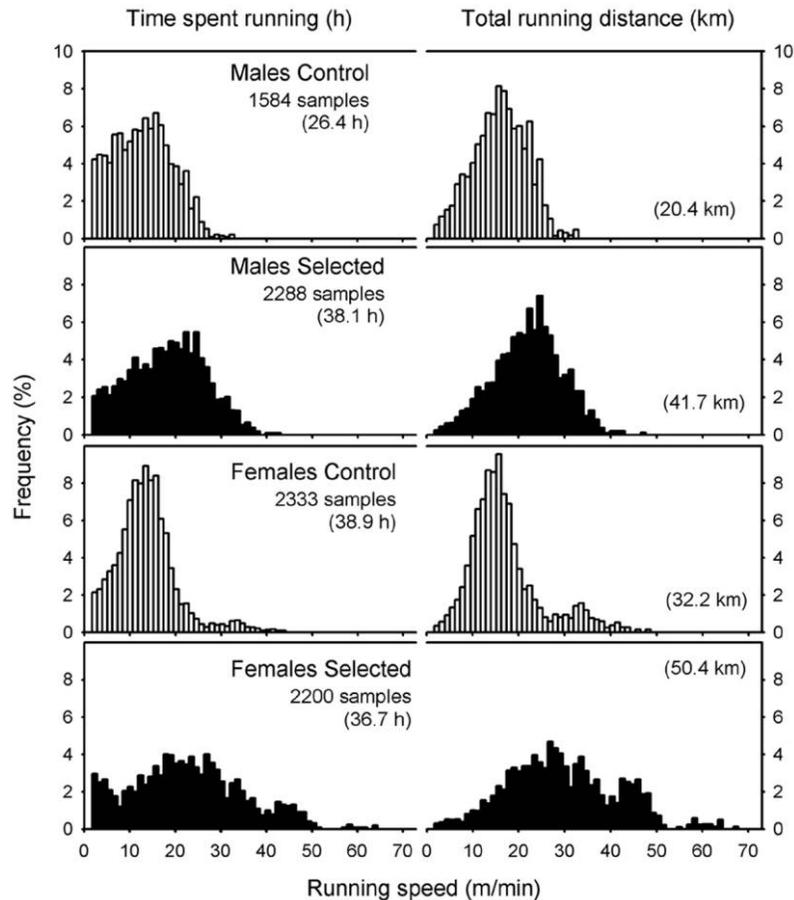


Figure 5. Distribution of voluntary running speeds in house mice selectively bred for high wheel-running behavior (S lines) and their nonselected control lines (C lines) expressed as percentages of time spent running and distance traveled at different speeds. Speed data are 1-min averages, based on samples from each animal, separated by at least 3 min to eliminate autocorrelation in the data (Chappell et al. 2004; Rezende et al. 2006b). The number of data points varied among individuals. Cumulative time and distance summed across all individuals are shown in parentheses. The bin size was 1.12 m/min (1 wheel rpm). For clarity and to avoid interpretive problems from electrical noise, speeds below 0.5 rpm are not shown.

higher in female S mice during peak running (highest 5 min; Girard et al. 2001), and in our sample both the mean duration and the maximum duration of bouts were higher in S lines (Table 2). Intermittency has been reported to improve some aspects of running performance (Weinstein and Full 1999). In contrast, one study of forced-exercise costs in house mice suggests that net costs of activity show an inverse relationship to bout duration because of excess postexercise oxygen consumption (Baker and Gleeson 1998). From that perspective, the longer bout durations in S mice might be expected to improve running economy, but this was not apparent in our data.

Sex Effects, Selection History, and Limits to Selection

After accounting for differences in running distances, the relative costs of activity and DCOT were significantly higher in males than in females, regardless of selection history (Table 4). The sexes differed in activity costs after controlling for differ-

ences in body mass, running time, running distance, mean speed, and N_{bouts} . Consistent with our results, incremental COT averaged 63% higher in the same males (Rezende et al. 2006b), and Koteja et al. (1999b) reported that the costs of both the activity measured per revolution and the mass-specific cost of locomotion tended to be higher in males. The cause of these sex differences is not known. There may be sex-specific differences in running behavior that cannot be detected by continuous records of wheel speed (e.g., coasting and intermittent activity; Eikelboom 2001; Girard et al. 2001). In the cohort of animals in this study, it is possible that some effects stem from methodological and age differences between sexes (see “Material and Methods”), but it is unclear how DCOT could be affected by age or reproductive experience (note that age effects on daily wheel-running distance are negligible within the range of ages studied here; Bronikowski et al. 2006). Videotape analyses from running males, as were obtained for females (Girard et al. 2001), will be necessary to resolve how sexes might differ in the details of running behavior. It is also important to note

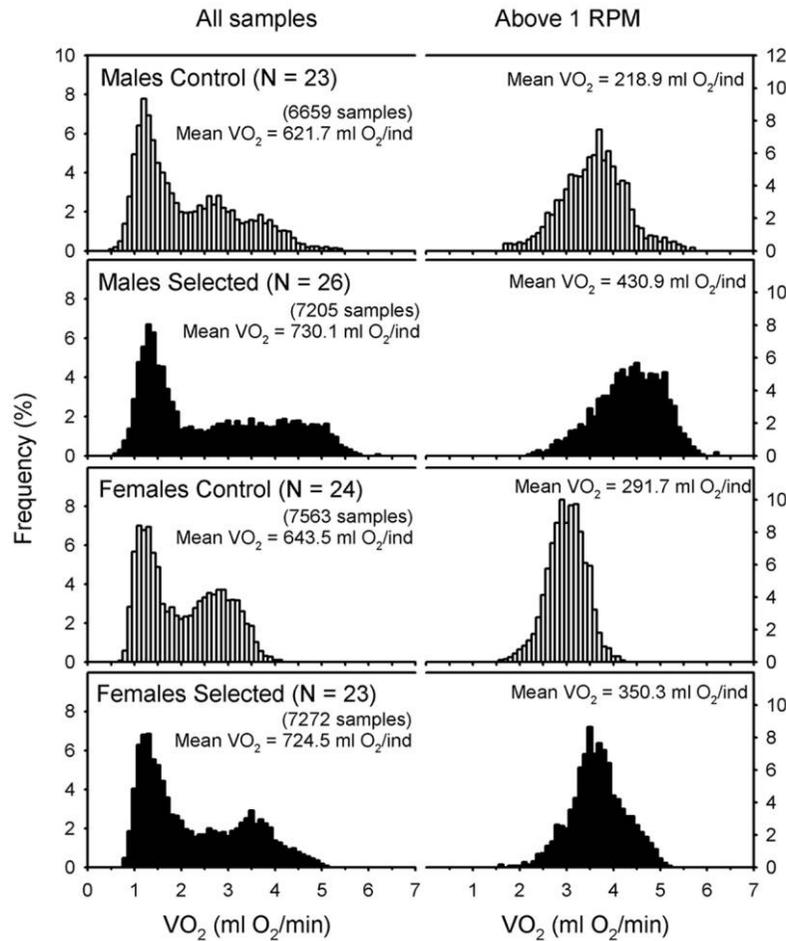


Figure 6. Distribution of oxygen consumption ($\dot{V}O_2$) during voluntary wheel-running trials for mice from lines selectively bred for high wheel running (S lines) and from nonselected control lines (C lines). Data are 1-min averages, with different points from each animal separated by 3 min, as in Figure 5. For males, $\dot{V}O_2$ values were adjusted to the mean body mass of 37.4 g by employing a scaling factor of $\text{mass}^{1.007}$ (the average of mass exponents for resting metabolic rate and daily energy expenditure; Fig. 1); for females the scaling factor was $\text{mass}^{0.864}$ and adjusted body mass was 25.3 g. Bin size was 0.1 mL O_2 /min. Despite size differences between the sexes, $\dot{V}O_2$ when wheels were not turning was similar for males and females. Males tended to have slightly higher $\dot{V}O_2$ at speeds above 1 rpm (probably because they were larger), and S mice attained higher $\dot{V}O_2$ than their C counterparts, regardless of sex (Table 1).

that behavioral observations (Koteja et al. 1999a) and pharmacological studies (Keeney et al. 2008) have suggested sex differences in the function of and/or motivation for wheel running.

Our results offer some insights into the apparent selection limit in wheel-running distance, which reached a plateau in S lines at approximately generation 16, despite continued selection for more than 30 additional generations (Garland 2003; T. Garland, unpublished data). Because their DCOT is higher than that of females, males may be more prone to energetic constraints during wheel running. Also, mean running speeds were positively correlated with DEE in males but not in females (Table 3). Previous studies show greater time spent running in S males than in C males despite the accompanying increase in postural costs (Thompson 1985; Kenagy and Hoyt 1989; Koteja et al. 1999b). Among the replicate selected lines, running time was the major factor determining differences in total running

distances for males (e.g., mean running speed in S males increased by about 40% above that of C males, but males from all four S lines converged to approximately the same mean running speed; Fig. 4). Taken together, these results suggest that even higher running speeds may not be possible for S males, which is particularly interesting because maximum metabolic rates during forced exercise evolved in response to selection primarily in males and to a lesser degree in females (Rezende et al. 2005, 2006a, 2006b). In females, selection has not significantly increased running times, and both S and C females spend about the same amount of time running per day as S males (Fig. 4; Tables 1, 2). The higher speeds in S females may explain a reduction in whole-animal DCOT in females but not males (Rezende et al. 2006b). These observations suggest that additional running time is in some way constrained for both sexes, possibly because of the need to sleep a given amount of time.

Implications for Ecological and Evolutionary Physiology

In summary, our results show that locomotion costs on wheels can form a large fraction of the energy budget of laboratory house mice. Our findings differ from allometric calculations of minimal locomotion expenditures (e.g., Garland 1983; Altmann 1987) for two primary reasons. First, daily running distances in our mice were much larger than published observations (or predictions) for wild small mammals, the reliability of which is uncertain. Our results emphasize the importance of accurate information on field behavior for calculating locomotion costs in nature. Second, our data include postural costs, which are not part of most COT estimates (DCOT, ECT). Because postural costs are severalfold greater than DCOT and seem to be unavoidable during locomotion, they are important to consider in comprehensive analyses of the role of locomotor costs in energy budgets.

The high proportion of postural costs in total transport expenditures leads to a somewhat counterintuitive conclusion: despite the importance of locomotion in the energy budget (30%–60% of DEE in our mice), large increases in running distance can be attained with little additional energy cost, provided that the increased distance is accomplished with faster speeds instead of increased running time. This suggests that the costs of increasing territory size, foraging distance, or mate-searching behavior may be relatively minor if accomplished by greater locomotor speed. Indeed, our sample of mice from lines bred for high voluntary wheel running showed little or no change in DEE or total locomotor costs compared with non-selected control lines (Tables 2, 5), despite running nearly twice as far per day.

Finally, selective breeding for increased daily running distance, although highly successful, had surprisingly few effects on the mass-specific economy of running. Despite expectations of energy-efficient cursorial adaptations in long-distance runners, we found no difference in the relationship between speed and power in S and C mice after accounting for differences in body mass (see also Rezende et al. 2006b). Selection also changed the pattern and duration of running bouts in ways that have been reported to save energy in other species, but these changes had little apparent effect on overall running economy.

From a broader perspective, our results are another example in which some expected outcomes of a selection experiment have not been realized (Bennett 2003; Garland 2003; Garland and Rose 2009). Other examples can be found in experiments on the evolution of desiccation resistance in *Drosophila* (Gibbs 1999; Bennett 2003), temperature adaptation in *Drosophila* (Huey and Rosenzweig 2009), and temperature adaptation in bacteria (Bennett and Lenski 1993, 2007).

Future Directions

To our knowledge, no study of rodents has yet attempted to partition energy expenditure between locomotion that occurs in wheels and that which occurs in the associated cage. Studies

with photobeams, force plates, and passive infrared detectors have shown that compared with those from the C lines, mice from the HR lines exhibit elevated cage activity when wheels are not available (Rhodes et al. 2001; Malisch et al. 2008, 2009; Vaanholt et al. 2008). Whether this occurs when wheels are available is unknown (see Rhodes et al. 2005 regarding the focal-animal observations in Koteja et al. 1999a). Also of interest is how activity in cages when wheels are available relates to the concept of nonexercise activity thermogenesis in human beings, defined in a recent review as “all energy expended due to activity excluding volitional exercise” (Novak and Levine 2007, p. 924). Those authors have argued further that “animal [sic] do not have volitional exercise *per se*.” To the contrary, we believe that voluntary wheel running in rodents may be a reasonable model of human “volitional exercise” (see also Eikelboom 1999). For example, it has been argued that exercise can be addictive in both human beings and rodents (references in Eikelboom 1999; Rhodes et al. 2005). It thus becomes of considerable interest to explore simultaneously the effects of various genetic, physiological, pharmacological, and environmental manipulations on both cage activity and wheel running. Again, these sorts of studies apparently have not yet been performed with any rodents. Recently, we have discovered that a high-fat diet can have remarkable stimulatory effects on both cage activity when wheels are absent (Vaanholt et al. 2008) and voluntary wheel running (Meek et al. 2009a) in the S lines—but not in the C lines. This demonstrates that genes and environment can affect activity levels in an interactive fashion (and that these effects can differ between the sexes; Vaanholt et al. 2008).

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Literature Cited

- Alexander R.M. 2003. Principles of Animal Locomotion. Princeton University Press, Princeton, NJ.
- Altmann S.A. 1987. The impact of locomotor energetics on mammalian foraging. *J Zool (Lond)* 211:215–225.
- Baker E.J. and T.T. Gleeson. 1998. EPOC and the energetics of brief locomotor activity in *Mus domesticus*. *J Exp Biol* 280: 114–120.
- Baudinette R.V. 1991. The energetics and cardiorespiratory correlates of mammalian terrestrial locomotion. *J Exp Biol* 160: 209–231.

- Belke T.W. and T. Garland Jr. 2007. A brief opportunity to run does not function as a reinforcer for mice selected for high daily wheel-running rates. *J Exp Anal Behav* 88:199–213.
- Bennett A.F. 2003. Experimental evolution and the Krogh principle: generating biological novelty for functional and genetic analyses. *Physiol Biochem Zool* 76:1–11.
- Bennett A.F. and R.E. Lenski. 1993. Evolutionary adaptation to temperature. II. Thermal niches of experimental lines of *Escherichia coli*. *Evolution* 47:1–12.
- . 2007. An experimental test of evolutionary trade-offs during temperature adaptation. *Proc Natl Acad Sci USA* 104: 8649–8654.
- Bronikowski A.M., P.A. Carter, J.G. Swallow, I.A. Girard, J.S. Rhodes, and T. Garland Jr. 2001. Open-field behavior of house mice selectively bred for high voluntary wheel running. *Behav Genet* 31:309–316.
- Bronikowski A.M., T.J. Morgan, T. Garland Jr., and P.A. Carter. 2006. The evolution of aging and age-related physical decline in mice selectively bred for high voluntary exercise. *Evolution* 60:1494–1508.
- Bronikowski A.M., J.S. Rhodes, T. Garland Jr., T.A. Prolla, T. Awad, and S.C. Gammie. 2004. The evolution of gene expression in the hippocampus in response to selective breeding for increased locomotor activity. *Evolution* 58:2079–2086.
- Carbone C., G. Cowlishaw, N.J.B. Isaac, and J.M. Rowcliffe. 2005. How far do animals go? determinants of day range in mammals. *Am Nat* 165:290–297.
- Carter P.A., J.G. Swallow, S.J. Davis, and T. Garland Jr. 2000. Nesting behavior of house mice selected for increased wheel-running activity. *Behav Genet* 28:227–237.
- Chappell M.A., T. Garland Jr., E.L. Rezende, and F.R. Gomes. 2004. Voluntary running in deer mice: speed, distance, energy costs, and temperature effects. *J Exp Biol* 207:3839–3854.
- Chappell M.A., T. Garland Jr., G.F. Robertson, and W. Saltzman. 2007. Relationships among running performance, aerobic physiology, and organ mass in male Mongolian gerbils. *J Exp Biol* 210:4179–4197.
- Clobert J., T. Garland Jr., and R. Barbault. 1998. The evolution of demographic tactics in lizards: a test of some hypotheses concerning life history evolution. *J Evol Biol* 11:329–364.
- Corp N., M.L. Gorman, and J.R. Speakman. 1999. Daily energy expenditure of free-living male wood mice in different habitats and seasons. *Funct Ecol* 13:585–593.
- Dewsbury D.A. 1980. Wheel-running behavior in 12 species of muroid rodents. *Behav Processes* 6:271–280.
- Dlugosz E.M., M.A. Chappell, D.G. McGillivray, D.A. Syme, and T. Garland Jr. 2009. Locomotor trade-offs in mice selectively bred for high voluntary wheel running. *J Exp Biol* 212:2612–2618.
- Dohm M.R., J.P. Hayes, and T. Garland Jr. 2001. The quantitative genetics of maximal and basal rates of oxygen consumption in mice. *Genetics* 159:267–277.
- Dumke C.L., J.S. Rhodes, T. Garland Jr., E. Maslowski, J.G. Swallow, A.C. Wetter, and G.D. Cartee. 2001. Genetic selection of mice for high voluntary wheel running: effect on skeletal muscle glucose uptake. *J Appl Physiol* 91:1289–1297.
- Eikelboom R. 1999. Human parallel to voluntary wheel running: exercise. *Anim Behav* 57:F11–F12.
- . 2001. Bins, bouts and wheel-running speed. *Anim Behav* 61:679–681.
- Gammie S.C., N.S. Hasen, J.S. Rhodes, I. Girard, and T. Garland Jr. 2003. Predatory aggression, but not maternal or intermale aggression, is associated with high voluntary wheel-running behavior in mice. *Horm Behav* 44:209–221.
- Garland T., Jr. 1983. Scaling the ecological cost of transport to body mass in terrestrial mammals. *Am Nat* 121:571–587.
- . 1999. Laboratory endurance capacity predicts variation in field locomotor behavior among lizard species. *Anim Behav* 58:77–83.
- . 2003. Selection experiments: an under-utilized tool in biomechanics and organismal biology. Pp. 23–56 in V.L. Bels, J.-P. Gasc, and A. Casinos, eds. *Vertebrate Biomechanics and Evolution*. BIOS Scientific, Oxford.
- Garland T., Jr., A.F. Bennett, and E.L. Rezende. 2005. Phylogenetic approaches in comparative physiology. *J Exp Biol* 208:3015–3035.
- Garland T., Jr., A.W. Dickerman, C.M. Janis, and J.A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. *Syst Biol* 42:265–292.
- Garland T., Jr., and P.A. Freeman. 2005. Selective breeding for high endurance running increases hindlimb symmetry. *Evolution* 59:1851–1854.
- Garland T., Jr., F. Geiser, and R.V. Baudinette. 1988. Comparative locomotor performance of marsupial and placental mammals. *J Zool (Lond)* 215:505–522.
- Garland T., Jr., and S.A. Kelly. 2006. Phenotypic plasticity and experimental evolution. *J Exp Biol* 209:2234–2261.
- Garland T., Jr., and M.R. Rose, eds. 2009. *Experimental Evolution: Concepts, Methods, and Applications of Selection Experiments*. University of California Press, Berkeley.
- Gibbs A.G. 1999. Laboratory selection for the comparative physiologist. *J Exp Biol* 202:2709–2718.
- Girard I. 2001. Field cost of activity in the kit fox, *Vulpes macrotis*. *Physiol Biochem Zool* 74:191–202.
- Girard I. and T. Garland Jr. 2002. Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. *J Appl Physiol* 92:1553–1561.
- Girard I., M.C. McAleer, J.S. Rhodes, and T. Garland Jr. 2001. Selection for high voluntary wheel-running increases speed and intermittency in house mice (*Mus domesticus*). *J Exp Biol* 204:4311–4320.
- Girard I., E.L. Rezende, and T. Garland Jr. 2007. Leptin levels and body composition of mice selectively bred for high voluntary activity. *Physiol Biochem Zool* 80:568–579.
- Girard I., J.G. Swallow, P.A. Carter, P. Koteja, J.S. Rhodes, and T. Garland Jr. 2002. Maternal-care behavior and life-history traits in house mice (*Mus domesticus*) artificially selected for high voluntary wheel-running activity. *Behav Processes* 57: 37–50.
- Gomes F.R., E.L. Rezende, J.L. Malisch, S.K. Lee, D.A. Rivas,

- S.A. Kelly, C. Lytle, B.B. Yaspelkis III, and T. Garland Jr. 2009. Glycogen storage and muscle glucose transporters (GLUT-4) of mice selectively bred for high voluntary wheel running. *J Exp Biol* 212:238–248.
- Gorman M.L., M.G. Mills, J.P. Raath, and J.R. Speakman. 1998. High hunting costs make African wild dogs vulnerable to kleptoparasitism by hyenas. *Nature* 391:479–481.
- Harestad A.S. and F.L. Bunnell. 1979. Home-range and body weight: a reevaluation. *Ecology* 60:389–402.
- Hartmann J., T. Garland Jr., R.M. Hannon, S.A. Kelly, G. Muñoz, and D. Pomp. 2008. Fine mapping of “mini-muscle,” a recessive mutation causing reduced hind-limb muscle mass in mice. *J Hered* 99:679–687.
- Healy S.D., S.R. de Kort, and N.S. Clayton. 2005. The hippocampus, spatial memory and food hoarding: a puzzle revisited. *Trends Ecol Evol* 20:17–22.
- Houle-Leroy P., H. Guderley, J.G. Swallow, and T. Garland Jr. 2003. Artificial selection for high activity favors mighty mini-muscles in house mice. *Am J Physiol* 284:R433–R443.
- Hoyt D.F. and C.R. Taylor. 1981. Gait and the energetics of locomotion in horses. *Nature* 292:239–240.
- Huey R.B. and F. Rosenzweig. 2009. Laboratory evolution meets Catch 22: balancing simplicity and realism. Pp. 671–707 in T. Garland Jr. and M. R. Rose, eds. *Experimental Evolution: Concepts, Methods, and Applications of Selection Experiments*. University of California Press, Berkeley.
- Husak J.F., S.F. Fox, M.B. Lovern, and R.A. Van Den Bussche. 2006. Faster lizards sire more offspring: sexual selection on whole-animal performance. *Evolution* 60:2122–2130.
- Irschick D.J. and T. Garland Jr. 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Annu Rev Ecol Syst* 32:367–396.
- Kane S.L., T. Garland Jr., and P.A. Carter. 2008. Basal metabolic rate of aged mice is affected by random genetic drift but not by selective breeding for high early-age locomotor activity or chronic wheel access. *Physiol Biochem Zool* 81:288–300.
- Karsov W.H. 1992. Daily energy expenditure and the cost of activity in mammals. *Am Zool* 32:238–248.
- Karsov W.H. and C. Martinez del Rio. 2007. *Physiological ecology: how animals process energy, nutrients, and toxins*. Princeton University Press, Princeton, NJ.
- Keeney B.K., D.A. Raichlen, T.H. Meek, R.S. Wijeratne, K.M. Middleton, G.L. Gerdeman, and T. Garland Jr. 2008. Differential response to a selective cannabinoid receptor antagonist (SR141716: rimonabant) in female mice from lines selectively bred for high voluntary wheel-running behavior. *Behav Pharmacol* 19:812–820.
- Kelly S.A., P.P. Czech, J.T. Wight, K.M. Blank, and T. Garland Jr. 2006. Experimental evolution and phenotypic plasticity of hindlimb bones in high-activity house mice. *J Morphol* 267:360–374.
- Kelt D.A. and D.H. Van Vuren. 2001. The ecology and macroecology of mammalian home range area. *Am Nat* 157:637–645.
- Kenagy G.J. and D.F. Hoyt. 1989. Speed and time-energy budget for locomotion in golden-mantled ground squirrels. *Ecology* 70:1834–1839.
- Koteja P. and T. Garland Jr. 2001. Forum: response to R. Eikelboom. *Anim Behav* 61:F25–F26.
- Koteja P., T. Garland Jr., J.N. Sax, J.G. Swallow, and P.A. Carter. 1999a. Behavior of house mice artificially selected for high levels of voluntary wheel running. *Anim Behav* 58:1307–1318.
- Koteja P., J.G. Swallow, P.A. Carter, and T. Garland Jr. 1999b. Energy cost of wheel running in house mice: implications for coadaptation of locomotion energy budgets. *Physiol Biochem Zool* 72:238–249.
- . 2001. Maximum cold-induced food consumption in mice selected for high locomotor activity: implications for the evolution of endotherm energy budgets. *J Exp Biol* 204:1177–1190.
- Malisch J.L., C.W. Breuner, F.R. Gomes, M.A. Chappell, and T. Garland Jr. 2008. Circadian pattern of total and free corticosterone concentrations, corticosteroid-binding globulin, and physical activity in mice selectively bred for high voluntary wheel-running behavior. *Gen Comp Endocrinol* 156:210–217.
- Malisch J.L., C.W. Breuner, E.M. Kolb, H. Wada, R.M. Hannon, M.A. Chappell, K.M. Middleton, and T. Garland Jr. 2009. Behavioral despair and home-cage activity in mice with chronically elevated baseline corticosterone concentrations. *Behav Genet* 39:192–201.
- Malisch J.L., W. Saltzman, F.R. Gomes, E.L. Rezende, D.R. Jeske, and T. Garland Jr. 2007. Baseline and stress-induced plasma corticosterone concentrations of mice selectively bred for high voluntary wheel running. *Physiol Biochem Zool* 80:146–156.
- McLoughlin P.D. and S.H. Ferguson. 2000. A hierarchical pattern of limiting factors helps explain variation in home range size. *Ecoscience* 7:123–130.
- Meek T.H., J.C. Eisenmann, B.K. Keeney, R.E. Hannon, and T. Garland Jr. 2009a. High fat diet increases wheel running in mice selectively bred for high voluntary wheel running. *Integr Comp Biol* 49:e113.
- Meek T.H., B.P. Lonquich, R.M. Hannon, and T. Garland Jr. 2009b. Endurance capacity of mice selectively bred for high voluntary wheel running. *J Exp Biol* 212:2908–2917.
- Middleton K.M., S.A. Kelly, and T. Garland Jr. 2008. Selective breeding as a tool to probe skeletal response to high voluntary locomotor activity in mice. *Integr Comp Biol* 48:394–410.
- Novak C.M. and J.A. Levine. 2007. Central neural and endocrine mechanisms of non-exercise activity thermogenesis and their potential impact on obesity. *J Neuroendocrinol* 19:923–940.
- Oufiero C.E. and T. Garland Jr. 2007. Evaluating performance costs of sexually selected traits. *Funct Ecol* 21:676–689.
- Perry G., K. LeVerger, I. Girard, and T. Garland Jr. 2004. Locomotor performance and social dominance in male *Anolis cristatellus*. *Anim Behav* 67:37–47.
- Pontzer H. and J. Kamilar. 2009. Greater ranging associated

- with greater reproductive investment in mammals. *Proc Natl Acad Sci USA* 106:192–196.
- Rezende E.L. 2005. Experimental Evolution of Exercise Physiology in House Mice Selectively Bred for High Locomotor Activity. PhD diss. University of California, Riverside.
- Rezende E.L., F. Bozinovic, and T. Garland Jr. 2004. Climatic adaptation and the evolution of maximum and basal rates of metabolism in rodents. *Evolution* 58:1361–1374.
- Rezende E.L., M.A. Chappell, F.R. Gomes, J.L. Malisch, and T. Garland Jr. 2005. Maximal metabolic rates during voluntary exercise, forced exercise, and cold exposure in house mice selectively bred for high wheel-running. *J Exp Biol* 208:2447–2458.
- Rezende E.L., T. Garland Jr., M.A. Chappell, J.L. Malisch, and F.R. Gomes. 2006a. Maximum aerobic performance in lines of *Mus* selected for high wheel-running activity: effects of selection, oxygen availability, and the mini-muscle phenotype. *J Exp Biol* 209:115–127.
- Rezende E.L., S.A. Kelly, F.R. Gomes, M.A. Chappell, and T. Garland Jr. 2006b. Effects of size, sex, and voluntary running speeds on costs of transport in lines of laboratory mice selected for high wheel-running activity. *Physiol Biochem Zool* 79:83–99.
- Rhodes J.S., S.C. Gammie, and T. Garland Jr. 2005. Neurobiology of mice selected for high voluntary wheel-running activity. *Integr Comp Biol* 45:438–455.
- Rhodes J.S., G.R. Hosack, I. Girard, A.E. Kelley, G.S. Mitchell, and T. Garland Jr. 2001. Differential sensitivity to acute administration of cocaine, GBR 12909, and fluoxetine in mice selectively bred for hyperactive wheel-running behavior. *Psychopharmacology* 158:120–131.
- Rhodes J.S. and T.J. Kawecki. 2009. Behavior and neurobiology. Pp. 263–300 in T. Garland Jr. and M.R. Rose, eds. *Experimental Evolution: Concepts, Methods, and Applications of Selection Experiments*. University of California Press, Berkeley.
- Rhodes J.S., P. Koteja, J.G. Swallow, P.A. Carter, and T. Garland Jr. 2000. Body temperatures of house mice artificially selected for high voluntary wheel-running behavior: repeatability and effect of genetic selection. *J Therm Biol* 25:391–400.
- Sherwin C.M. 1998. Voluntary wheel running: a review and novel interpretation. *Anim Behav* 56:11–27.
- Sockol M.D., D.A. Raichlen, and H. Pontzer. 2007. Chimpanzee locomotor energetics and the origin of human bipedalism. *Proc Natl Acad Sci USA* 104:12265–12269.
- Storey J.D. and R. Tibshirani. 2003. Statistical significance for genome-wide experiments. *Proc Natl Acad Sci* 100:9440–9445.
- Swallow J.G., P.A. Carter, and T. Garland Jr. 1998. Artificial selection for increased wheel-running behavior in house mice. *Behav Genet* 28:227–237.
- Swallow J.G. and T. Garland Jr. 2005. Selection experiments as a tool in evolutionary and comparative physiology: insights into complex traits: an introduction to the symposium. *Integr Comp Biol* 45:387–390.
- Swallow J.G., P. Koteja, P.A. Carter, and T. Garland Jr. 1999. Artificial selection for increased wheel-running activity in house mice results in decreased body mass at maturity. *J Exp Biol* 202:2513–2520.
- . 2001. Food consumption and body composition in mice selected for high wheel-running activity. *J Comp Physiol* 171B:651–659.
- Swallow J.G., J.S. Rhodes, and T. Garland Jr. 2005. Phenotypic and evolutionary plasticity of organ masses in response to voluntary exercise in house mice. *Integr Comp Biol* 45:426–437.
- Taylor C.R., N. Heglund, and G.M.O. Maloiy. 1982. Energetics and mechanics of terrestrial locomotion. I. Metabolic energy consumption as a function of speed and body size in birds and mammals. *J Exp Biol* 97:1–21.
- Thompson S.D. 1985. Bipedal hopping and seed-dispersion selection by heteromyid rodents: the role of locomotion energetics. *Ecology* 66:220–229.
- Vaanholt L.M., B. De Jong, T. Garland Jr., S. Daan, and G.H. Visser. 2007a. Behavioral and physiological responses to increased foraging effort in male mice. *J Exp Biol* 210:2013–2024.
- Vaanholt L.M., I. Jonas, M. Doornbos, K.A. Schubert, C. Nyakas, T. Garland Jr., G.H. Visser, and G. van Dijk. 2008. Metabolic and behavioral responses to high-fat feeding in mice selectively bred for high wheel-running activity. *Int J Obes* 32:1566–1575.
- Vaanholt L.M., P. Meerlo, T. Garland Jr., G.H. Visser, and G. van Dijk. 2007b. Plasma adiponectin is increased in mice selectively bred for high wheel-running activity, but not by wheel running per se. *Horm Metab Res* 39:377–383.
- Vásquez R.A., L.A. Ebersperger, and F. Bozinovic. 2002. The influence of habitat on travel speed, intermittent locomotion, and vigilance in a diurnal rodent. *Behav Ecol* 13:182–187.
- Waters R.P., K.J. Renner, R.B. Pringle, C.H. Summers, S.L. Britton, L.G. Koch, and J.G. Swallow. 2008. Selection for aerobic capacity affects corticosterone, monoamines and wheel-running activity. *Physiol Behav* 93:1044–1054.
- Weibel E.R., L.D. Bacigalupe, B. Schmitt, and H. Hoppeler. 2004. Allometric scaling of maximal metabolic rate in mammals: muscle aerobic capacity as determinant factor. *Respir Physiol Neurobiol* 140:115–132.
- Weinstein R.B. and R. Full. 1999. Intermittent locomotion increases endurance in a gecko. *Physiol Zool* 72:732–739.