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

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Accepted 11/30/2007; Electronically Published 3/18/2008

ABSTRACT

The study of correlated evolution can lead to new insights about the inheritance patterns of complex traits. In order to better understand the evolution of metabolic rate, we tested whether voluntary activity levels and basal metabolic rate are genetically correlated in 90-wk-old mice (*Mus domesticus*) from replicated lines of the sixteenth generation of an artificial selection experiment for high early-age wheel-running activity. We measured basal rates of oxygen consumption and carbon dioxide production and also computed the respiratory exchange ratio. Half of the individuals from both selected and control lines had been allowed free access to running wheels since 4 wk of age, while the other half were in standard cages. This design allowed testing of hypotheses about (1) genetic correlations between voluntary activity and metabolic rate and (2) lifetime training effects on metabolic traits. Selection group did not have a significant effect on metabolic traits; therefore, this study does not support some of the implicit assumptions of the aerobic capacity model for the evolution of vertebrate energetics. Activity group also did not affect metabolic rate, indicating that lifetime training does not alter basal metabolism in these mice. However, strong replicate line-within-selection-group differences were detected, indicating the occurrence of random genetic drift. In females, this divergence in metabolic traits attributable to drift was independent of body mass, but in males it was probably caused by a correlated response to selection involving body mass. This study is the first to show such effects of random genetic drift on metabolic traits.

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Physiological and Biochemical Zoology 81(3):288–300. 2008. 1522-2152/2008/8103-07050$15.00
DOI: 10.1086/587093

Introduction

A major goal of evolutionary biology is to understand the processes involved in the historical evolution of complex traits. The evolutionary history of one such trait, endothermy in birds and mammals, has been debated for decades (reviews in Else et al. 2004; Frappel and Butler 2004; Grigg et al. 2004; Hillenius and Ruben 2004; Koteja 2004; Pörtner 2004; Seymour et al. 2004; Watanabe 2005). A key feature of endothermy in these lineages is high resting or basal metabolic rate (BMR). Another key feature of these groups is relatively high activity levels (e.g., as indicated by large home ranges and long daily movement distances). Thus, activity levels and BMR seem to have evolved in tandem during the origin of birds and mammals.

Correlated evolution of activity and BMR could have occurred in several ways (e.g., see Hayes and Garland 1995). One possibility is that selection simultaneously favored increases in both traits. Another possibility is that the two traits exhibited a positive genetic correlation. A genetic correlation might exist because the traits shared some sort of physiological mechanism. Such a mechanism might involve alterations in mitochondrial structure or changes in neurotransmitters or hormones that affect both activity and BMR (Hulbert and Else 2004). Another possibility is that selection for high activity levels entailed an increase in maximal aerobic metabolic rate, which in turn entailed an increase in BMR, as in the aerobic capacity model for the evolution of endothermy (Bennett and Ruben 1979; Hayes and Garland 1995). Thus, the aerobic capacity hypothesis is also based on the underlying assumption that metabolic rate responded to selection on activity because of positive genetic correlations among activity, metabolic rate, and Darwinian fitness.

Several studies have tested the aerobic capacity hypothesis (see Hayes and Garland 1995). Most of these studies have attempted to establish phenotypic correlations between maximum and basal metabolic rates in intraspecific (e.g., Chappell and Bachman 1995) or interspecific studies (e.g., Bozinovic 1992; Hinds and Rice-Warner 1992; Duttenhoffer and Swanson 1996). Though studies of this type are useful, phenotypic correlations cannot distinguish between genetic and environmental components of the correlation and are thus generally limited in their ability to predict evolutionary relationships among traits.

Discerning the historical evolution of a complex trait is empirically difficult (e.g., see Garland et al. 1997), and assessing whether a complex trait evolved through correlated responses to selection on a genetically correlated trait is burdened with all the complications of determining whether the genetic covariance is stable over long spans of evolutionary time (e.g., Steppan et al. 2002; Jones et al. 2003). However, understanding the current genetic relationships among related traits and testing their ability to evolve in a correlated fashion may lead to insights into whether a particular evolutionary path is genetically possible. Replicated selection experiments are an excellent technique for studying evolution because they can identify genetic correlations and because the environment can be carefully manipulated in order to test for the effects of different environments on correlations among characters (Hill and Caballero 1992; Garland and Carter 1994; Garland 2003; Fuller et al. 2005). With this technique, one trait can be directly selected; after several generations, replicate selected lines can be compared to replicate control lines to test for consistent correlated responses to selection in a variety of traits (e.g., Swallow et al. 1998*b*; Koteja et al. 1999; Carter et al. 2000; Feder et al. 2000; Houle-Leroy et al. 2000; Koteja et al. 2001; Malisch et al. 2007; Rezende et al. 2006*a*, 2006*b*). In addition, potentially correlated traits may be measured in different environments to test for interactions between the genetic history of lines (genotype) and the environment (e.g., Carter et al. 2000). Finally, the use of replicate lines within each selection group provids a way to test for the effects of random genetic processes, such as founder effects and genetic drift (Lynch 1994; Swallow et al. 1998*a*; Garland et al. 2002).

In this experiment, we tested for correlated responses to selection (indicative of genetic correlations), interactions between selection history and housing environment (genotype by environment, or G × E), and differences among replicate lines (random genetic processes) in metabolic traits of the house mouse (*Mus domesticus*). We studied four replicate lines that had been selectively bred for high voluntary wheel running for 16 generations and their four replicate control lines (Swallow et al. 1998*a*). In this model system of mice, differences in daily wheel-running activity were substantial after 10 generations of selection: mice from the selected lines ran, on average, over 70% more than individuals from the unselected control lines; this difference was due to the fact that mice from selected lines ran faster, though not for longer periods, than those from the control lines (Swallow et al. 1998*a*). In addition, correlated responses to this selection have been measured for several physiological traits. For example, mice from selected lines weighed 14% less than mice from the control lines after 14 generations of selection (Swallow et al. 1999). Selection significantly increased exercise-induced maximal oxygen consumption ($\dot{V}O_2 max$) and maximal carbon dioxide production (mass-adjusted values; Swallow et al. 1998*b*; Rezende et al. 2006*a*), increased baseline circulating corticosterone (Malisch et al. 2007) and adiponectin (Vaanholt et al. 2007) levels, and reduced body fat and increased glucose uptake in some muscles (Dumke et al. 2001; Swallow et al. 2001); selection in conjunction with long-term access to wheels increased muscle aerobic capacities compared with capacities of control mice without wheel access (Houle-Leroy et al. 2000). These studies indicate the existence of genetic correlations between wheel-running behavior and at least some metabolic pathways, providing support for the hypothesis that activity levels and aspects of metabolism may have evolved in tandem. Furthermore, Houle-Leroy et al. (2000) and Swallow et al. (2005) demonstrated interactions between selection group and wheel access, indicating that the presence of some correlated responses to selection depends on the environment that the animals experience. However, there have been no published studies on this system of mice that have looked for a correlation between evolution of basal metabolism and selection for wheel-running activity and no studies that look at the effect of early-age selection on the metabolism of aged mice.

Many studies have shown that exercise training can alter metabolism in nongenetic ways. For example, exercise training in humans can alter BMR (Dolezal and Potteiger 1998; Hunter et al. 2000). Wheel-running exercise has been shown to prevent an age-related decline in BMR (Ichikawa et al. 2000) and to increase $\dot{V}O_2 max$ in both rats and mice (Gleeson et al. 1983; Cartee and Farrar 1987; Lambert and Noakes 1990; Swallow et al. 1998*b*). In addition, prolonged moderate exercise has been shown to increase fat metabolism in opossums (Weber and O'Connor 2000), which might affect the respiratory exchange ratio (RER). Because of these results, the manipulated environmental variable in this experiment was the presence or absence of a running wheel over a period of 22 mo.

The metabolic traits measured in this experiment were BMR (represented by oxygen consumption), CO_2 production during BMR, and the RER during BMR. BMR, which applies to endotherms, measures energy expenditure in a resting, nongrowing, postabsorptive state within the thermoneutral zone (Schmidt-Nielsen 1997) and is one of the most widely used ways to quantify organismal metabolic status. By measuring carbon dioxide production, we were able to calculate RER for the mice. RER depends on substrate utilization; that is, RER changes depending on whether the organism is metabolizing fat, protein, or carbohydrates (Schmidt-Nielsen 1997).

We hypothesized that both genotype (selection group) and environment (wheel-access group) would affect the metabolic traits. We predicted that mice from the lines selected for high wheel-running activity would have a higher BMR than control individuals, based on the aerobic capacity hypothesis and that mice with access to wheels would have a higher BMR than sedentary mice because of age and training effects (Ichikawa et al. 2000). In addition, we hypothesized an interaction between genotype and environment, with the expectation that mice in the wheel-access environment would demonstrate stronger correlated responses to selection.

Methods

Experimental Design

The individuals used here were part of a larger experiment to measure ontogenetic changes of behavioral and physiological traits in mice that have experienced selection for high early-age voluntary wheel running and lifelong access to running wheels (Morgan et al. 2003; Bronikowski et al. 2006). Briefly, mice from each of the eight lines (four selected for high wheel-running activity, four nonselected as controls) of the sixteenth generation of the selection experiment were randomly assigned to either the active (wheel access) or sedentary (no wheel access) treatment group at 4 wk of age. Of these, 115 individuals (58 males, 57 females) were used for the metabolic comparisons. Because only a few individuals could be in the metabolic chambers each day, measurements lasted from week 90 through week 97. Thus, age was used as a covariate in the statistical model (see "Data Analysis").

Animal Care

Mice in this experiment were produced in a full-sib design from generation-15 breeders of the artificial selection experiment for increased early-age voluntary wheel-running activity (see Swallow et al. 1998*a*). Mice from the fifteenth generation of the selection experiment were sent from the University of Wisconsin to Washington State University and bred in the same manner as at Wisconsin. The selection protocol for this experiment is described elsewhere (Swallow et al. 1998*a*) but summarized here. Mice were provided wheel access, and the number of wheel revolutions was recorded when the mice were 6–8 wk old. The trait under selection was average number of wheel revolutions on days 5 and 6 of wheel exposure. Breeders for each generation in the selected lines were chosen by within-family selection to reduce inbreeding and maternal effects: the male and female offspring from each family with the highest average number wheel revolutions on those days were selected to breed. In addition, six additional individuals (the three males and the three females with the second-highest average wheel revolutions) were chosen from each line to ensure 10 families per line. To reduce inbreeding effects, sib mating was not allowed. In the control lines, one male and one female were randomly chosen from each family.

The pups produced were weaned at age 21 d and housed singly in standard polyurethane mouse cages. Pine shavings (1.5 cm) were provided; standard rodent chow (Teklad) and water were given ad lib. The mice in the active treatment were in cages (35 cm × 20 cm × 14 cm) with a wheel attached to the lid that was inset in the cage (0.7215-m circumference, 24-cm diameter). The number of revolutions was recorded on a calculator (Math Explorer, Texas Instruments); running totals were noted weekly. Mice in the sedentary treatment were housed in cages (28 cm × 18 cm × 13 cm) without an attached wheel. The photoperiod regime was 12L : 12D, with lights on at 0700 hours PST. All animal care protocols were approved by Washington State University Laboratory Animal Resource Center in accordance with Institutional Animal Care and Use Committee standards, protocol 2682.

Metabolic Measurements

BMR was calculated by measuring O_2 consumed in a flow-through respirometry apparatus. Because of concerns about the ability of aged mice to withstand long-term food deprivation (P. A. Carter, unpublished data), animals were fasted for only 3 h before metabolic measurements. However, as the mice were well into their inactive period when measurements were taken (all but three animals achieved BMR after the first 2 h of measurement, between 1200 and 1800 hours), animals were post-absorptive (Speakman and McQueenie 1996; Hammond et al. 1999; Mueller and Diamond 2001). Mice were selected randomly with respect to activity, selection line, sex, and family for each day's run, weighed, and placed in plastic respiration chambers (500 mL; Nalgene) that were then placed in an incubator at 33°C. The 33°C temperature is considered to be in the thermoneutral zone for laboratory mice (Jakobson 1981; Dohm et al. 2001). The experiment lasted 8 h, beginning at approximately 1000 hours PST and ending at approximately 1800 hours PST. Four to seven individuals were measured each day of the experiment. Room air was pumped through both a Drierite column (to remove water vapor) and a CO_2 scrubber (Ascarite) before passing into a mass flow controller (Cole-Palmer). Flow rate out of the mass flow controller was 1.6 L/min. The air stream was then split by a manifold chamber (Sable Systems), and each of the eight excurrent streams had a flow rate of approximately 200 mL/min (exact flow rates were

Table 1: *P* values from nested ANOVA for basal metabolic rate, CO_2 production, and respiratory exchange ratio for females, with body mass included as a covariate in the analysis

	BMR	CO_2	RER
Activity	.2293	.5063	.1956
Linetype	.7441	.3327	.6929
Line (linetype)	**.0450**	**.0350**	**.0070**
Linetype × activity	.6048	.3463	.5364
Line (linetype) × activity	.1490	**.0061**	.0541
Body mass	**.0001**	**.0001**	**.0011**
Age	.0921	**.0005**	.0706
Age^2	**.0020**	**.0002**	.2672
Time	.0606	.2857	.7186
$Time^2$	.5915	**.0420**	.3011
N	50	43	45
Active: ctrl. vs. sel.	.5224	.2494	.4411
Sedentary: ctrl. vs. sel.	.9465	.9009	.9215

Note. BMR = basal metabolic rate, RER = respiratory exchange ratio, ctrl. = control, sel. = selected. Bold type indicates *P* values that are significant at the 0.05 level. *N* = sample size.

Table 2: *P* values from nested ANOVA for basal metabolic rate, CO_2 production, and respiratory quotient for males, with body mass included as a covariate in the analysis

	BMR	CO_2	RER
Activity	.0734	**.0160**	.1171
Linetype	.6859	.3536	.2236
Line (linetype)	.1491	.5631	.0999
Linetype × activity	.8811	**.0144**	.0592
Line (linetype) × activity	.7227	.9258	.5915
Body mass	**.0062**	.0814	.2089
Age	.3216	**.0156**	.1009
Age^2	.2434	.3625	**.0020**
Time	.9129	.7503	**.0307**
$Time^2$	.6197	.3140	.0647
N	57	43	42
Active: ctrl. vs. sel.	.4848	.3105	**.0240**
Sedentary: ctrl. vs. sel.	.7172	**.0092**	.9029

Note. BMR = basal metabolic rate, RER = respiratory exchange ratio, ctrl. = control, sel. = selected. Bold type indicates *P* values that are significant at the 0.05 level. *N* = sample size.

recorded for the purpose of BMR calculations). Excurrent air from the chambers was sent through a TR-RM8 respirometer multiplexer (Sable Systems) and a second Drierite column before being subsampled at a rate of 100 mL/min before entering the CO_2 analyzer. The CO_2 analyzer was calibrated each morning with air from a known gas mixture (5% CO_2). The sample air was then scrubbed of CO_2 in a second Ascarite column before moving to the O_2 analyzer. Room air that had been scrubbed of water vapor and CO_2 was pumped through an O_2 analyzer as a control; the difference between the two values (sample air and control air) was the datum actually recorded.

The experiment was run for 8 h, cycling through all eight chambers (seven experimental chambers and a control chamber) every hour; thus, 7.5 min h^{-1} of data were recorded for each chamber, with a total of 60 min of data for each mouse chamber over the course of the 8 h. Data were collected with the Datacan software package (Sable Systems). Each chamber's 7.5-min data recording was examined in Datacan, and the interchamber transition (wash-out period) was removed. The mean of the resulting interval was used to calculate oxygen consumption and CO_2 production. BMR was considered to be an individual's lowest mean interval of the eight total measurement intervals per individual per day. RER was calculated by dividing the amount of oxygen consumed by the amount of CO_2 produced while BMR was achieved. To ensure that the BMR values did not represent an anomaly, the repeatability between the lowest 1-min period of oxygen consumption (BMR) and the second-lowest period of oxygen consumption was estimated for all individuals in the population. The repeatability was very high ($r = 0.938$, $P < 0.0001$), indicating that the estimates of BMR were not anomalies.

Data Analysis

Raw data (in mV) were converted to milliliters of O_2 consumed per hour or milliliters of CO_2 produced per hour by the Datacan analysis program (Sable Systems). These data files (in ASCII format) were then converted to an Excel file (Microsoft). We used the GLM procedure in SAS (ver. 6.12, SAS Institute) to conduct a split-plot nested ANOVA using Type III sums of squares. Males and females were analyzed separately because sex had a significant effect on wheel running even before the imposition of selection (Swallow et al. 1998*a*), and one to two individuals of each sex from the same family were used. The fixed main effects were linetype (selected vs. control) and activity group (sedentary vs. active). Line within linetype was a random main effect. Covariates used in all the analyses were age and the time of day that BMR was achieved. The square of the *z* score of age (age^2) and time of day ($time\ of\ day^2$) were used to allow for nonlinear effects of those variables. We included these variables to account for variation explained by these traits; however, we are primarily interested in the main effects of the experiment.

Because body mass has been shown to respond in a negative correlated fashion to selection for high wheel-running activity in these mice (Swallow et al. 1999), we performed each analysis twice, once with body mass as a covariate and once without, as we did in several previous studies with these mice (e.g., see Houle-Leroy et al. 2000; Thomson et al. 2002). The analyses that included body mass as a covariate statistically removed the effect of body mass's response to selection and thus estimated the evolutionary correlated response of each metabolic trait to changes in wheel running only. The analyses that do not include body mass as a covariate estimated the combined correlated

Table 3: *P* values from nested ANOVA for basal metabolic rate, CO_2 production, and respiratory exchange ratio for females, with body mass not included as a covariate in the analysis

	BMR	CO_2	RER
Activity	.4053	.2051	.8615
Linetype	.7634	.3865	.9864
Line (linetype)	.2244	.1038	.2893
Linetype × activity	.7606	.2782	.4261
Line (linetype) × activity	.2239	.5848	.2451
Age	.5207	.2582	**.0046**
Age^2	.1754	.0797	.9527
Time	.1517	.3749	.5717
$Time^2$	.4298	.5202	.1809
N	51	45	45
Active: ctrl. vs. sel.	.6790	.1138	.5720
Sedentary: ctrl. vs. sel.	.9959	.8556	.5426

Note. BMR = basal metabolic rate, RER = respiratory exchange ratio, ctrl. = control, sel. = selected. Bold type indicates *P* values that are significant at the 0.05 level. *N* = sample size.

Table 4: *P* values from nested ANOVA for basal metabolic rate, CO_2 production, and respiratory exchange ratio for males, with body mass not included as a covariate in the analysis

	BMR	CO_2	RER
Activity	.3477	.6503	.3928
Linetype	.5875	.2077	.2652
Line (linetype)	**.0001**	.1411	.1488
Linetype × activity	.7262	**.0413**	.1271
Line (linetype) × activity	.0735	.9288	.3079
Age	.1471	.2227	.0883
Age^2	.1212	.9252	**.0015**
Time	.1978	.3740	**.0434**
$Time^2$	.7566	.4952	**.0426**
N	53	44	42
Active: ctrl. vs. sel.	.6251	.5368	.0750
Sedentary: ctrl. vs. sel.	.3981	**.0041**	.7475

Note. BMR = basal metabolic rate, RER = respiratory exchange ratio, ctrl. = control, sel. = selected. Bold type indicates *P* values that are significant at the 0.05 level. *N* = sample size.

response of each trait to selection on wheel running itself and the negative response of body mass to selection on wheel running. We also conducted an analysis using body mass as the dependent variable, thereby testing for the effect of each of the covariates on body mass.

In all analyses, statistical outliers (identified as standardized residual values greater than 3.0) were removed from the analysis. Adjusted means and standard errors were calculated by the LSMeans command in the SAS GLM procedure, using all the covariates. Sample sizes for each of the analyses are given in Tables 1–5. In most analyses, one selected line (line 6) had to be removed because of low sample sizes.

Post hoc statistical power analyses (Dean and Voss 1999) were conducted. Given the sample sizes and observed within-group standard deviations, and setting the power at 0.94, the power analysis indicated that tests for the genotype × environment interaction analysis of BMR could be expected to detect group differences of 3.74 and 4.08 mL O_2 h^{-1} in females and males, respectively (a 7% and 6% difference, respectively). The analyses of CO_2 consumption could detect differences of 4.67 and 2.00 mL CO_2 h^{-1} in females and males, respectively (a 10% and 4% difference, respectively), and the analyses of RER could detect differences of 0.05 and 0.02 mL CO_2 mL^{-1} O_2 in females and males, respectively (a 6% and 3% difference, respectively).

Results

BMR

Descriptive statistics and sample sizes of all traits for all four experimental groups are presented for females (Table 6) and males (Table 7). The analysis of BMR including body mass as a covariate in the statistical model revealed no significant effects of either selection or wheel access in either sex (Tables 1, 2; Fig. 1, *top*). A significant effect of line within each selection group was found in females ($P = 0.045$; Table 1) but not in males (Table 2). As expected, body mass was a statistically significant covariate in both females ($P = 0.0001$; Table 1) and males ($P = 0.0062$; Table 2). Age^2 also had a significant effect on BMR in females ($P = 0.002$; Table 1) but not in males (Table 2). In females, when body mass was not included as a covariate in the statistical model, no independent variables were significant (Table 3). In males, a significant line-within-selection-group effect was detected ($P = 0.0001$; Table 4) for both selection groups.

Carbon Dioxide Production

The analysis of CO_2 production that included body mass as a covariate revealed no significant effects of selection or wheel access in females (Table 1; Fig. 1, *middle left*). A significant line-within-selection-group effect was measured in females; this result is entirely attributed to differences among control lines, with line 2 being significantly different from the other three control lines (Table 8). In males, the selection group × activity interaction was significant ($P = 0.0144$; Table 2; Fig. 1, *middle right*). Although selection groups did not differ significantly in the active environment, they did differ in the sedentary environment, with active mice having significantly lower CO_2 production than the control mice. Body mass was a significant covariate for females ($P = 0.0001$; Table 1) and marginally nonsignificant for males ($P = 0.0814$; Table 2). A significant effect of age in both females ($P = 0.005$; Table 1) and males ($P = 0.0156$; Table 2) was also detected. When body mass was removed from the analysis, no variables had significant effects on CO_2 production in females (Table 3). In males, the

Table 5: *P* values of nested ANOVA of body mass as a dependent variable

	Females	Males
Activity	.0767	.1259
Linetype	.5757	.0971
Line (linetype)	**.0009**	**.0038**
Linetype × activity	.9544	.4503
Line (linetype) × activity	.2748	.3000
Age	**.0035**	.7048
Age^2	**.0156**	.6083
Time	.8609	.6589
$Time^2$	.4545	.5230
N	51	57
Active: ctrl. vs. sel.	.4623	.1472
Sedentary: ctrl. vs. sel.	.3807	**.0448**

Note. RER = respiratory exchange ratio, ctrl. = control, sel. = selected. Bold type indicates *P* values that are significant at the 0.05 level. *N* = sample size.

Table 6: Descriptive statistics for females ($N = 52$)

	Control Active	Control Sedentary	Selected Active	Selected Sedentary
Mass mean (g)	32.92	34.98	31.40	33.09
Mass SD	4.29	3.33	3.60	3.52
Mass range (g)	26.18–43.31	30.63–42.26	25.72–37.50	28.35–39.24
Mass *N*	14	14	13	11
Age mean (d)	678.93	689.86	689.31	691.18
Age SD	6.21	12.21	9.70	10.04
Age range (d)	670–694	672–705	672–704	675–704
Age *N*	14	14	13	11
O_2 consumption *N*	14	14	13	11
CO_2 production *N*	11	11	12	11
RER *N*	11	11	12	11

Note. RER = respiratory exchange ratio.

significant genotype × environment interaction remained ($P = 0.0413$; Table 4).

RER

When body mass was included in the statistical analysis, no significant effects of selection or wheel access on RER were detected in either sex (Tables 1, 2; Fig. 1, *bottom*). Significant effects of line within each selection group were measured in females ($P = 0.005$; Table 8) but not in males. In males, a marginally nonsignificant G × E interaction for RER was measured ($P = 0.0592$; Fig. 1, *bottom right*), with the near-significant difference between selection group occurring in the active environment only. Interestingly, this effect was in the environment opposite the one for the significant G × E interaction for CO_2. Within the sedentary environment, control individuals had higher CO_2 production and O_2 consumption than selected individuals, but the ratio of CO_2 production to O_2 consumption (i.e., the RER, represented by the slopes) was not different between selection groups (Fig. 2, *bottom*). In the active environment, individuals in both selection groups had similar values of CO_2 production and O_2 consumption, but the ratio of CO_2 to O_2 differed, causing the marginally nonsignificant difference in RER (Fig. 2, *top*). Finally, body mass had a significant effect on RER in females ($P = 0.0011$; Table 1) but not in males ($P = 0.2089$; Table 2). In males, age^2 and time of day also had significant effects on RER (Table 2). When body mass was removed from the analysis, no main effects or G × E interactions were significant for either females or males (Tables 3, 4). Age was significant in females ($P = 0.0046$; Table 3); age^2, time of day, and time of day^2 were significant in males (Table 4).

Body Mass

When body masses among treatment groups were compared, no significant differences between the selected and control lines or between the active and sedentary groups were identified for either females or males (Table 5). A significant line-within-selection-group effect was measured in both females and males ($P = 0.0009$ and 0.0038, respectively; Table 5); this effect in both sexes was caused by differences among lines within both the selected and control groups (Tables 8, 9). Age and age^2 had a significant effect in females but not in males (Table 5).

Discussion

We found no significant differences in BMR in this study comparing four lines of mice selectively bred for high locomotor activity for 16 generations and their four replicate control lines, strongly suggesting that no genetic correlation between BMR and voluntary wheel running existed in the base population. Several hypotheses may be proposed to explain a lack of a genetic correlation between BMR and activity. First, BMR may now have insufficient genetic variation to evolve in these mice, an explanation that is difficult to assess because of conflicting evidence. On the one hand, enough genetic variance in BMR was present in the mice studied here to allow significant random genetic changes among lines within selection groups. On the other hand, previous work suggests that additive genetic variation for BMR in the base population from which these mice were derived may be very low (depending on the genetic model fitted; Dohm et al. 2001), and a recent study of a wild rodent also reported low heritability of BMR (Nespolo et al. 2005). In this case, genetic drift could occur by affecting alleles that had dominance effects, even if few or no alleles with additive effects were present in the population. Second, genetic correlations themselves can evolve, so a genetic correlation that existed many generations ago, such as when endothermy evolved, might no longer exist (Turelli 1988; Wilkinson et al. 1990; Shaw et al. 1995; Roff 2000). This hypothesis is unlikely in this case (although it cannot be ruled out), because a basic assumption of the aerobic capacity hypothesis is that maximal metabolic rates and BMRs share biochemical or physiological pathways. Under such circumstances, the underlying genetic correlations are not expected to change very much over time (Riska 1989; Stearns 1989; Hayes and Garland 1995). In addition, studies in other species have found a genetic correlation between basal

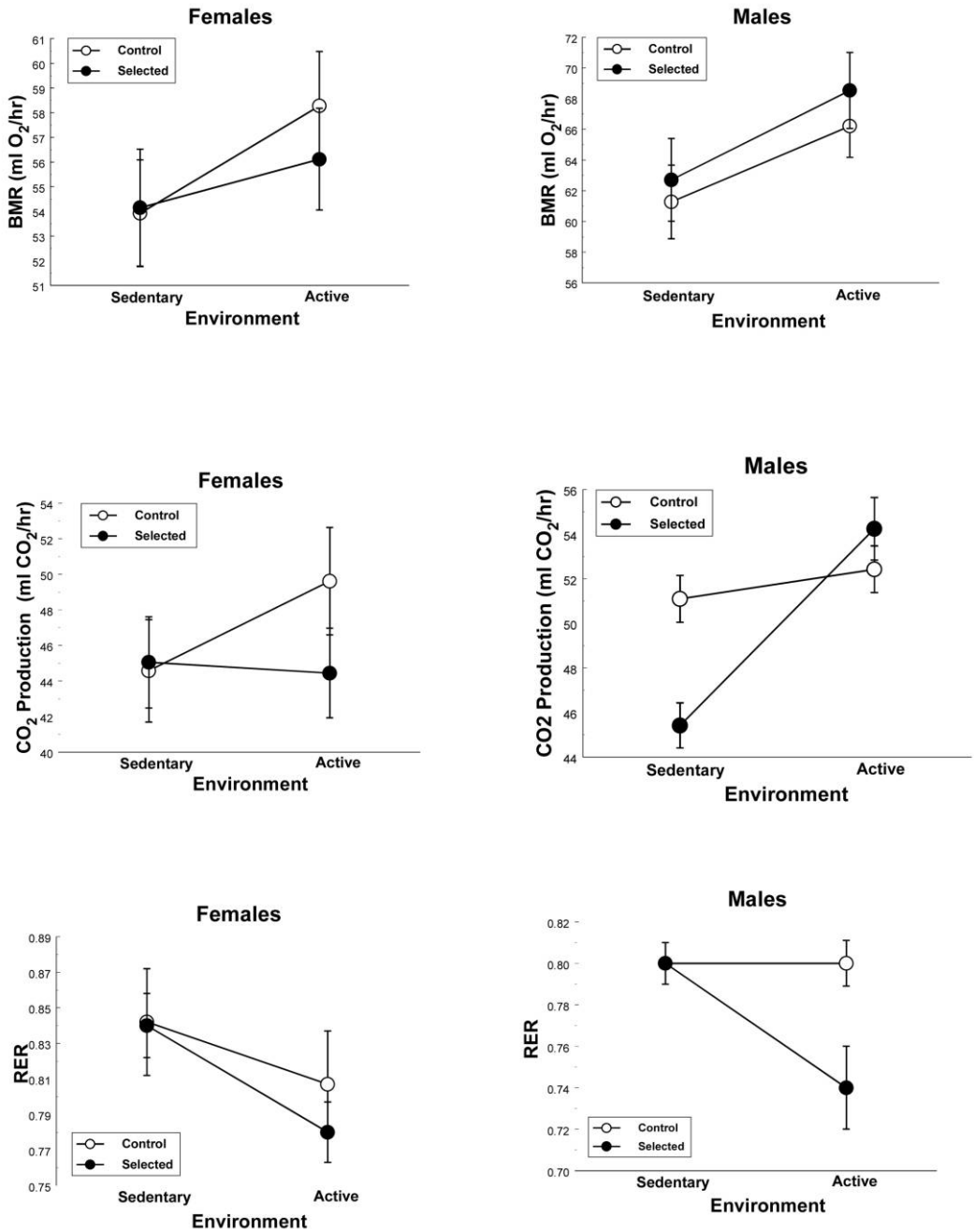

Figure 1. Least squared means and standard errors of basal metabolic rate (*BMR*) in females (*top left*) and males (*top right*), carbon dioxide production in females (*middle left*) and males (*middle right*), and respiratory exchange ratio (*RER*) in females (*bottom left*) and males (*bottom right*).

and maximal metabolic rates in wild populations (Sadowska et al. 2005). Third, previous work comparing wild and lab mice (Dohm et al. 1994) suggests that BMR may evolve more slowly than the behavioral trait under selection, high wheel-running activity. This result could occur if the genetic correlation between the two traits is low, and thus, a correlated response in metabolism would take longer to become apparent. However, this explanation seems unlikely, because wheel running in the selected lines now exceeds that measured in wild mice and because limits of selection for wheel running were within a few generations of being reached (Garland 2003). If BMR could evolve in a correlated fashion to selection for high wheel-running activity, then it would most likely have begun to do so by the generation at which these mice were measured. Finally, young animals may show a correlated response of BMR to selection, but older animals may not; indeed, McCarter and

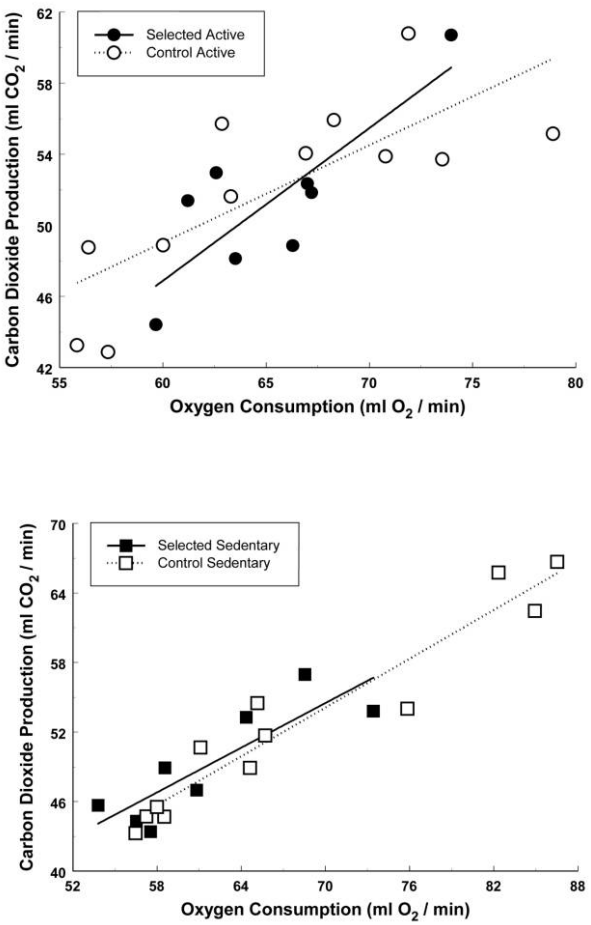

Figure 2. Scatterplot of carbon dioxide production and oxygen consumption with trend lines for males in the active environment (wheel access; *top*) and sedentary environment (cages without wheels; *bottom*).

Palmer (1992) demonstrated that metabolic profiles change over the lifetime of an animal. However, other results from this model system of selected mice indicate that BMR does not show an early-age response to selection either (T. Garland Jr., J. G. Swallow, and P. A. Carter, unpublished data), and a study of minimum resting (not fasted) metabolic rate found no difference between the selected and control lines at 2–4 mo of age (Rezende et al. 2006*b*). Thus, the simplest explanation is that no genetic correlation between activity and BMR currently exists in these mice.

One advantage to studying evolution with selection experiments is that the environment can be carefully manipulated; here, the effect on metabolic traits of the presence or absence of a running wheel was tested. Surprisingly, metabolic traits in these mice did not show a strong training effect when animals had wheel access, and wheel-running activity was not significantly correlated with any metabolic traits. This result is somewhat surprising because exercise has been shown to have profound effects on other aspects of metabolism, such as $\dot{V}O_2$max (e.g., Gleeson et al. 1983; Lambert and Noakes 1990). However, few studies have looked at the effect of training throughout ontogeny as this study did. Wheel running decreased over the life span of these animals, with steeper declines at older ages (Morgan et al. 2003; Bronikowski et al. 2006), so presumably any training effect seen in young mice might be stronger than that seen in 22-mo-old mice, although it may also be the case that early-life training may have persistent effects, given that the individuals continue to have access to wheels.

A possible reason for the presence of wheels to not have a strong effect may be that mice in both environments had similar activity levels but that their method of activity differed. Although non-wheel-running activity levels were not measured in this study, a study by Rhodes et al. (2001) found that selected mice from generations 23 and 24 had significantly higher levels of spontaneous cage activity than control individuals on the second day of testing (wheels were not provided), and this result has recently been confirmed for both sexes after 6 d of testing (T. Garland Jr., J. G. Swallow, and P. A. Carter, unpublished results). However, an earlier study of activity differences from generation 13 found that the only consistent difference in activity levels between mice in cages with freely rotating wheels and mice in cages with locked wheels was specifically wheel-related activity (Koteja et al. 1999). While it is difficult to make direct comparisons between these studies and ours, as control animals did not have access to running wheels in our study, it seems unlikely that cage activity itself affected BMR. Therefore, we believe that the lack of an effect of activity group probably was not due to similar activity levels between mice housed with and without wheels.

Another advantage to manipulating the environment in artificial selection studies is that the effects of G × E interactions can be measured. A strong G × E interaction was measured for CO_2 production in males: CO_2 production did not differ between selection groups with wheel access, but within the sedentary cages, individuals from the selected lines had significantly lower CO_2 production than those in the control lines (Fig. 1, *middle right*). Hence, CO_2 production showed a negative correlated response to selection for high wheel-running activity, but it was only in the sedentary environment.

We also detected a marginally nonsignificant selection group by environment interaction in male RER ($P = 0.0592$); however, in this case, the difference between selection groups was in the cages with wheels (Fig. 1, *bottom right*) and was not being driven by the significant G × E interaction for CO_2 production, which occurred in the sedentary environment. Low RER (approaching 0.7) indicates a greater percentage of fat metabolism, relative to protein and carbohydrate metabolism (Schmidt-Nielsen 1997). This difference cannot be attributed to diet, because all individuals ate the same food. Therefore, selected active mice may have a tendency to use fat stores to a greater degree than control active mice. This hypothesis is consistent with a previous study on metabolic enzymes in these groups of mice; selected active mice were found to have generally higher carnitine palmitoyltransferase activity levels than

Table 7: Descriptive statistics for males ($N = 52$)

	Control Active	Control Sedentary	Selected Active	Selected Sedentary
Mass mean (g)	37.10	40.37	34.93	38.62
Mass SD	2.91	3.49	1.79	4.41
Mass range (g)	32.33–42.59	35.52–49.56	32.71–37.70	30.59–56.06
Mass *N*	16	14	10	12
Age mean (d)	683.38	681.43	684.70	684.67
Age SD	7.64	7.83	10.49	8.58
Age range (d)	673–699	671–697	672–701	675–705
Age *N*	16	14	10	12
O_2 consumption *N*	16	14	10	12
CO_2 production *N*	14	13	8	11
RER *N*	14	13	8	11

Note. RER = respiratory exchange ratio.

sedentary mice, suggesting a higher rate of lipid oxidation (Houle-Leroy et al. 2000). Other studies also suggest that metabolic profiles may change with chronic activity levels and that moderate exercise leads to higher lipid oxidation rates (Treuth et al. 1995; Roberts et al. 1996; Weber and O'Connor 2000).

The strongest effect on metabolic traits seemed to be caused by random genetic factors, independent of selection group. In this experiment, we were working with a finite number of populations (four within each treatment group) each with a relatively small effective population size ($N_e \sim 34$; T. Garland Jr., J. G. Swallow, and P. A. Carter, unpublished data). Although in most cases only one line was significantly different from others within its selection group, the variation suggests that random genetic factors can influence metabolic traits. Differences among lines within a selection group could be the result of genetic drift, founder effect, or variable selection within the high-activity selected lines (Garland and Carter 1994; Lynch 1994). In both males and females, most of the statistically significant variation among lines was within the control group, suggesting that variable selection did not have a strong effect within the selected lines. In females, significant line effects when body mass was included as a covariate suggest that random genetic changes in metabolic traits are independent of body mass. In males, on the other hand, a line difference in BMR was detected only when body mass was not included as a covariate. This result indicates that the difference in BMR in males among the lines is primarily driven by founder effect or genetic drift in mass among the lines and that the metabolic trait evolved in a correlated fashion because of the strong genetic correlation between metabolic traits and body mass.

To compare these results with those expected under standard population genetic theory, we applied Lande's estimate of drift of a quantitative trait from the mean (Lande 1976):

$$V = \frac{V_a}{N_e}.$$

Earlier work on this system of mice indicated that the heritability of BMR is 0.10 or less (Dohm et al. 2001). This heritability estimate, in conjunction with the estimate of effective population size described above and the phenotypic variance measured in this study, provides an estimate of among-line variance of 4%–8%, with more variation in control lines and females. The actual amount of divergence among lines from

Table 8: Least squares means of each line for metabolic traits in females, with body mass either included as a covariate or excluded from the model, and of body mass as the dependent variable

	BMR		CO_2 Production		RER		
Line	Mass Included	Mass Excluded	Mass Included	Mass Excluded	Mass Included	Mass Excluded	Mass
1	*60.69*	63.46	50.03	52.78	.85	.82	*37.09*
2	53.87	53.22	**41.97**	42.76	*.78*	.78	*32.57*
4	52.39	56.93	49.23	48.48	.85	.82	*36.18*
5	57.44	54.51	47.13	45.51	.81	.82	*31.11*
3	55.21	59.56	44.75	45.53	.79	.78	33.84
7	59.42	55.07	46.00	42.60	.76	.79	**30.07**
8	50.75	53.59	43.49	46.26	**.88**	.85	34.79

Note. Lines 1, 2, 4, and 5 are control lines; lines 3, 7, and 8 are selected lines. Values in bold are significantly different from all other lines in their selection group ($P < 0.05$). Values in italics are significantly different from values in at least two other lines in their selection group ($P < 0.05$). BMR = basal metabolic rate. RER = respiratory exchange ratio.

Table 9: Least squares means of each line for metabolic traits in males, with body mass either included as a covariate or excluded from the model, and of body mass as the dependent variable

	BMR		CO_2 Production		RER		
Line	Mass Included	Mass Excluded	Mass Included	Mass Excluded	Mass Included	Mass Excluded	Mass
1	70.94	**76.78**	54.32	57.81	.80	.80	39.38
2	62.51	61.19	49.59	48.47	.78	.78	36.95
4	58.39	63.00	50.37	53.36	.83	.82	*41.29*
5	63.10	56.69	52.77	51.81	.79	.80	36.75
3	62.67	63.18	51.73	50.65	.76	.77	38.30
6	62.61	52.07					**28.62**
7	71.58	63.72	48.23	45.82	.74	.75	34.90
8	65.61	65.59	49.53	48.56	.81	.82	37.24

Note. Lines 1, 2, 4, and 5 are control lines; lines 3, 6, 7, and 8 are selected lines. Values in bold are significantly different from all other lines in their selection group ($P < 0.05$). Values in italics are significantly different from values in at least two other lines in their selection group ($P < 0.05$). BMR = basal metabolic rate. RER = respiratory exchange ratio.

the mean was between 1% and 11.3%, indicating that the putative drift measured in this study is not substantially different from what is expected, given the observed phenotypic variance and effective population size.

Direct comparisons of our finding to those of other studies are difficult. A replicated selection study of *Drosophila* attributed 13.5% of the variation among selected lines for ethanol tolerance to drift, versus 64.1% attributed to selection after 24 generations and an effective population size of 154 (Cohan and Hoffman 1986). A study using replicate lines of mice selected for high or low epididymal fat pad and hind carcass weight also found significant divergence among lines within a selection protocol (Eisen and Pomp 1990). While many studies have used artificial selection on metabolic traits, these studies did not replicate the selected and control lines and thus cannot tease apart the relative effects of selection and genetic drift (Koch and Britton 2001; Ksiazek et al. 2004). Indeed, we know of no other studies in mammalian systems that have selected for metabolic traits using replicated selection lines. Thus, this study is the first to both test and demonstrate the potential of genetic drift to influence BMR within populations.

When body mass was analyzed as a dependant variable, a highly significant effect of line within selection group was measured in both males and females in both selection groups. The divergence in body mass among these lines over ontogeny has been documented (Morgan et al. 2003). Other studies also suggest that significant genetic variation for body size exists in both laboratory (e.g., Dohm et al. 2001 for our base population) and natural populations of rodents and that natural selection plays an important role in maintaining this variation (Hastings 1996; Speakman 1996). However, many of the factors thought to influence selection on body mass in wild populations of small mammals (e.g., thermal conductance, competition, noradrenaline-induced metabolic rate) may play little or no role in a laboratory population, where animals are housed in relatively constant temperatures with ad lib. food. In the absence of selection and in relatively small populations, such as those represented by the eight different closed lines, founder effects and, especially, genetic drift are expected to have large effects on gene frequencies and, thus, genetically correlated phenotypic traits (Falconer and Mackay 1996). A clear example of the effects of genetic drift on a gene of major effect in these lines is presented in Garland et al. (2002).

Taken together, the results of this experiment suggest that neither early-age selection for high wheel-running activity nor 22 mo of wheel-running activity have strong effects on basal metabolic rates of house mice. This study does suggest that selected active mice may rely on fat metabolism to a greater degree than active controls, indicating that genotype and exercise training can interact to affect metabolic profiles (see also Houle-Leroy et al. 2000). In addition, random genetic changes over relatively few generations (16) have caused divergence in metabolic traits among lines within a selection group. In females, this change occurred largely independent of body mass divergence among the lines, but in males, those changes in metabolic traits seem to have been driven by drift in body mass. Further studies that examine correlated changes in body composition among selected and control groups (e.g., Dumke et al. 2001; Swallow et al. 2001, 2005), as well as studies that closely examine changes in metabolic profiles over the lifetime of these animals, will help elucidate the correlated evolution of activity levels and metabolic traits. In addition, more work is necessary to examine effect and degree of genetic drift among replicated selection lines in mammalian systems.

Acknowledgments

We are grateful for the many hours of assistance with colony care from T. Morgan, S. Hall, S. Thomson, L. Jenkins, M. Baze, B. Irwin, M. Schmit, A. Poopatanapong, and D. Baker and also to the veterinarians of Laboratory Animal Resources at Washington State University (WSU) for their technical assistance.

This research was supported by grants from the WSU College of Sciences (to P.A.C.) and National Science Foundation grants DEB-0083638 and DEB-0105079 (to P.A.C.) and IBN-9728434 and IOB-0543429 (to T.G.).

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