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Artificial Selection for Increased Wheel-Running Behavior in House Mice

John G. Swallow,[1] Patrick A. Carter,[1,2] and Theodore Garland, Jr.[1,3]

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Replicated within-family selection for increased voluntary wheel running in outbred house mice (*Mus domesticus*; Hsd:ICR strain) was applied with four high-selected and four control lines (10 families/line). Mice were housed individually with access to activity wheels for a period of 6 days, and selection was based on the mean number of revolutions run on days 5 and 6. Prior to selection, heritabilities of mean revolutions run per day (rev/day), average running velocity (rpm), and number of minutes during which any activity occurred (min/day) were estimated by midparent–offspring regression. Heritabilities were 0.18, 0.28, and 0.14, respectively; the estimate for min/day did not differ significantly from zero. Ten generations of selection for increased rev/day resulted in an average 75% increase in activity in the four selected lines, as compared with control lines. Realized heritability averaged 0.19 (range, 0.12–0.24 for the high-activity lines), or 0.28 when adjusted for within-family selection. Rev/day increased mainly through changes in rpm rather than min/day. These lines will be studied for correlated responses in exercise physiology capacities and will be made available to other researchers on request.

KEY WORDS: Artificial selection; correlated response; genetic correlation; heritability; *Mus domesticus*; wheel-running activity.

INTRODUCTION

Selective breeding is one of the most useful analytical tools in behavior genetics and is emerging as an important tool in evolutionary physiology (Garland and Carter, 1994). We are using artificial selection for increased wheel-running behavior to study the genetics and evolution of locomotor behavior and underlying physiological capacities for exercise in house mice (*Mus domesticus*). We chose wheel running, as opposed to other measures of behavioral activity, such as open-field behavior (Defries *et al.*, 1978) or locomotor play (Walker and Byers, 1991), because wheel running has greater potential to tax exercise capacities. Here, we report the results of 10 generations of selection for high levels of voluntary wheel running, which resulted in an average 75% increase in activity levels. The primary goals of this paper are (1) to estimate the (realized) narrow-sense heritability of voluntary wheel-running behavior and (2) to determine how higher levels of wheel running evolve by direct monitoring of its components, duration and velocity of activity.

For several reasons, voluntary wheel running offers an excellent model for the study of locomotor behavior and exercise physiology. First, wheel running shows substantial individual variation in rodents, including both laboratory and wild

[1] Department of Zoology, 430 Lincoln Drive, University of Wisconsin, Madison, Wisconsin 53706-1381.
[2] Present address: Department of Zoology, Washington State University, Pullman, Washington 99164.
[3] To whom correspondence should be addressed. e-mail: tgarland@macc.wisc.edu

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house mice (Dohm *et al.*, 1994). For example, Friedman *et al.* (1992) report a range of 24 to nearly 14,000 revolutions run in a 24-h period (12-h light cycle) by 4-month-old male house mice ($N = 35$; coefficient of variation = 70%).

Second, previous studies of *Mus* and *Rattus* indicate that individual variation in wheel running is heritable. By creating lines of rats divergent for activity in rotating-drum cages, Rundquist (1933) became the first to demonstrate additive genetic variance for wheel-running behavior. Subsequently, a variety of studies on inbred strains and hybrids has confirmed a genetic basis for wheel-running activity (e.g., Bruell, 1964; Oliverio *et al.*, 1972). Apparently, however, an estimate of narrow-sense heritability is reported only by Oliverio *et al.* (1972), who indicated a value of 0.2 ± 0.04 (SE) based on crosses of only two inbred lines of house mice. The present study provides the first estimate of narrow-sense heritability, estimated both by midparent–offspring regression and by regression of selection response on the cumulative selection differential, in a genetically variable, outbred strain of house mice [Hsd:ICR (Hauschka and Mirand, 1973; Carter *et al.*, unpublished)].

Third, Bruell (1964) demonstrated a substantial amount of dominance genetic variance in the direction of higher levels of wheel running, based on crosses of inbred lines of house mice. Similarly, based on crosses of lab and wild house mice, Dohm *et al.* (1994) demonstrated net dominance in the direction of higher activity. The presence and direction of dominance genetic variance may indicate the direction of past selection; thus, the foregoing results suggest that activity level, as measured by voluntary wheel running, is a selectively important trait.

Finally, studies of prolonged exposure to wheels indicate that wheel running is potentially physiologically taxing. Prolonged access to running wheels has been shown to elicit a variety of physiological adaptations to training (for review see Harpur, 1980), including increases in maximal aerobic capacity (Lambert and Noakes, 1990; Swallow *et al.*, 1998) and increases in aerobic muscle enzyme activities (Rodnick *et al.*, 1989). As well, prolonged access to running wheels may enhance average longevity in rats (e.g., Holloszy, 1993).

All previous quantitative genetic analyses of wheel running focused solely on one factor, total distance run per day. However, wheel-running activity is a complex trait that can be broken down into several component parts. Most simply, total activity, measured as revolutions run per day, can be decomposed into the product of mean velocity and duration of activity. Thus, the evolution of increased total activity levels could be accomplished by an increase in mean velocity, an increase in the amount of time spent running, or a combination of both.

MATERIALS AND METHODS

Strain History and Founder Population. We used the outbred Hsd:ICR strain of house mice (*Mus domesticus*) as the founder population. The ICR strain is genetically heterogeneous (Hauschka and Mirand, 1973; Carter *et al.*, unpublished) and has served as a model system for numerous quantitative genetic analyses (for references see Dohm, 1994). Hsd:ICR mice are descendents of 100 female and 30 male Swiss–Webster albino mice purchased in 1947 by the Institute for Cancer Research in Philadelphia, Pennsylvania (Hauschka and Mirand, 1973). Following a selection and husbandry program to increase litter size and weaning success, the ICR lines were established. Charles River Laboratories acquired and subsequently developed additional lines. In 1983, Harlan Sprague Dawley purchased ICR mice from Charles River Laboratories. The ICR strains are currently maintained in 10 separate closed colonies, consisting of several hundred to thousands of individuals, at Harlan Sprague Dawley facilities across the United States. The colony in Indiana from which we received mice is maintained with 1000 breeder females per generation. Additional details of strain history are given by Dohm (1994).

Animal Husbandry. In our laboratory, mice were housed in standard clear plastic cages (27 × 17 × 12.5 cm) with metal or wire tops and wood shavings. Room temperature was controlled at approximately 22°C. Photoperiod was a constant 12:12, centered at 1400 (CST). Except during breeding or measurements of behavioral (e.g., wheel running) and physiological traits, animals were housed in same-sex groups of up to four individuals. Water and food [Harlan Teklad Rodent Diet (W) 8604] were available ad libitum.

Establishment of Lines. Male and female (112 of each) laboratory house mice (*Mus domesticus*) of the Hsd:ICR strain were purchased from Harlan

Sprague Dawley, Indianapolis, IN (Building 202, Barrier A). These founder animals were designated generation −2. They were paired randomly, and their offspring, designated generation −1, were assigned to eight closed lines. Lines were established as follows. One male and one female were chosen randomly from each litter. These individuals were then paired randomly except that sib-mating was disallowed; 10 pairs were then assigned randomly to each line and 4 lines were assigned to each linetype (selection or control). Their offspring were designated generation 0. From generation 0 to 10, 13 pairs were established within each line every generation; within each line, the first 10 litters weaned with at least two pups of each sex were used to maintain that line. The three extra pairings per line were made to ensure that each line was propagated with 10 families per generation.

Pairing and Weaning. Breeder mice were paired at approximately 10 weeks of age (except generation −2, which was paired at 7 weeks, and generations −1 and 0, which were paired at 8.5 weeks of age) by placing one male and one female together in a fresh cage. Males were removed and weighed 15–18 days after pairing; births began 19 days after pairing. From 19 to 33 days after pairing, pregnant females were checked daily between 1600 and 1800; on the day of parturition, the number of pups was recorded. At 21 days of age, offspring were weaned from the dam, weighed, toe clipped for individual identification, and housed in groups of four by sex. All offspring of selection families were kept, but only a random subsample of two males and two females was kept from each control family. At weaning, families were arbitrarily assigned to one of three wheel-running measurement batches such that each batch contained approximately 200 mice from nearly equal numbers of families from each line.

Voluntary Wheel Running. Voluntary wheel running was measured on 200 Wahman-type activity wheels (1.12-m circumference, 35.7-cm diameter, stainless-steel and plexiglass construction; Lafayette Instruments, Lafayette, IN). Wahman-type activity wheels were chosen for two reasons; preliminary studies used wheels of this same diameter (see Friedman *et al.*, 1992, Dohm *et al.*, 1994), and we plan on using these wheels for interspecific comparative studies of animals of variable body size (cf. Dewsbury, 1980). Furthermore, Collier and Leshner (1967) found that although mice may run more with respect to both revolutions and total distance covered in smaller wheels, work done is independent of wheel diameter. Activity wheels were divided equally between two rooms in the University of Wisconsin—Madison, Department of Zoology, Birge Hall Animal Care Facility. Normal housing cages were attached to the wheels via a 5.5-cm-long stainless-steel tube inserted through a 7.7-cm-diameter hole in the side of the cage, so that a mouse in a cage would have continuous access to a wheel. Attached to each wheel was a photocell counter, which was interfaced to an MS-DOS personal computer. Software from San Diego Instruments (San Diego, CA) measured the number of revolutions during every 1-min interval over a period of 6 days for each of the 200 wheels.

Each generation, approximately 600 mice of 6–8 weeks of age were monitored for wheel running activity for 6 consecutive days (testing occurred at 5–7 weeks of age in generation −1 and 0). Three batches of approximately 200 mice each (see previous paragraph) were measured during 3 successive weeks. Mice from a given batch were weighed and placed randomly on wheels during the morning of the first day; data collection was started at 1300. Data were downloaded every 24 h, at which time wheels were checked to remove food pellets and wood shavings and to ensure freedom of movement. On the sixth day, mice were removed from the wheels and weighed.

Selection of Breeders. Within-family selection was used both to reduce the rate of inbreeding and to avoid possible complications of common environmental effects, such as maternal effects (e.g., see, Defries *et al.*, 1970; Falconer, 1973; Lynch, 1980). The selection trait was the average number of wheel revolutions run on days 5 and 6 on the activity wheels or the residual value for this trait (see below). From each family in selection lines, the highest-running male and the highest-running female were chosen to breed. Three additional males and three additional females from each line were also chosen for breeding to provide extra families for each line to ensure that 10 families were produced. These additional mice were chosen on the basis of being the second-highest runners from the highest-running families, but with the stipulation that no two of the six additional mice came from the same family. The 13 males and 13 females from each line were paired randomly, with the provision of no sibling mating.

From each family in the control lines, one male and one female were chosen randomly for breeding. An additional three females and three males were chosen randomly from each line, with the stipulation that no two of the six could come from the same family. The 13 males and 13 females from each line were paired randomly, with the provision of no sibling mating.

Prior to choosing breeders, a multiple regression approach was used to control statistically for effects on wheel running of various background variables. The dependent variable was always mean number of revolutions run on days 5 and 6 of exposure to activity wheels, transformed as necessary to improve normality. Wheel freeness was measured for each of 200 wheels after each washing and prior to each generation as the number of free-spinning revolutions following acceleration to a given velocity. Wheels that did not spin a minimum number of revolutions (20) were replaced or repaired. Each generation, this inverse measure of wheel resistance was used as a covariate in the analyses (cf. Blizard, 1983) and was transformed as needed to improve normality. The z-transform of wheel freeness was squared and used as an additional covariate. Dummy variables were used to code for measurement block (batches 1–3 and rooms 1–2), whether an animal was measured for oxygen consumption [beginning in generation 1, maximum rate of oxygen consumption (see Swallow *et al.*, 1998) was measured on a subset of animals on 2 consecutive days during the week prior to wheel running], family, and the four sex × linetype combinations. Age was not used as a covariate because whole families were measured within a single batch; thus, family and measurement block accounted for variation in age.

Ordinary least-squares multiple regression was used. Family and the sex × linetype dummy variables were first forced into the model, then forward entry (significance level set at $P < 0.05$) was used to determine the best model. For regressions in which wheel freeness squared was found to be statistically significant, but wheel freeness was not, wheel freeness was forced into the equation prior to computation of residuals (Montgomery and Peck, 1992). In generations in which one or more variables (other than family or sex × linetype) were statistically significant, breeders were chosen based on residuals calculated from the regression equation.

Data Analysis. Parent–offspring regression was used to estimate the heritability for the number of revolutions run per day (rev/day), the number of 1-min intervals during which any running occurred (min/day), and the average number of revolutions per 1-min interval (rpm; calculated using only intervals in which activity occurred) using individuals from generation -1 as the parental generation (N = 158 individuals; 79 families) and generation 0 as the offspring generation (N = 557). Generation 0 included all offspring of families that would subsequently be assigned to selected lines (mean N = 10.15; range, 6–13), but only a random subsample of two males and two females from each family that would subsequently be assigned to control lines (mean N = 3.88; range, 3–4). Residuals were calculated separately for parents and offspring using sex, number of toes cut, age, z-transformed age squared, wheel freeness, z-transformed wheel freeness squared, and dummy variables coding for measurement block. Unweighted regressions were calculated from the mean of the offspring of each set of parents. Weighted regressions were also calculated. The weighing factor was calculated in a two step process following Falconer (1963). First, the quantity T was computed as follows:

$$T = [t - 0.5\ (b^2)]/(1 - t)$$

The intraclass correlation of wheel running scores, t, was calculated as the among-family component of variance divided by the sum of the among-family component of variance plus the within-family component of variance. This was done using ANOVA in the SAS GLM procedure and was determined in generation 0 separately for males and females and then averaged, yielding a value of 0.246. The slope from the unweighted midparent offspring regression was used as b. Then the weighting factor (W_n) appropriate to families with n offspring measured was calculated as

$$W_n = (n + nT)/(1 + nT)$$

Estimates of realized heritabilities were obtained for each of the four selected lines as the regression of response to selection on cumulative selection differential based on data from generations 0 to 10. Regressions were tried with a quadratic term, as well, to check for evidence of reaching a selection limit. Selection differentials were calculated, separately within litter and sex, as the difference of selected mice from their respective litter means,

Table I. Wheel Running on Days 5 and 6 in Generation 0[a]

	Female	Male
Day 5	4,490 ± 2,098.8	3,818 ± 1,922.4
Range	(421–13,029)	(139–11,999)
Day 6	5,098 ± 2,349.8	4,213 ± 2,032.8
Range	(564–13,186)	(206–12,046)
Paired t	7.01	6.37
P	<0.0001	<0.0001
r	0.787	0.868
P	<0.0001	<0.0001
n	287	273

[a] Means ± 1 SD.

and then averaged within line. Response to selection was taken as the deviation between the selected line and the mean of the four control lines. Standard errors of the heritability estimates of each line were calculated according to Hill's (1972) method.

Following Lynch (1980), the estimate of realized heritability from regression was adjusted for within-family selection by the following formula:

$$h^2 = h^2_R(1 - t)/(1 - r)$$

where h^2 is that expected from mass selection or a randomly mating population, h^2_R is the realized heritability obtained from a within-family selection protocol, r is the coefficient of relationship of full sibs (0.5), and t is the intraclass correlation of full sibs for wheel-running scores (calculated as above).

At generation 10, nested ANCOVA was used to compare selected and control lines. The number of revolutions run per day (rev/day), the number of 1-min intervals during which running occurred (min/day), and the average number of revolutions per 1-min interval (rpm; calculated using only intervals in which activity occurred) were analyzed using Type III Sums of Squares in the SAS GLM procedure. All traits were scored as the mean of days 5 and 6 and transformed as needed to meet the assumptions of normality. Family was nested within line, and line was nested within linetype (selected vs. control); cofactors [measurement block, measurement animal (whether measured for oxygen comsumption or not)], covariates (wheel freeness, the square of the z-transform of wheel freeness, body mass, number of toes clipped for identification), and interactions (linetype and line by all cofactors and covariates) were included in the model. Because whole families were measured at the same age, family accounted for variation in age. Models were analyzed iteratively to remove nonsignificant ($p > .05$) interactions; nonsignificant interactions with covariates were removed before nonsignificant interactions with cofactors. Procedure GLM in SAS calculated regression coefficients for covariates and least-squares adjusted means for selected and control lines. Adjusted means were calculated using all cofactors and covariates, regardless of significance levels. Only the significant ($p < .05$) linetype × cofactor and/or covariate interactions were retained in the final models.

RESULTS

Repeatability of Measures. An average of 577 (range = 547–595) individuals was tested each generation (0–10) for wheel running. Measurement of wheel-running activity was repeatable between days of exposure. In generation 0, the Pearson product–moment correlation between the number of revolutions run on day 5 and that on day 6 was .79 and .87 for females and males, respectively (Table I). A two-tailed paired t test indicated that wheel-running activity was significantly higher on day 6 (Table 1).

Response to Selection. An initial analysis of mean revolutions run on days 5 + 6 (rev/day) in Generation 0 included all measured individuals. Analysis was performed as described under Materials and Methods except that sex was also used as a cofactor. Data were transformed to improve normality. Mice assigned to selected and control lines did not differ significantly in rev/day ($F = .05$, df 1,6, $p = .825$). Similarly, no significant differences existed among lines within linetype ($F = 1.49$, df. 6,72, $p = .193$). However, rev/day differed significantly between sexes; females ran more than males [4745 rev/day for females vs. 3817 rev/day for males (mean values were backtransformed from LSMEANS in SAS); $F = 10.8$, df 1,466, $p = .001$]. Sex differences were apparent not only for the selection criterion but also for components of wheel running. Females were active for a longer period of the day, as measured by the number of 1-min intervals during which any running occurred on days 5 and 6 [402.7 min/day for females vs. 355.0 min/day for males (backtransformed means from LSMEANS in SAS); $F = 13.48$, df 1,466, $p < .001$]. Females also ran at higher average revo-

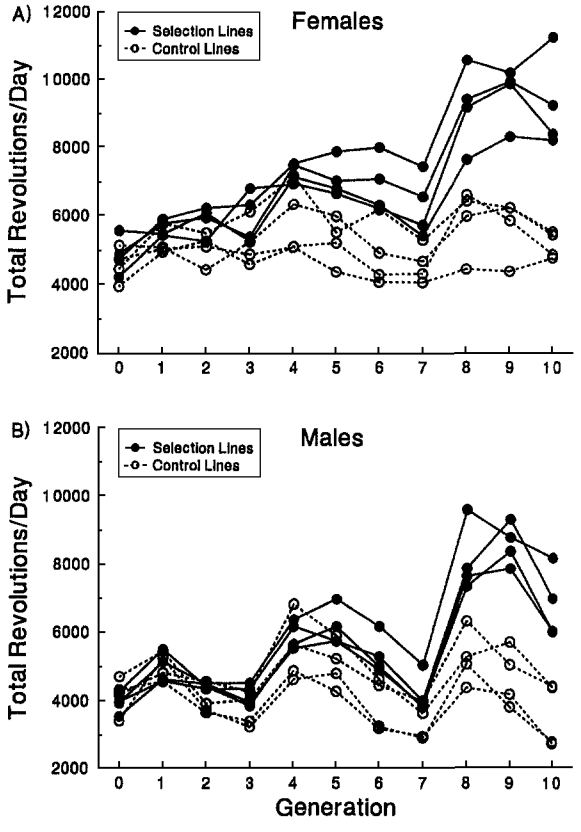

Fig. 1. Unadjusted average running distance for (A) female and (B) male mice from each of the eight lines plotted against generation. Values are the mean number of revolutions run on days 5 and 6 of a 6-day test. Each generation total $N \leq 300$ for each sex (approximately 25/selected line and 10/control line). Wheel circumference is 1.12 m; diameter is 35.7 cm.

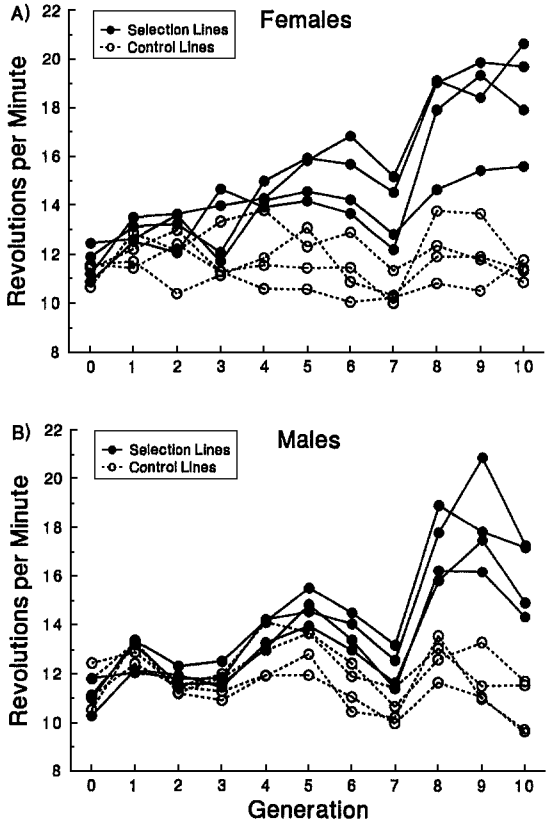

Fig. 2. Unadjusted mean velocity during activity for (A) female and (B) male mice in each of the eight lines plotted against generation. Velocity is the average revolutions per minute during activity on days 5 and 6 of a 6-day test.

lutions per 1-min interval (during which any running occurred) on days 5 and 6 than males [11.7 rpm for females vs. 10.6 rpm for males (backtransformed means from LSMEANS in SAS); $F = 4.94$, df 1,466, $p = .027$].

In generation 0, no statistically significant differences existed between selection and control lines for rev/day, min/day, or rpm ($F < 1.4$, df 1,6, $p > 0.2$; see Figs. 1–2) for either sex. No significant differences existed among lines within linetype for rev/day or rpm, but min/day did differ significantly in males ($F = 2.25$, df 6,72, $p = .048$; for comparison, results of analysis of min/day for females were as follows, $F = 1.88$, df 6,72, $p = .096$).

Data were split by sex for analysis of generation 10 for several reasons. First, we found consistent differences between sexes in generation 0

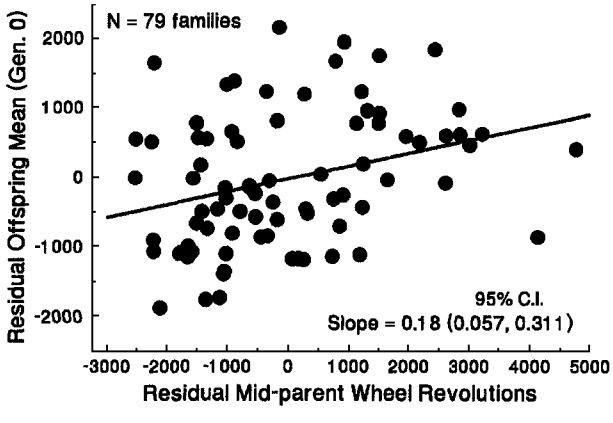

Fig. 3. Plot of mean revolutions per day (rev/day) (residuals calculated by regression) of offspring from 79 families against the mean rev/day of their parents. The slope of the unweighted regression is 0.18 (CI = 0.06–0.31).

Table II. Adjusted Means (Backtransformed from Least-Squares Adjusted Means Produced by SAS) for Wheel Running in Generation 10 (Mean of Days 5 + 6)

		Adjusted mean		
	Sex	Selection	Control	Ratio
Total revolutions[a]	F	8774	5077	1.73
	M	6056	3437	1.76
No. 1-min intervals with any running[b]	F	486.2	434.7	1.12
	M	392.0	327.2	1.20
Revolutions/min[c]	F	17.76	11.07	1.60
	M	15.26	10.44	1.46

[a] Square root transform used in analyses for both females and males.

[b] Square root transform used in analysis for females. Significant linetype × wheel freeness interaction in analysis of females. Untransformed values used for males.

[c] Cube root transform used in analyses for both females and males.

for wheel running and both its components. Second, we have also found sexual dimorphism for numerous traits which may influence wheel-running behavior, such as locomotor performance abilities (Dohm *et al.*, 1996), energy cost of locomotion (unpublished), and food consumption (unpublished). Third, the motivational basis for wheel-running activity may differ between sexes (see Mather, 1981; Perrigo and Bronson, 1985). Thus, we reasoned that males and females might respond differently to selection.

The change in rev/day across 10 generations of selection is shown in Fig. 1. The extent to which the environment influenced wheel running is suggested by intergenerational variation in rev/day by mice in the control lines (Fig. 1). Figure 1 also suggests an effect of season because the repeating pattern of low values occurs every fourth generation during measurements made in the late summer months. These fluctuations were also mirrored in the selected lines. As of generation 10 postselection, females from the selected lines were running, on average, 73% more than females from the control lines; for males, the difference was 76% (Table II). These differences were statistically significant (Table III). In generation 10, significant differences existed among lines within linetype for both sexes (Table III).

Mean values for min/day slowly increased over the course of the experiment in selected lines. By generation 10, females and males from selected lines were running, on average, 51.5 (12%) and 64.8 (20%) more min/day, respectively, than mice from control lines (Table II; backtransformed adjusted means). However, the difference was not statistically significant for either sex (Table III). Line within linetype was statistically significant only for males (Table III).

Average number of revolutions per minute during wheel running (rpm) increased rapidly in the selected lines over 10 generations of selection (Fig. 2). At generation 10, mice from selected lines were running approximately 0.33 m/s (60% increase over control lines) for females and 0.29 m/s (46% increase over control lines) for males (Table II; backtransformed adjusted means); these differences were statistically significant (Table III). Line within linetype was significant for both sexes (Table III).

As noted above, significant line-within-linetype effects (i.e., replicate effects) were detected at generation 10 for mean rev/day and its two components (Table III). Line differences may be the result of founder effects, random genetic drift, and/or differential responses to selection. Although interesting, these genetic effects are not the focus of this paper and are not discussed further.

Midparent–Offspring Regression. Mean rev/day (residuals calculated by regression) of offspring from 79 families are plotted against mean rev/day of their parents in Fig. 3. The slope of the regression line, which provides an estimate of heritability, is 0.18 ± 0.064 (SE). The slope of the midparent offspring regression for rpm equals 0.28 ± 0.074. However, the slope of the midparent offspring regression for min/day is shallower and does not differ significantly from zero (0.14 ± 0.088). Because the number of individuals measured in each family differed (mean = 7.0, range = 3–13), the regressions were repeated weighted by number of offspring per family (see Materials and Methods). However, none of the estimates differs to the second significant digit; therefore, they are not reported.

Realized Heritability. Response to selection, measured as deviation from controls, is plotted against the cumulative selection differential in Fig. 4. Selection differential averaged 0.94 phenotypic standard deviations per generation. Estimates of realized heritabilities and their standard errors are listed by line in Table IV. The quadratic term in the regression of response to selection on the selection differential was not statistically significant

Table III. Significant Effects from Analysis of Variance at Generation 10 (Mean of Days 5 + 6)

Trait	Linetype	Line (Linetype)	Family	Body Mass	Toescut	Wheel Freeness	Wheel Freeness²
Total revolutions							
Females	$F_{1,6} = 16.67^{**}$	$F_{6,72} = 4.65^{***}$	$F_{72,189} = 1.45^{*}$	$F_{1,189} = 2.72$	$F_{1,189} = 0.22$	$F_{1,189} = 0.07$	$F_{1,189} = 0.06$
Males	$F_{1,6} = 13.41^{**}$	$F_{6,72} = 5.38^{***}$	$F_{72,224} = 0.98$	$F_{1,224} = 0.24$	$F_{1,224} = 5.32^{*}$	$F_{1,224} = 0.09$	$F_{1,224} = 1.24$
No. 1-Minute Intervals							
Females[a]	$F_{1,6} = 4.23$	$F_{6,72} = 0.48$	$F_{72,182} = 1.41^{*}$	$F_{1,182} = 3.01$	$F_{1,182} = 0.94$	$F_{1,182} = 2.78$	$F_{1,182} = 0.16$
Males	$F_{1,6} = 4.26$	$F_{6,72} = 5.58^{***}$	$F_{72,224} = 1.24$	$F_{1,224} = 0.72$	$F_{1,224} = 4.41^{*}$	$F_{1,224} = 2.72$	$F_{1,224} = 2.12$
Revolutions/min							
Females	$F_{1,6} = 28.77^{**}$	$F_{6,72} = 2.78^{*}$	$F_{72,189} = 1.59^{**}$	$F_{1,189} = 0.39$	$F_{1,189} = 1.42$	$F_{1,189} = 0.23$	$F_{1,189} = 0.27$
Males	$F_{1,6} = 22.84^{**}$	$F_{6,72} = 2.62^{*}$	$F_{72,224} = 1.48^{*}$	$F_{1,224} = 0.00$	$F_{1,224} = 4.93^{*}$	$F_{1,224} = 0.54$	$F_{1,224} = 0.51$

[a] Significant linetype × wheel freeness interaction; $F_{1,6} = 12.75$.

$^{*}p < .05$.

$^{**}p < .01$.

$^{***}p < .001$.

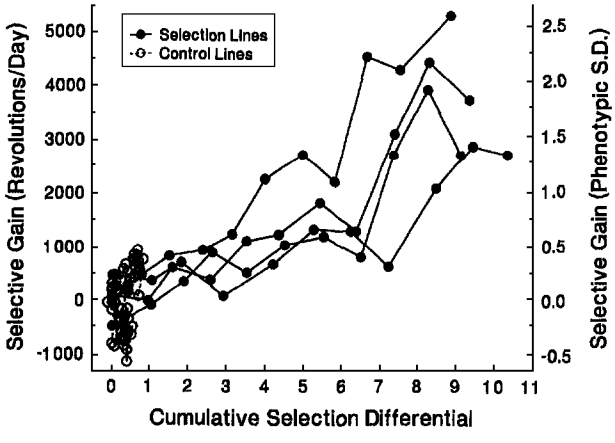

Fig. 4. Plot of the response to selection, measured as the deviation between each line and the mean of the control lines, against the cumulative selection differential. The left axis shows the response to selection plotted as revolutions per day; the right axis shows phenotypic standard deviations.

(all four, two-tailed $p > .05$), and moreover, all had a positive sign. Therefore, as of generation 10, response to selection is linear, and no evidence of reaching a selection limit exists for any of the four selected lines.

The average realized heritability of rev/day, adjusted for within-family selection, was 0.28 versus 0.19 for the unadjusted estimate (Table IV). A higher average adjusted value, compared to the unadjusted, indicates that within-family selection is less efficient than truncation selection, at least in the short term. Heritability estimates from intraclass correlations of full sibs ($h^2 = 2\ t$) in generation 0 averaged 0.49 (0.53 for females and 0.46 for males).

Table IV. Realized Heritability Through Generation 10

Line	Realized $h^2 \pm$ SE	Adjusted $h^2 \pm$ SE[a]
High 3	0.21 ± 0.036	0.32 ± 0.042
High 6	0.24 ± 0.038	0.36 ± 0.045
High 7	0.17 ± 0.033	0.26 ± 0.039
High 8	0.12 ± 0.028	0.18 ± 0.033
Mean	0.19	0.28

[a] Adjusted for within-family selection (See Materials and Methods).

DISCUSSION

Ten generations of artificial selection for increased voluntary activity on running wheels resulted in the creation of four replicate lines of mice that ran approximately 75% more, on average, than individuals from four control lines (Table II). This increase corresponds to nearly two standard deviations of selective gain relative to the control lines (Fig. 4). By generation 8, no overlap in mean activity levels existed between any of the selected and control lines (Fig. 1).

Estimates of realized heritability for the four selected lines ranged from 0.12 ± 0.028 to 0.24 ± 0.038 (SE), with an average of 0.19 (Table IV). When adjusted for within-family selection, the heritability averaged 0.28. This value is higher than the value of 0.18 ± 0.064 (SE) estimated from our

midparent–offspring regression in the base population (Fig. 3) and the value of 0.20 ± 0.04 estimated by Olivario *et al.* (1972), based on crosses of two inbred lines. The heritability of wheel-running activity is comparable to the heritability of open-field activity in house mice [0.22 ± 0.09 (Defries *et al.*, 1978)]. The fact that the intraclass correlation estimate of heritability (0.49 in generation 0) is nearly double our estimate of realized heritability (0.28) suggests a substantial amount of nonadditive genetic variance and/or common environmental effects (e.g., maternal effects) for wheel-running activity. Both Bruell (1964) and Dohm *et al.* (1994) demonstrated substantial nonadditive genetic variance for wheel-running activity. To our knowledge, possible maternal effects on voluntary wheel running have not been studied in house mice.

Dohm *et al.* (1994, unpublished) found that female wild house mice from a Wisconsin population ran 68% more revolutions per day (days 5 & 6) than did female house mice from the Hsd:ICR strain. The wild mice did not run significantly more minutes per day but, instead, ran at significantly higher velocity than the laboratory mice. Based on these results, we had hypothesized that artificial selection for increased activity would occur primarily by an increase in velocity. Through 10 generations, our results are consistent with this hypothesis. At generation 10, mice from the selected lines did not run significantly more minutes per day ($p > .05$) than did mice from the control lines. Rather, mice from the selected lines ran at a 53% higher velocity than mice from control lines ($p < .01$; Table II). The lower response in duration of activity is not surprising given that the midparent–offspring regression estimate of heritability in the base population did not differ significantly from zero [slope = 0.14 ± 0.088 (SE)].

As of generation 10, mice from our selected lines exceed the activity levels (measured in revolutions/day) of wild house mice tested in our laboratory (Dohm *et al.*, 1994). Moreover, the difference in activity between mice from our selected and control lines is comparable with variation among some species of muroid rodents (Dewsbury, 1980). Contrary to our previous hypothesis (Dohm *et al.*, 1994), the activity levels of wild mice do not seem to correspond to an upper selection limit for activity. In fact, at generation 10, we find no evidence of approaching a selection limit, as indicated by positive (but statistically nonsignificant, $p > .05$) quadratic terms in each of the four regressions of response to selection on cumulative selection differential (see Fig. 4). Eventually, of course, continued selection may exhaust genetic variance for activity, thus causing a limit. Based on our measured quantitative genetic parameters, and assuming that they remain constant, a predicted theoretical selection limit may be approached by about generation 27 [(Hill and Rasbash, 1986); assuming $N_e = 35$, selection differential = 0.94 phenotypic SD, SD of wheel running = 2050 rev/day, corrected realized $h^2 = 0.28$], at which time the selected lines would be running almost three times more than controls.

The present selected lines of mice offer a unique model system for studying the genetics and evolution of voluntary locomotor behavior in relation to physiological capacities for exercise. For example, maximal aerobic capacity, typically measured as maximal oxygen consumption ($\dot{V}O_2max$) during forced treadmill exercise (e.g., Friedman *et al.*, 1992; Dohm *et al.*, 1994), sets an upper limit to the intensity of activity that can be sustained aerobically (Hayes and Garland, 1995, and references therein). Three lines of evidence suggest a positive genetic correlation between wheel-running activity and $\dot{V}O_2max$. First, multivariate analyses of males from the base population indicate a weak positive phenotypic correlation between wheel running and $\dot{V}O_2max$ (Friedman *et al.*, 1992). Second, Dohm *et al.* (1994) found that wild house mice exceeded lab mice not only in voluntary wheel running but also in $\dot{V}O_2max$. Third, a preliminary study of mice older (77–86 days) than the age at which wheel running is measured for selection (e.g., 39–57 days in generation 10) showed a significantly higher $\dot{V}O_2max$ in mice from selected lines (Swallow *et al.*, 1998). If activity levels and aerobic capacities are indeed genetically correlated, then these aspects of behavior and physiology are not entirely free to evolve as independent traits. We are monitoring $\dot{V}O_2max$ to test this hypothesis.

The present lines may also be useful for elucidating relationships among measures of activity in different situations, such as wheel running and distance moved in open-field tests. Open-field activity was the subject of a previous artificial selection experiment with laboratory house mice (Defries *et al.*, 1970). After 10 generations of selection, no significant correlated response in wheel

running had occurred (DeFries *et al.*, 1970). Moreover, among 12 species of muroid rodents, Dewsbury (1980) found no correlation between voluntary wheel running and open-field activity. Therefore, we hypothesize that open-field activity will not show a correlated response in our selected lines.

We have yet to study the control of activity in our selected lines, but recent research on lines of house mice selected for thermoregulatory nest-building behavior (Lynch, 1994) suggests some possibilities. Bult *et al.* (1992) showed a correlated response in the number of arginine–vasopressin (AVP)-immunoreceptive neurons in the superchiasmatic nuclei (SCN), with low-nesting lines having 1.5 times the number of AVP neurons as the high-nesting lines. Mean daily activity on running wheels showed a similar relationship; the low-nesting lines were 1.5 times more active than the high-nesting lines (Bult *et al.*, 1993). Based on the foregoing results, we hypothesize that mice from our selected lines will show lower levels of thermoregulatory nest-building behavior and higher levels of AVP neurons in the SCN, compared with mice from control lines.

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