ARTIFICIAL SELECTION FOR INCREASED WHEEL-RUNNING ACTIVITY IN HOUSE MICE RESULTS IN DECREASED BODY MASS AT MATURITY

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Summary

To test the hypothesis that body size and activity levels are negatively genetically correlated, we conducted an artificial selection experiment for increased voluntary wheel-running activity in house mice (*Mus domesticus*). Here, we compare body masses of mice from control and selected lines after 14 generations of selection. In both groups, beginning at weaning and then for 8 weeks, we housed half of the individuals with access to running wheels that were free to rotate and the other half with wheels that were locked to prevent rotation. Mice from selected lines were more active than controls at weaning (21 days) and across the experiment (total revolutions during last week: females 2.5-fold higher, males 2.1-fold higher). At weaning, mice from selected and control lines did not differ significantly in body mass. At 79 days of age, mice from selected lines weighed 13.6 % less than mice from control lines, whereas mice with access to free wheels weighed 4.5 % less than 'sedentary' individuals; both effects were statistically significant and additive. Within the free-wheel-access group, individual variation in body mass of males was negatively correlated with amount of wheel-running during the last week ($P<0.01$); for females, the relationship was also negative but not statistically significant ($P>0.40$). The narrow-sense genetic correlation between wheel-running and body mass after 8 weeks of wheel access was estimated to be −0.50. A negative genetic correlation could account for the negative relationship between voluntary wheel-running and body mass that has been reported across 13 species of muroid rodents.

Key words: activity level, body mass, evolution, house mice, *Mus domesticus*, quantitative genetics, selection, wheel-running.

Introduction

The relationship between body size and physical activity is of interest from a variety of perspectives. In the laboratory, the body size of rodents responds to regimens of chronic daily exercise, as can be implemented by providing access to running wheels. The effects of voluntary wheel-running on body size of rats and mice depends somewhat on sex and age (e.g. Pitts, 1984), but some generalities emerge. Males with access to running wheels gain less mass than do animals without, regardless of age when first introduced to wheels (for mice, see Dupont-Versteegdon et al., 1994; Hayes and Williams, 1996; Swallow et al., 1998b; for rats, see Goodrick, 1980; Pitts, 1984; Cortright et al., 1997). Differences in body mass between sedentary and wheel-access individuals are the result of changes in body fat (Pitts, 1984) and, when given access to wheels as juveniles (3–5 weeks of age), changes in fat-free body mass (Pitts, 1984; Cortright et al., 1997). In contrast, female mice and rats given wheel access generally experience no change in body mass (for mice, see Bell and McGill, 1991; for rats, see Pitts, 1984; Cortright et al., 1997) or actually increase body mass (Yano et al., 1997) relative to controls.

Among species of terrestrial mammals observed in the wild, body size is positively correlated with activity, as indexed by home range area or daily movement distance (Garland, 1983; Goszczynski, 1986). In the laboratory, however, body size and voluntary wheel-running activity were weakly negatively correlated ($r=-0.21$) across 13 species of muroid rodents (data from Table 1 in Dewsbury, 1980; body mass of *Rhabdomys pumilio* assumed to be 50 g). Associations of traits observed among species can be caused by selection acting on both traits and/or genetic coupling (Garland and Carter, 1994). Data concerning how natural or sexual selection may act jointly on size and activity are unavailable. Quantitative geneticists, however, have found indications of a possible genetic relationship between body size and voluntary activity in mammals.

Anecdotal evidence from two separate artificial selection experiments on body size of house mice *Mus domesticus* (MacArthur, 1944; Falconer, 1953) has led to the widely held belief that body size and activity are negatively genetically correlated. MacArthur (1944, p. 224) noted that "individuals

of the small race of mice are very active and aggressive, and rather savage, as compared with the tamer and more docile large race". Similarly, Falconer (1953, p. 490) observed that his "large mice became slow and phlegmatic, the small quick in movement and difficult to handle". To date, however, no studies of mice divergently selected for body size have measured wheel-running or other long-term indicators of activity. Moreover, selection for divergent activity levels in the open field (DeFries et al., 1970; Frankova et al., 1987) was not reported to have changed body mass.

If body size and activity are negatively genetically correlated, then selection for high activity should result in reduced body size. We conducted an artificial selection experiment to increase activity levels of house mice (Swallow et al., 1998a), then tested for a negative correlated response in body mass. Data from generation 10, when selected mice were running approximately 70 % more revolutions per day than controls, suggested that selected mice might be evolving smaller body masses, but the differences were small and/or not statistically significant (Swallow et al., 1998a,b; Koteja et al., 1999a). In the present study, we used mice from generation 14 in a two-way experimental design to test simultaneously the effects of genetic selection for high activity, access to running wheels for 8 weeks (an environmental factor), and a possible interaction between the two (genotype-by-environment interaction).

Materials and methods

Animal husbandry and breeding design

Outbred, genetically variable Hsd:ICR mice *Mus domesticus* L. (Schwartz and Schwartz, 1943; Hauschka and Mirand, 1973; Dohm et al., 1996; Carter et al., 1999) were used as the base population for an artificial selection experiment for increased activity. Eight closed populations were established. In four of the lines, mice were selected for high levels of voluntary wheel-running; the other four were bred randomly and used as controls. See Swallow et al. (1998a) for full details of the selection protocol. Throughout the selection experiment and during the present study, water and food (Harlan Teklad Laboratory Rodent Diet [W] 8604) were available *ad libitum*, photoperiod was a constant 12 h:12 h L:D centered at 14:00 h (CST), and room temperature was controlled at approximately 22 °C. Mice were sampled from generation 14, when a more than twofold difference in activity (average of both sexes) existed between the selected and control lines. The mice studied herein were from second litters; their siblings (first litters) were part of the routine selection protocol (Swallow et al., 1998a). The parents had first been mated at 15 weeks of age and were then remated at approximately 35 weeks of age by placing each female with its mate from the first pairing.

At weaning (21 days of age), two males and two females were chosen at random from each of five families from each of the eight lines. Mice were weighed and toe-clipped for identification. Mice were housed with three siblings until the following day when they were housed individually with access to running wheels. For the 'sedentary' group, the wheels were prevented from rotating by the use of a wire tie. Within each family and sex, one individual was assigned to a free wheel (active group) and one was assigned to a locked wheel (sedentary). Therefore, each line (four selected and four control) was represented by five active males, five active females, five sedentary males, and five sedentary females. A total of 160 animals were given access to running wheels. However, during the course of the study two mice died: one control female and one control male. To maintain a balanced design for statistics, siblings of the mice that died were also excluded from the analyses. Thus, 156 animals were used in the final analyses (78 of each sex).

Voluntary wheel-running behavior

In the wheel-access group, activity was monitored every day for each mouse from 22 days of age until the day prior to measurement of body mass (mean age 78.9 days; range 75–82 days). Activity was measured on the same Wahman-type wheels used in the normal selection protocol (1.12 m circumference, 10 cm wide running surface of 10 mm wire mesh bounded by clear acrylic walls; Lafayette Instruments, Lafayette, IN, USA; model 86041 with modifications). Normal housing cages were attached to the wheels, so mice had continuous access to activity wheels. A photocell counter, interfaced to a personal computer, was attached to each wheel. Customized software from San Diego Instruments (San Diego, CA, USA) measured the number of revolutions during every 1 min interval. Data were downloaded every 24 h, at which time wheels were checked to remove food pellets and wood shavings and to ensure freedom of rotation. As recorded in this study, wheel-running (revs day^{-1}) can be broken into two components: the number of 1 min intervals during which any activity occurred (min day^{-1}) and the mean number of revolutions per minute (revs min^{-1}) during those active minutes.

Estimation of genetic correlations

We used Falconer's (1989) formula 19.6 to estimate genetic correlations from the correlated response to selection:

$$CR_y = ih_x h_y r_G SDp_y, \hspace{2em} (1)$$

where y is body mass and x is wheel-running. h_x is the square root of the corrected realized heritability of wheel-running, as in Table IV of Swallow et al. (1998a), but updated through 14 generations to yield a value of 0.26. h_y is the square root of the estimated heritability of body mass measured at the end of 6 days of wheel access. This was taken from our offspring-on-parent regression for generations −1 and 0 [mean age was 49 days and estimated heritability was 0.31; similar values were obtained by Dohm et al. (1996) for mice of the same strain but with no wheel access]. i is the cumulative standardized selection differential, averaged for the four selected lines (13.23 through 14 generations). The standardized selection differentials were computed based on the within-line phenotypic standard deviations (adjusted for sex) of wheel-

running (means of days 5+6) for each generation, and not the actual within-family standard deviations. This was done mainly because some families had only one or two individuals of a given sex. SDp_y is the standard deviation of final body mass in the present experiment (2.21 g). The standard deviation was computed from residuals with line (which also accounts for linetype), activity group, number of toes clipped and age in the model. CR_y is the difference in body mass between selected and control lines (−4.38 g at the end of the present experiment). r_G, the only unknown in the equation, is the genetic correlation between wheel-running and body mass. Methods for computing a standard error for this estimate of a genetic correlation have not been developed.

Statistical analysis

The general linear models (GLM) procedure in SAS was used to estimate three different types of analysis of covariance (ANCOVA) models. First, a cross-nested two-way ANCOVA model was used to test effects of linetype (selected *versus* control) and activity group (sedentary *versus* free-wheel-access) on body mass measured at the end of the trial. Second, a nested one-way ANCOVA model was used to test for effects of linetype on mass at weaning (mice were weaned before animals were assigned to an activity group). Similar models were used to test the effect of linetype within a given activity group. For instance, we tested the effect of linetype on average $revs\,day^{-1}$, $min\,day^{-1}$ and $revs\,min^{-1}$ (separately for each of the 8 weeks), as well as final body mass within the wheel-access group. Third, repeated-measures ANCOVA models were used to test simultaneously effects of linetype and changes in wheel-running activity across 8 consecutive weeks (trial factor). All the analyses were performed separately for females and males.

The two main grouping factors, linetype and activity, were considered fixed effects. Replicate line (N=8 total), nested within linetype, was a random effect. In the two-way ANCOVA models, family, nested within line, was also included (random effect). Family level does not appear explicitly in the analyses within separate activity groups because each individual represents a different family. In the foregoing mixed models (i.e. with both random and fixed effects), we tested the effects over appropriate error terms as follows: in the two-way ANCOVA models, effects of linetype were tested over the mean squares of line, and effects of line were tested over the mean squares of family. Effects of activity and the activity×linetype interaction were tested over the mean squares of the activity×line interaction. In the one-way ANCOVA models, effects of line were tested over the error sum of squares.

A variety of covariates were used in the ANCOVA models. Mass at weaning, age, time of day, (z-transformed time of day)^2 and number of toes clipped for identification were included as covariates in all models of body mass. Within the wheel-access group, models of body mass were examined both with and without mean $revs\,day^{-1}$ measured during the last week of the trial. In addition, models of body mass were also

estimated without mass at weaning as a covariate. A measure of wheel freeness (see Swallow et al., 1998a), (z-transformed wheel freeness)^2 and number of toes clipped for identification were used as covariates in all models of wheel-running.

Covariates were chosen to remove variation unrelated to treatments. Body mass and wheel-running both vary linearly across small age ranges. Differences in initial body mass (mass at weaning) might persist and influence final body mass; however, because weaning mass may be influenced by linetype, models were tried both with and without this covariate. Mice are diurnal with most activity occurring at night, including feeding. Time of day and (z-transformed time of day)^2 were included to control for gastric or bladder emptying and related mass loss during the day. Number of toes clipped for identification has been shown to have significant effects on sprinting ability as well as body mass (Dohm, 1994; Dohm et al., 1996). Finally, wheel freeness and (z-transformed wheel freeness)^2 were included to control for any friction differences in the running wheels (Swallow et al., 1998a).

Results

Wheel-running

In both sexes, repeated-measures ANCOVA indicated statistically significant effects of linetype and week on mean number of $revs\,day^{-1}$ ($P<0.001$, Fig. 1A). The difference between selected and control lines was apparent in the first week (age 22–29 days of age) of wheel access (2150 and 1120 $revs\,day^{-1}$ for females and males, respectively). The absolute difference in $revs\,day^{-1}$ between selected and control lines increased and peaked at approximately 50 days of age, at which time selected females and males were running approximately 2.6 times more than controls. This peak difference was 8396 $revs\,day^{-1}$ for females (week 5) and 6546 $revs\,day^{-1}$ for males (week 4). By week 8, the difference had declined to 7470 $revs\,day^{-1}$ for females and 4490 $revs\,day^{-1}$ for males.

Across the entire 8 weeks, selected females were active for significantly longer ($min\,day^{-1}$) than were controls ($P<0.025$); in males, the difference approached significance ($P<0.10$; Fig. 1B). Similarly, selected mice of both sexes ran at significantly higher $revs\,min^{-1}$ compared with controls ($P<0.01$; Fig. 1C). The temporal increase in total $revs\,day^{-1}$ resulted from increases in both $min\,day^{-1}$ and $revs\,min^{-1}$ (Fig. 1B,C). The difference in $revs\,min^{-1}$ between selected and control lines increased with time and peaked during weeks 4 and 5 (Fig. 1C). As reported for generation 10 (Swallow et al., 1998a), $revs\,day^{-1}$ has increased in the selected lines primarily by increases in $revs\,min^{-1}$ (during last week: 2.0- and 1.7-fold increase for selected females and males, respectively) rather than in $min\,day^{-1}$ (during last week: 1.3-fold increase in both sexes).

Body mass
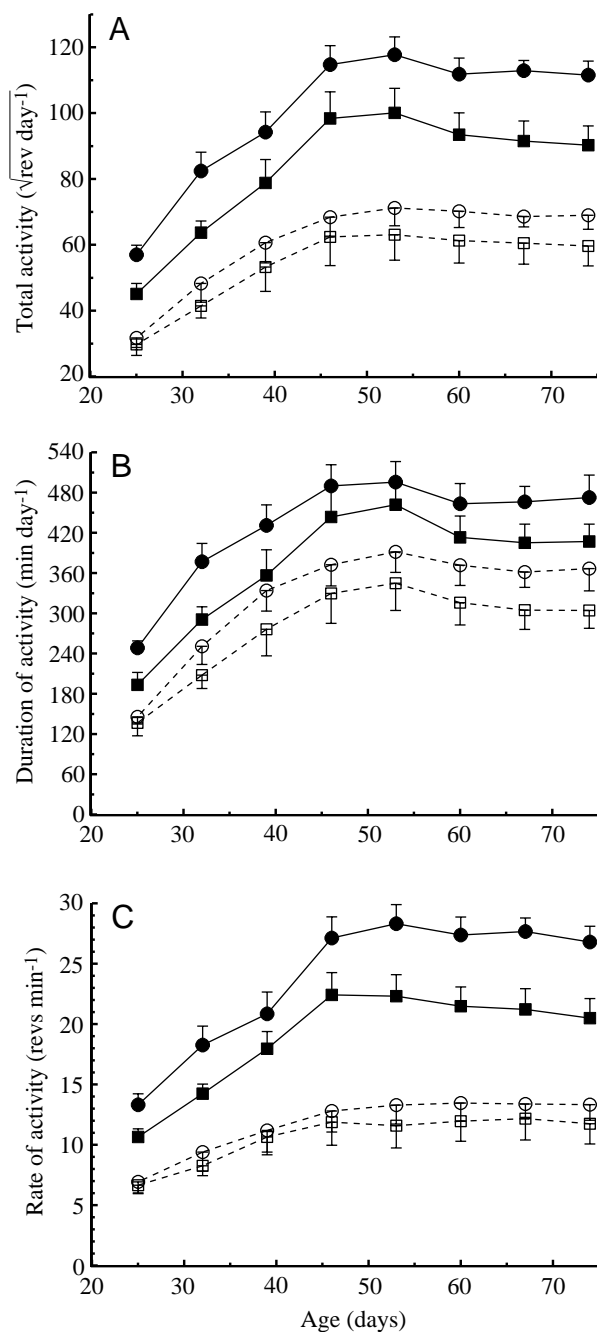
Weaning mass (21 days of age) was measured for a total of

Fig. 1. Least-squares adjusted means (from the SAS GLM procedure) of total daily running (square root-transformed) activity (A) and it components, number of 1 min intervals during which any activity occurred (B) and average revs min⁻¹ during those active intervals (C) for males (squares) and females (circles) from the selected (filled symbols) and control (open symbols) lines. Wheel circumference is 1.12 m. Values are weekly averages +1 least-squares S.E. (N=19–20 per group).

Table 1. *Body mass (g) of mice from selected and control lines at weaning (21 days of age)*

	Females		Males	
	Control	Selected	Control	Selected
N	97	106	109	98
Mean	12.1±0.60	11.6±0.42	12.2±0.60	12.1±0.57
Range	4.5–17.8	3.5–17.2	6.1–19.0	4.5–18.4
Adjusted mean	12.5±0.60	11.8±0.42	13.1±0.60	12.6±0.57

Values are means + S.E.M.; N=number of animals.

Adjusted means are least-squares means (LSMEANS command) from SAS GLM procedure; calculations are based on the ANOVA model that nested family within line, and line within linetype (no covariates were used).

access and selection resulted in a significant decrease in final mass in both sexes. With weaning mass as a covariate, wheel-access females were 1.16 g (4.3 %) lighter than sedentary females; wheel-access males were 1.58 g (4.7 %) lighter than sedentary males (Table 2). Selected females were 3.66 g (12.9 %) lighter than control females; selected males were 5.10 g (14.3 %) lighter than control males (Table 2). The activity×linetype interaction was not statistically significant for either sex. Analyses were repeated without weaning mass as a covariate (Table 2) with qualitatively equivalent results for both sexes. Thus, even after accounting for differences in initial (weaning) body mass, differences in final mass between selected and control lines persisted.

Effects of selection on final body mass were also analyzed separately within the sedentary and wheel-access groups. Within the sedentary group, selected females averaged 3.2 g (11.2 %) lighter than controls (P<0.05); selected males were 5.2 g (14.2 %) lighter than controls (P<0.01). Within the wheel-access group, selected females averaged 3.9 g (14.0 %) lighter than controls (P<0.01); selected males were 4.5 g (12.9 %) lighter than controls (P<0.01).

Within the wheel-access group, models with a covariate of average revs day⁻¹ measured during the last week of wheel access were also tested. In these analyses, amount of wheel-running had a significant negative effect on body mass for males (Table 3; Fig. 2B), but not for females (Table 3; Fig. 2A). Final body mass adjusted for variation in amount of wheel-running was 3.2 g (11.6 %) less in selected females than in controls (Table 3); in males, the difference was 3.00 g (8.8 %; Table 3). Thus, even after accounting for variation in wheel-running, the differences in final mass were substantial. The difference was statistically significant for males but not females (Table 3).

Genetic correlation between wheel-running and body mass

Based on Falconer's (1989) equation 19.6, the genetic correlation between wheel-running (the trait selected for 14 generations) and body mass at the end of the 8 weeks of wheel access was estimated to be −0.50.

410 individuals from 39 families. Weaning mass did not differ statistically between selected and control lines for either sex (P>0.5; Table 1). By the end of the 8-week wheel trial, however, a two-way ANCOVA indicated that both wheel

Table 2. *Body mass of mice from the four measurement groups after 8 weeks of access to running wheels either free to rotate (wheel-access) or locked (sedentary)*

| Variable	Body mass (g)			
	Sedentary control	Wheel-access control	Sedentary selected	Wheel-access selected
Females				
N	19	19	20	20
Body mass at 75–81 days				
Mean	28.4±0.54	28.1±0.53	25.6±0.52	24.1±0.49
Range	23.8–32.3	25.1–33.9	21.7–31.3	20.1–28.2
Adjusted mean (with weaning mass as a covariate)*,‡	28.6±0.41	28.2±0.40	25.7±0.41	23.8±0.38
Adjusted mean (without weaning mass as a covariate)*,‡	29.0±0.35	28.6±0.34	25.3±0.34	23.5±0.34
Males				
N	19	19	20	20
Body mass at 75–82 days				
Mean	36.1±0.65	34.7±0.65	31.7±0.59	29.9±0.56
Range	32.1–42.3	30.9–42.6	26.2–37.3	24.4–34.2
Adjusted mean (with weaning mass as a covariate)*,§	36.3±0.52	35.1±0.47	31.5±0.48	29.6±0.45
Adjusted mean (without weaning mass as a covariate)**,§	36.6±0.46	35.2±0.44	31.3±0.42	29.5±0.44

Adjusted means are presented for models with and without weaning mass (see Table 1) as a covariate.

Values are means + S.E.M.; N=number of animals.

*Effect of linetype $P<0.05$; **effect of linetype $P<0.01$.

‡Effect of activity group $P<0.05$; §effect of activity group $P<0.01$.

In addition to weaning mass, time (z-transformed time)^2, age and number of toes clipped for identification were used as covariates in the model. Age was a significant covariate in models both with and without weaning mass for females. No covariates were statistically significant for males.
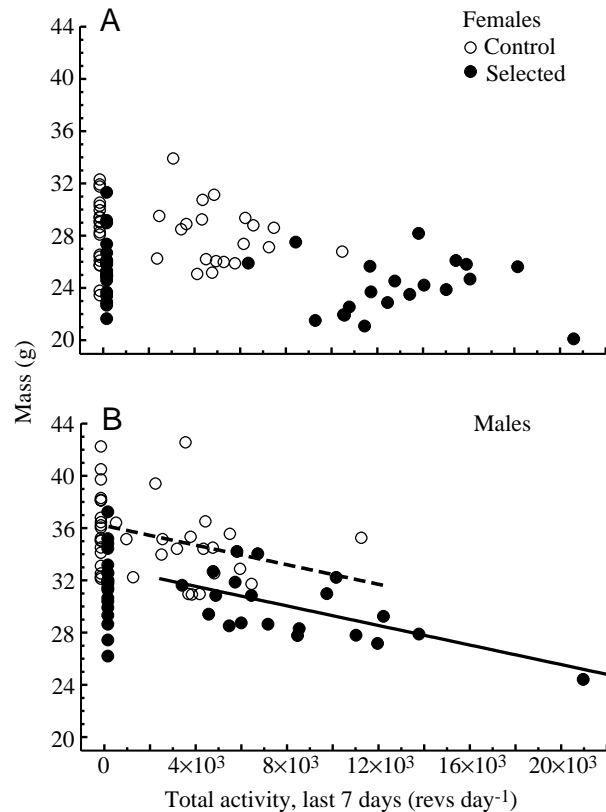
Discussion

Wheel-running

Mice from the selected lines ran significantly more revs day^{-1} immediately after weaning (week 1 of the trial) and a difference persisted for the entire 8-week study (Fig. 1). These results indicate that the phenotype upon which we base our selection (i.e. amount of running on days 5+6 at 5.5–8 weeks of age) is positively genetically correlated with wheel-running at all ages between 3 and 11 weeks. As might be expected, the largest absolute difference between the selected and control lines occurred during the fourth and fifth week of wheel access, which is approximately the same age (7–8 weeks

Fig. 2. Body mass of selected and control mice measured at the end of wheel access (75–82 days of age) in relation to revs day^{-1} (mean over last week of access to wheels) for females (A) and males (B). Mice with non-rotating wheels ('sedentary') are plotted with zero revs day^{-1} (values offset slightly for clarity). The regression line shown in B is the partial regression slope from one-way ANCOVA (within the wheel-access group only) of final body mass with revs day^{-1} over the last week as one of the covariates (see Table 3). The regression lines were forced through mean body mass and average daily running distance of the selected and control groups. The estimates of the slope for the effect of revs day^{-1} on body mass were −0.093 g 1000 revs^{-1} ($P>0.40$; not shown in A) and −0.374 g 1000 revs^{-1} ($P<0.01$) for females and males, respectively.

Table 3. *Results of nested ANCOVA of final body mass of female and male mice with access to running wheels ('sedentary' mice excluded from analysis)*

Source	d.f.	Body mass (g) Type III SS	MS	*F* value	*P*
Females					
Linetype	1	19.51	19.51	2.12	0.1955
Line(linetype)	6	55.18	9.20	3.64	0.0099
Weaning mass	1	49.96	49.96	19.76	0.0002
Time of day	1	0	0	0	0.9958
Time^2	1	1.52	1.52	0.60	0.4449
Age	1	6.07	6.07	2.40	0.1340
Number of toes clipped	1	0.02	0.02	0.01	0.9322
Revs day^{-1} (final week)	1	1.34	1.34	0.53	0.4726
Model	13	279.98	21.54	8.52	0.0001
Error	25	63.21	2.53		
Corrected total	38	343.19			
Males					
Linetype	1	47.21	47.21	7.01	0.0382
Line(linetype)	6	40.43	6.74	1.55	0.2037
Weaning mass	1	30.53	30.53	7.02	0.0138
Time of day	1	2.33	2.33	0.54	0.4711
Time^2	1	0.48	0.48	0.11	0.7432
Age	1	1.73	1.73	0.40	0.5344
Number of toes clipped	1	8.47	8.47	1.95	0.1753
Revs day^{-1} (final week)	1	41.70	41.70	9.58	0.0048
Model	13	383.82	29.52	6.79	0.0001
Error	25	108.78	4.35		
Corrected total	38	492.602			

Tests of hypotheses concerning linetype were performed using the type III MS with line(linetype) as an error term.

d.f., degrees of freedom; SS, sum of squares; MS, mean square.

of age) that we normally select for increased wheel-running (see Swallow et al., 1998a).

Body mass as a genetically correlated trait

This experiment clearly demonstrated that selection for increased activity results in decreased body mass at maturity. By 11 weeks of age, mice from genetically selected lines averaged 13.6 % lighter than controls (based on adjusted means from nested two-way ANCOVA; Table 2). Even when the analyses were repeated only within the sedentary group in which, presumably, differences in activity would be less, mice from the selected lines were significantly lighter than mice from control lines (11.2 % and 14.2 % lighter for females and males, respectively; Table 2).

Thus, we selected for high voluntary activity and produced lines of mice that are smaller in body mass: the genetic correlation between adult body mass and wheel-running was estimated to be −0.50. At first glance, these results seem

consistent with the anecdotal observations of MacArthur (1944) and Falconer (1953). However, apparent behavioral differences between lines of mice selected for body size, as reported by these authors, may have been more indicative of reactivity to disturbance than general activity level; in particular, they described their small lines of mice as 'elusive' (MacArthur, 1944) and 'jumpy' (Falconer, 1989, p. 199). Indeed, Fowler (1962) found no difference in spontaneous home-cage activity between Falconer's large and small lines. Moreover, in a different set of lines selected for large and small body size, Holmes and Hastings (1995) also found no difference in open-field activity.

As Mather (1981; see also Sherwin, 1998) points out, activity levels measured in different ways (e.g. wheel-running, open-field activity, spontaneous home-cage activity) are not interchangeable and, therefore, cannot be directly compared (see also Koteja et al., 1999b). Alternative measures of activity often reflect different traits rather than different manifestations of a single trait, general activity level. For example, selection on open-field activity did not cause changes in voluntary wheel-running behavior (De Fries et al., 1970). Thus, our study constitutes the first clear evidence that activity levels are negatively genetically correlated with body size in house mice.

What is the mechanism for a negative genetic correlation between voluntary activity levels and body mass? One possibility is that selection may be affecting body mass entirely through the intermediate phenotype of activity: mice from selected lines might be smaller because they were more active even in normal housing cages. After 8 weeks of wheel access, however, even after the effects of wheel-running and weaning mass had been accounted for by ANCOVA (Table 3), mice from selected lines were still lighter than mice from control lines (females 11.6 %, males 8.8 %; Fig. 2). Therefore, we conclude that the difference in mass between selected and control lines is not mediated solely through the intermediate phenotype of activity. In future studies, we will investigate the mechanistic basis of this apparent correlated response, including studies of food consumption and body composition (Swallow, 1998; Koteja et al., 1999a).

Effect of wheel access on body mass

8 weeks of access to running wheels also had a negative effect on final body mass, but the magnitude of the effect was much less than the difference between selected and control lines (Table 2). At 11 weeks of age, after 8 weeks of access to wheels, wheel-access females were 4.3 % lighter than sedentary females and wheel-access males were 4.7 % lighter than sedentary males.

Within the wheel-access group, the final body mass of males depended on activity levels (Fig. 2B, Table 3). The slope describing the relationship between final body mass and wheel-running was −0.374 g 1000^{-1} revs (partial regression slope from the ANCOVA model with mean revs day^{-1} during the last week as one of the covariates). Two previous studies on mice from generation 10 also indicated that, in males, individual

variation in wheel-running activity was negatively correlated with final body mass (Swallow et al., 1998b; Koteja et al., 1999a).

Consistent with a study from generation 10 (Koteja et al., 1999a), the relationship between total revs day^{-1} and final body mass in females was not statistically significant (-0.093 g 1000^{-1} revs, Fig. 2A). Notably, this slope is less than $\frac{1}{4}$ the estimate for males, suggesting that females protect against loss of body mass better than do males, as is also true of female rats (Cortright et al., 1997). This result is supported by our finding that female mice maintain body mass better than males when challenged by cold exposure (P. Koteja, J. G. Swallow, P. A. Carter and T. Garland Jr, unpublished data).

Lack of genotype-by-environment interaction

In our analysis, the effect on body mass of the activity×linetype interaction was not significant ($P>0.05$). Lack of an interaction implies that the effects of linetype and activity are independent and additive. *A priori*, we expected the difference in activity, and therefore in body mass, between selected and control animals to be greater in the wheel-access group as compared to the sedentary group. The trend was in this direction in both sexes (Table 2). Lack of statistical significance may reflect the low power of ANOVA to detect interactions (Wahlsten, 1990). Alternatively, selection for wheel-running activity might also have influenced activity levels in general, even without access to free wheels. However, focal-animal observations at generation 13 indicate that differences in activity other than wheel-running (e.g. grooming, non-wheel-running locomotion, sleep) are not statistically significant (Koteja et al., 1999b). A previous study of males at generation 10 also found no significant activity×linetype interaction for body mass (Swallow et al., 1998b).

Evolutionary implications

Genetic correlations have the potential to constrain (or facilitate) the rate and direction of phenotypic evolution in response to selection (e.g. see Falconer, 1989; Garland and Carter, 1994; Lynch, 1994; Dohm et al., 1996). In the absence of selection, or if selection acts on only one of two genetically correlated traits, evolutionary divergence of populations and species should occur along a trend dictated by the genetic correlation. Thus, the negative genetic correlation we observed between size and activity could account for the negative, although not statistically significant, relationship ($r=-0.21$) between body size and voluntary wheel-running observed across 13 species of muroid rodents (Dewsbury, 1980). However, across species of mammals ranging in size and phylogenetic position from mice to elephants, both home range area and daily movement distance increase with body size (see Garland, 1983; Goszczynski, 1986 and references therein). Thus, evolutionary diversification of size and activity levels in mammals must have entailed parallel selection on both. Therefore, over long time scales, the negative genetic correlation between body size and activity

did not impose an ineluctable evolutionary constraint. To test experimentally whether the negative genetic correlation between size and activity imposes a constraint on the evolution of house mice, selection could be applied simultaneously to both traits (Weber, 1990; Garland and Carter, 1994).

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