Selection for high voluntary wheel-running increases speed and intermittency in house mice (*Mus domesticus*)

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Summary

In nature, many animals use intermittent rather than continuous locomotion. In laboratory studies, intermittent exercise regimens have been shown to increase endurance compared with continuous exercise. We hypothesized that increased intermittency has evolved in lines of house mice (*Mus domesticus*) that have been selectively bred for high voluntary wheel-running (wheel diameter 1.12 m) activity. After 23 generations, female mice from four replicate selection lines ran 2.7 times more revolutions per day than individuals from four random-bred control lines. To measure instantaneous running speeds and to quantify intermittency, we videotaped mice ($N=41$) during a 5-min period of peak activity on night 6 of a 6-day exposure to wheels. Compared with controls (20 revs min^{-1} while actually running), selection-line females (41 revs min^{-1}) ran significantly faster. These instantaneous speeds closely matched the computer-recorded speeds over the same 5-min period. Selection-line females also ran more intermittently, with shorter (10.0 s bout^{-1}) and more frequent (7.8 bouts min^{-1}) bouts than controls (16.8 s bout^{-1}, 3.4 bouts min^{-1}). Inter-bout pauses were also significantly shorter in selection-line (2.7 s) than in control-line (7.4 s) females. We hypothesize that intermittency of locomotion is a key feature allowing the increased wheel-running performance at high running speeds in selection-line mice.

Key words: activity, evolution, exercise, intermittent locomotion, selection, wheel-running, house mouse, *Mus domesticus*.

Introduction

In laboratory studies of terrestrial locomotion, researchers usually attempt to control the influence of behavior by testing animals under fixed locomotor regimens. One example is the testing of animals at constant treadmill speeds to determine steady-state rates of oxygen consumption and, hence, energetic costs (e.g. Autumn et al., 1999; Full et al., 1990; Taylor et al., 1982). As another example, studies of locomotor performance abilities attempt to motivate animals maximally and thus eliminate variation in 'willingness to perform' (for a review, see Garland and Losos, 1994). Free-ranging animals, however, can choose to move at a range of speeds and for varying durations (D. J. Irschick and T. Garland, in preparation) (Blumstein, 1992; Christian et al., 1997; Irschick and Jayne, 1999; Kenagy and Hoyt, 1989; Weinstein, 1995). Moreover, contrary to the conditions imposed in most laboratory studies, many species from a wide range of taxa exhibit intermittent locomotion in the field (Kramer and McLaughlin, 2001). For instance, ghost crabs observed in the field spent 64% of overall activity time in motionless pauses (Weinstein, 1995). Similarly, anuran hopping locomotion is typically intermittent (for references, see Anderson et al., 1991). Terrestrial rodents, such as gray squirrels and chipmunks, spend up to 41% of foraging time motionless (McAdam and Kramer, 1998), and heteromyid rodents typically spent more than 90% of surface activity in slow, intermittent movement and stops (Thompson, 1985). Some species of bird use flap-bounding, an intermittent pattern of flying (Tobalske et al., 1999). Intermittent swimming and gliding is characteristic of many deep-diving mammals, such as dolphins, Weddell seals and blue whales (Pennisi, 2000; Williams et al., 2000). Many human exercise activities also include periodic rest periods. For example, many occupational tasks are performed intermittently (Mathiassen, 1993; Mathiassen and Winkel, 1992; Mathiassen and Winkel, 1996), and several sports are characterized by intermittent, high-intensity efforts (e.g. soccer and American football).

Intermittent exercise has been studied most thoroughly in humans (e.g. Donnelly et al., 2000; Essen, 1978; Rossouw et al., 2000; Spriet et al., 1988). Several studies have shown that endurance can be increased and/or performance can be improved when heavy work is performed in short periods of exercise interrupted by pause periods of low-intensity or no work. In early studies of intermittent exercise in humans, researchers demonstrated that, under a heavy work load, exercise duration was increased when the work was performed intermittently (Astrand et al., 1960). Endurance-trained athletes could remain at maximal oxygen uptake 2.9 times longer and traveled 1.9 times farther before exhaustion during intermittent running at speeds associated with maximal oxygen

uptake compared with continuous running at slightly lower speeds (Billat et al., 2000). An intermittent protocol improved work-tolerance time and decreased the rate of core body temperature increase for heavy exercise in Canadian Forces soldiers wearing medium or heavy protective clothing (McLellan et al., 1993). Trained swimmers maintained higher swimming velocities and lower blood lactate levels in a highly intermittent regimen compared with steady swimming, although some intermittent patterns did not alter swimming performance (Olbrecht et al., 1985). In studies of isolated muscle groups, endurance time was enhanced and perceived fatigue reduced in protocols involving intermittent isometric elbow (Hagberg, 1981), shoulder–neck (Mathiassen, 1993) and hand-grip (Bystrom et al., 1991) exercises.

Intermittent locomotion is relatively unstudied in non-human mammals. In an exploration of the rabbit (New Zealand white) as a model for exercise, Meng and Pierce (1990) tested animals during low-speed ($1.2\,\mathrm{km\,h^{-1}}$) continuous and moderate-speed ($2.4\,\mathrm{km\,h^{-1}}$) intermittent treadmill-running. Distance endurance was not increased by intermittent (30 s run, 30 s rest) running, although the rabbits tolerated higher plasma lactate levels and 'appeared to be more motivated to run' during the intermittent protocol (Meng and Pierce, 1990). Wistar rats actually demonstrated reduced distance capacity in an intermittent (alternating 1-min intervals at 0.86 and $2.6\,\mathrm{km\,h^{-1}}$) compared with a continuous treadmill-running protocol (Brooks and Gaesser, 1980). However, one study suggests that intermittent running could improve performance in rodents: Thompson (1985) found that untrained kangaroo rats ran intermittently on a treadmill, which caused a plateau in the relationship between oxygen consumption and treadmill speed.

An endurance benefit of intermittent locomotion has also been demonstrated in two species of ectotherm. In the ghost crab *Ocypode quadrata*, distance capacity (the absolute distance covered until fatigue) was increased 2.2-fold during intermittent locomotion (120 s of exercise, 30 s pause) at speeds of 170 % of maximal aerobic speed (MAS) compared with continuous locomotion at the same average speed (Weinstein and Full, 1998). In a similar study of the frog-eyed gecko *Teratoscincus przewalskii*, distance capacity was increased 1.7-fold during intermittent locomotion (15 s of exercise, 30 s pause) at speeds of 270 % of MAS (Weinstein and Full, 1999). It is important to note that, in both studies, only speeds well above MAS were used in the intermittent regimens and some intermittent protocols failed to increase (gecko) (Weinstein and Full, 1999) or actually decreased (ghost crab) (Weinstein and Full, 1998) distance endurance.

We are studying the correlated evolution of locomotor behavior and physiology through a replicated selective-breeding experiment with house mice (Swallow et al., 1998a). The selection criterion is the total number of revolutions accrued on days 5 and 6 of a 6-day exposure to running wheels, as recorded by an automated system. Sixteen generations of selection increased wheel-running by approximately 2.6-fold in four replicate replicate selection lines compared with four

replicate control lines, and this differential has been maintained through generation 26 (see Results) (see also Koteja and Garland, 2001; Rhodes et al., 2000). On the basis of the computer-recorded data, which are obtained in 1-min bins, the increase in distance (number of revolutions per day) has been achieved mainly by an increase in average running speed rather than by an increase in the amount of time spent running (Koteja and Garland, 2001; Rhodes et al., 2000; Swallow et al., 1998a). However, automated counting systems may not accurately reflect instantaneous running speeds, nor can they provide reliable estimates of the duration or frequency of actual running bouts (Eikelboom, 2001).

In the present study, we used videotaped observations to characterize the patterns of wheel-running in selection and control mice. We hypothesized that mice from the lines bred for high revolutions per day would also exhibit increased intermittency of wheel-running. Specifically, we expected that, during peak wheel-running, selection-line mice would exhibit a shorter bout duration, a greater frequency of bouts and shorter interbout pauses.

Materials and methods

Animals

Subjects were female laboratory house mice (*Mus domesticus*) from the twenty-third generation of an artificial selection experiment for high voluntary wheel-running (Swallow et al., 1998a). Because female mice of both selection and control lines demonstrate higher levels of wheel-running than do males (Koteja and Garland, 2001), we presumed that differences in patterns of wheel-running would be easier to detect and were, perhaps, of greater importance in females. In the beginning of the selection experiment, eight closed lines of mice were derived from outbred, genetically variable Hsd:ICR house mice. Four lines were selected for high voluntary wheel-running, while four others were bred randomly as controls. At 21 days of age, offspring were weaned from the dam, weighed, toe-clipped and housed in groups of four, by sex, until measurement of voluntary wheel-running at 35–55 days old. In the selection lines, breeders were chosen from the male and female with the highest running activity in each of 10 families per line. The selection criterion was the number of revolutions on days 5 and 6 of a 6-day exposure to wheels, as recorded by an automated system. In control lines, breeders were chosen randomly. Inter-sibling mating was disallowed in all lines.

Exposure to wheels

As part of the routine propagation of the selection experiment, wheel-access trials were conducted in three consecutive batches, with approximately 195 animals per batch in two wheel rooms. Six females from each of the four control and four selected lines (*N*=48 total; each from a different family) were chosen at random from the three wheel-access batches, with 16 females videotaped per batch. Videotaped subjects were placed in a single room on designated wheels

that had initial wheel freenesses (an inverse measure of rotational resistance) within 5 % of the mean initial freeness (range 95–105 revolutions) and a deceleration rate of 37–41 revs min^{-2} from an initial speed of 40 revs min^{-1}. Wheel freeness was measured as the number of revolutions following acceleration of the wheel to a velocity of 40 revs min^{-1}. Wheel freeness was measured at the beginning and end of the 6-day trials, with the measure of final freeness typically 80–120 % of that of initial freeness. Red lights and video equipment were placed in the room during the first day of wheel access and remained on through day 6. All mice were weighed immediately before wheel access and again at the end of the 6-day exposure.

Running was measured on Wahman-type activity wheels (1.12 m circumference; Layfayette Instruments, Layfayette, Indiana, USA) attached to a standard home cage by a short tube. The number of wheel revolutions was recorded at 1-min intervals by an automated system (San Diego Instruments, 1992). This yielded data over the 24 h of day 6, including total number of revolutions, number of active 1-min intervals (intervals with one or more revolutions), maximum number of revolutions in a 1-min interval and mean speed (total number of revolutions divided by the number of active 1-min intervals). The number of revolutions and mean speed over the 5-min observation period were also extracted from the 24-h dataset. In addition, characteristics of wheel bouts were calculated from the computer-recorded data using a computer program written in C++. Wheel bouts were defined as sets of continuously active 1-min intervals, bracketed by one or more intervals without any revolutions. Reversals in the direction of rotation (e.g. clockwise to counterclockwise) were not counted as ending a wheel bout. The total number of wheel bouts, the maximum length (in 1-min intervals) of any wheel bout and the mean length (in 1-min intervals) of all wheel bouts were calculated from the computer-recorded data.

Videotaping

Mice were videotaped on day 6. Each mouse was videotaped for 1 h, with up to six animals being videotaped simultaneously. Videotaping began 1 h after lights off and continued over the next 3 h, which corresponds to the period of peak wheel-running activity in both selection and control lines. The circadian pattern of wheel-running over day 6 appeared to be generally similar for mice from selection and control lines, although controls exhibited more wheel-running in the first hours after lights on (Fig. 1).

We wished to analyze periods when mice were running near their peak speeds, so only the fastest 5 min of running during the 1 h of videotape available for each mouse was analyzed. For each mouse, the 5-min period with the greatest number of revolutions during the 1-h tape was determined using the computer data. For this 5-min period, the duration of running bouts (to the nearest 0.5 s), the number of revolutions (to the
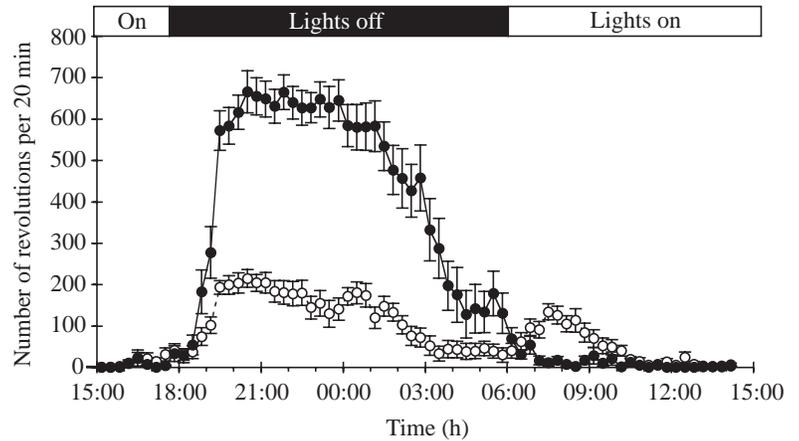

Fig. 1. Circadian pattern of wheel-running on day 6 of a 6-day exposure to wheels in selection (filled circles) and control (open circles) lines of house mice (females) included in a videotape microanalysis of wheel-running. Values are means ± S.E.M. The graph shows the number of revolutions for each 20-min period as collected by an automated system in 1-min bins.

nearest 0.5 revolution) run in each bout and the times of wheel entries and exits were recorded by one of four observers. The observation period began at the start of a running bout and continued to completion of the last running bout closest to 300 s. Running bouts were defined as periods of continuous running. A running bout ended when an animal coasted (i.e. was holding on inside a rotating wheel) or exited from the wheel. From these data, the following variables were calculated: the duration of each running bout (bout duration in s), the number of running bouts (bout frequency in number per min of observation), the times between running bouts (interbout pause in s), the speed during each bout (bout speed in revs min^{-1}), the proportion of revolutions run (number of revolutions actually spent running as a percentage of the total number of running and non-running revolutions accrued during the observation period), the total time spent running over the observation period (time spent actually running as a percentage of the total observation time), the total time spent off the wheel (time off the wheel as a percentage of the observation time), the number of exits from the wheel (exits per min of observation) and the total time of videotape analyzed (observation time in s). Adjustment for observation time was necessary because the observation period for each mouse was not exactly 300 s (range 298–378 s; excluding the maximum time of 378 s for one mouse, observation time ranged from 298 to 307 s in the other 40 mice included in analyses below). Mean bout duration, bout speed and interbout pause were calculated for each mouse and used in analyses.

Examples of the wheel-running activity during videotaping, as recorded automatically by computer, and of the 5-min segment chosen for micro-analysis, are shown in Fig. 2 for a representative selection and control female. From informal visual inspection of similar graphs for every animal, the wheel-activity patterns illustrated in Fig. 2 appeared to be typical of both selection and control mice.
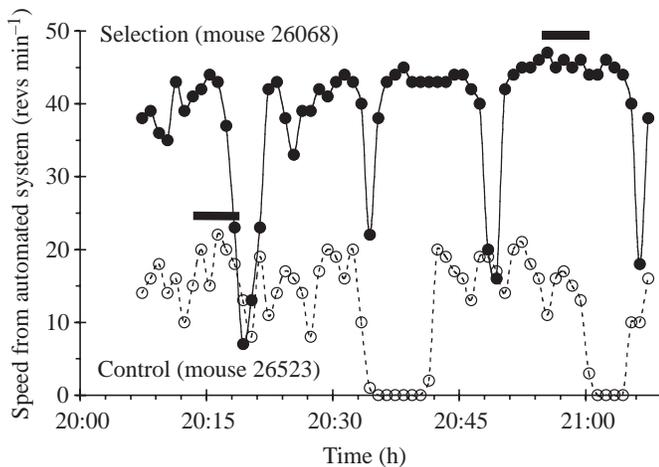
Fig. 2. Example of wheel-running as collected by the automated system for a selection-line female (filled circles) and a control female (open circles) during an hour of peak wheel-running activity and the 5-min observation period used for videotape microanalysis (filled bars). The four deceleration events from the wheel of the selection-line female suggest that she may have stopped running during these times, although the automated system recorded continuous activity over the hour. The wheel of the control-line animal clearly stopped rotating at least twice during this hour.

Statistical analyses

As characteristics of high-speed running were of primary interest, we included only data from females that ran close to their maximum speed. We implemented an arbitrary criterion that data were included only if the mean bout speed from videotape analysis was at least 75 % of the maximum speed in any 1-min interval, as recorded by the automated system over the entire day 6 (24 h). Data were therefore excluded for four animals (one control and three selection) that happened not to run close to maximum speed during videotaping. The total number of revolutions and mean running speeds (computer data) of these four mice did not differ from the mean number of revolutions and speeds of their respective lines ($t<1.5$, $P>0.18$); rather, they simply did not exhibit peak running activity over the hour during which they were videotaped. In addition, one control mouse did not run at all during videotaping, and two (one control and one selection) were not visible on the videotape because of an error in camera placement. Thus, data for 41 mice were ultimately analyzed. For all analyses, data were transformed as necessary for normality of residuals, and statistical outliers (studentized residual >2.5) were excluded.

Analyses of the effect of selection history were performed using a mixed-model analysis of covariance (ANCOVA) [PROC MIXED in SAS (6.12)], in which random line effects were nested within a fixed effect of genetic selection history. The effect of selection history on the characteristics of running in videotaped females was tested with models including age, batch, observer, wheel freeness measured at the end of the trials, time of day of the highest 5-min period and (z-transformed time of day)^2, to account for a possible non-linear

trend with time, as covariates or cofactors. Analyses were repeated including final body mass as a covariate. The effect of selection history on characteristics of wheel activity, as recorded by the automated system, was tested in models including wheel freeness, age, batch and room as covariates or cofactors. Comparisons among videotaped subjects ($N=41$) and the remaining females ($N=227$) tested concurrently on wheels were made within a selection group only: the main effect of videotaping was tested in a model including random line effects with wheel freeness, age, batch and room as covariates or cofactors.

Relationships between characteristics of running from videotape analysis and total number of revolutions on day 6 (reflecting the trait of selection) were examined in two ways. In one set of analyses, bout characteristics (speed, duration, frequency, interbout pause) were included in an ANCOVA model to predict the total number of revolutions on day 6. The ANCOVA models also included the main effect of selection history with wheel freeness and age as covariates. Five models were tested: one included all four bout characteristics simultaneously as independent variables, and four models included only one of each of the bout characteristics. In a second set of analyses, each bout characteristic was the dependent variable in an ANCOVA including the main effect of selection history with total revolutions on day 6 as a covariate. These four models also included the interaction term (total number of revolutions × selection history) with wheel freeness and age as covariates.

Results
Automated system day 6

Videotaped mice from the selection lines ran 2.8 times more total revolutions than controls. The mean speed of selection mice was 2.5 times higher than that of controls, with a small, statistically non-significant difference in the time spent running (Table 1; selection 1.1 times higher than controls).

Considering all mice, selection females in generation 23 ($14\,083\pm991$ revolutions; $N=193$, least-square mean ± S.E.M.) ran 2.7 times more revolutions on day 6 than did controls (5270 ± 35 revolutions; $N=75$). Selection and control mice included in videotape analyses did not differ from their respective populations as a whole in total number of revolutions, number of 1-min intervals or maximum speed in any 1-min interval (effect of videotaping, $F_{1,252}<0.6$, $P>0.5$; interaction of selection history × videotaping, $F_{1,6}<0.15$, $P>0.7$). All mice in the room containing the video equipment tended to run more than mice in the second room: in the above statistical model containing dummy variables for room and videotaping, room ($F_{1,252}>6.3$, $P<0.012$) was the only significant predictor of number of revolutions and speed on night 6 other than selection history. Although we cannot rule out the possibility that the process of videotaping disturbed all the mice, room has had a statistically significant effect of wheel-running during normal test exposures in past generations (J. G. Swallow, P. A. Carter, J. S. Rhodes and

Table 1. *Wheel-running activity on day 6 in control and selection lines of house mice*

	Selection (N=20)		Control (N=21)			Selection history	
	LSM	95 % CI	LSM	95 % CI	Ratio*	$F_{1,6}$	P
Automated system: 24 h							
Number of revolutions×10^{-3} (day^{-1})	16.0	(11.9, 20.6)	5.65	(4.57, 6.85)	2.83	32.0	0.001
1-min intervals (day^{-1})	538	(429, 673)	476	(353, 641)	1.13	3.4	0.11
Mean speed (revs min^{-1})	28.5	(24.8, 32.3)	11.6	(10.2, 13.1)	2.46	43.6	<0.001
Maximum speed (revs min^{-1})	43.0	(37.9, 47.5)	22.4	(17.7, 26.3)	1.92	40.3	<0.001
Automated system: 5 min							
Mean speed (revs min^{-1})	39.8	(36.7, 43.2)	17.3	(15.9, 18.6)	2.30	46.3	<0.001
Videotape: 5-min							
Bout speed (revs min^{-1})	40.8	(37.1, 44.9)	20.4	(19.5, 21.3)	2.01	70.8	<0.001
Bout duration (s)	9.95	(2.8, 34.8)	16.8	(4.2, 67.8)	0.59	9.5	0.022
Bout frequency (min^{-1})	7.82	(5.5, 10.5)	3.37	(1.9, 5.2)	2.32	10.4	0.018
Interbout pause (s)	2.73	(1.6, 4.5)	7.44	(4.5, 12.3)	0.37	55.1	<0.001
Number of revolutions run (% of total)	70.9	(67.6, 74.2)	78.3	(70.1, 86.4)	0.90	2.4	0.17
Time run (% of total)	70.7	(64.2, 77.1)	66.0	(57.6, 74.4)	1.07	0.8	0.40
Exits (min^{-1})	0.40	(0.23, 0.69)	0.77	(0.32, 1.8)	0.57	7.7	0.032
Time off (% of total)	1.96	(0.05, 5.0)	5.74	(2.4, 11.2)	0.34	9.3	0.022
Body mass on wheels (g)	24.1	(23.0, 25.3)	26.7	(25.6, 27.8)	0.90	10.9	0.016
Body mass off wheels (g)	23.8	(22.8, 24.8)	25.9	(25.0, 26.8)	0.92	10.8	0.016

Values are back-transformed least-square means (LSM) with 95 % confidence intervals (lower, upper).

Data were collected by an automated system over 24 h and for the 5-min videotaped observation period and from videotape microanalysis of a 5-min observation period during peak wheel-running activity.

Body mass was measured immediately prior to wheel exposure and following removal from the wheel at the end of the 6-day exposure.

Analyses of wheel-running data included random line effects nested within a fixed effect of selection history and covariates or cofactors: age, batch, room (automated data only), wheel freeness, observer, time of day of the highest 5-min period and (*z*-transformed time of day)^2 (videotape data only).

*Ratio is the ratio of the mean selection value to the mean control value.

I. Girard, unpublished observations). The level of wheel-running exhibited by the videotaped mice is not unusual: the value for mean number of revolutions on day 6 has ranged from 11 551 to 16 903 over the last eight generations in selection-line females. Final wheel freeness (all mice, 90.9±2.8 revolutions, mean ± S.E.M.; range 10–221 revolutions, N=268) was not a significant predictor of total number of revolutions, intervals or maximum speed (all $F_{1,252}$<1.09, P>0.29). Analyses repeated with final body mass as a covariate yielded similar results.

5-min videotape data

Mean bout speeds of individual mice ranged from 26.4 to 49.6 revs min^{-1} among selection-line animals and from 16.3 to 27.3 revs min^{-1} in controls (Table 1). In the former, the mean bout speed obtained from videotape analysis did not differ significantly from the average speed recorded by the automated system over the same period (paired *t*-test; *t*=1.0, d.f.=19, P=0.32). In animals from control lines, however, mean videotape bout speed was significantly higher than the average speed recorded by the automated system over the same period (paired *t*-test; *t*=4.5, d.f.=20, P=0.0002).

Bouts of wheel-running were generally much shorter than the 1-min bins used to collect data automatically. Although every wheel rotated continuously over the 5-min observation period, no mouse ran continuously over this period. Among selection females, the longest single bout was 124 s; among controls, the longest bout was 135 s. All mice ran at least five bouts over the observation period, with a maximum of 128 bouts per 367 s among selection mice and 32 bouts per 301 s among controls. For both selection and control lines, many animals were running at or near the maximal possible combination of bout duration and frequency (Fig. 3). No animal spent less than 50 % of the 5-min observation time in actual running, reflecting our criterion of using periods of high activity (total revolutions) for mice included in statistical analyses of videotape data.

During the 5 min of videotaping, selection females ran in bouts that were significantly faster, shorter and more frequent than those of controls (Table 1). Selection females also exited the wheel less often and spent less time out of the wheel than controls. The proportion of revolutions actually run and the proportion of observation time spent running were not significantly different between groups. The proportion of total revolutions accrued during coasting was significantly higher ($F_{1,6}$=23.0, P=0.003) in selection females (27.9±1.8 %) than in controls (15.5±1.9 %). Control mice tended to accrue more revolutions while they were outside the wheel (5.5±0.2 % of
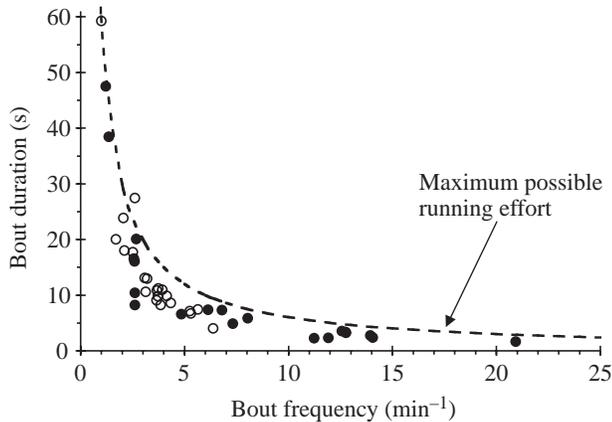
Fig. 3. Selection-line females (filled circles) ran bouts that were shorter and more frequent than those of control females (open circles). In both groups, the frequency and duration of running bouts show a strong negative relationship. The dashed line represents the maximum possible combination of bout frequency and duration if pause durations approach zero (individual points can lie above this line because of measurement error).
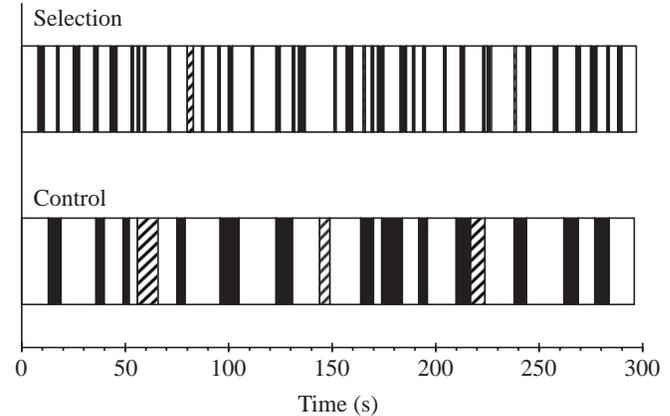

Fig. 4. Diagrammatic depiction of average patterns of intermittent wheel-running for selection-line (top) and control (bottom) female mice over the 5-min videotaped observation: open columns represent periods of running, filled columns represent pause periods of coasting and hatched columns represent periods when the mouse had exited from the wheel.

total revolutions) than selection mice (3.6±1.2%), but this difference was not statistically significant ($F_{1,6}=2.8$, $P=0.14$). Wheel freeness (100.4±2.2 revolutions, mean ± S.E.M.; range 62–154 revolutions, $N=41$) measured at the end of the trials never had a significant effect on any measure of wheel-running (all $F_{1,26}<1.8$, $P>0.19$). Analyses repeated with final body mass as a covariate yielded statistically similar results because body mass did not have a significant effect on any running parameter (body mass effect, $F_{1,29}<1.1$, $P<0.3$ in all analyses). The average pattern of wheel-running over the 5-min observation for selection and control females is represented in Fig. 4.

A 1-min QuickTime movie of wheel-running in one selection and one control female of a later generation (generation 27) filmed during activity under white light is included for illustrative purposes. The movie can be viewed at: http://www.biology.ver.edu/faculty/Garland/Girard01.mov.

Relationship between bout characteristics and total number of revolutions on day 6

All four bout characteristics (speed, duration, frequency, interbout pause) appeared to be related to the total number of revolutions accrued over day 6. Of these, bout speed and interbout pause appeared to be the most strongly related to the total number of revolutions (Fig. 5). In an ANCOVA model including all four bout characteristics as well as selection history, both bout speed and interbout pause were significant predictor of the number of revolutions on day 6 (bout speed, $F_{1,22}=14.2$, $P=0.001$; interbout pause, $F_{1,22}=5.8$, $P=0.025$), and the effect of selection history was not significant ($F_{1,6}=0.01$, $P=0.98$). In similar ANCOVAs with only one of the four bout variables entered at a time, bout speed ($F_{1,22}=52.4$, $P<0.0001$; selection history, $F_{1,6}=1.0$, $P=0.35$), bout frequency ($F_{1,22}=12.5$, $P=0.0015$; selection history, $F_{1,6}=19.1$, $P=0.004$) and interbout pause ($F_{1,22}=5.83$, $P=0.023$; selection history,

$F_{1,6}=11.2$, $P=0.014$) were significant independent predictors of the total number of revolutions over day 6, but bout duration was not ($F_{1,22}=1.2$, $P=0.28$; selection history, $F_{[1,6]}=26.4$, $P=0.002$). Wheel freeness never had a significant effect in any of the above ANCOVA models (all $F_{1,25}<1.3$, $P>0.26$).

In a second set of ANCOVA models, each of the four bout characteristics was included separately as the dependent variable, with selection history as a main effect and including total number of revolutions, wheel freeness, age and batch as covariates or cofactors. The interaction term (total number of revolutions × selection history) was never significant ($F_{1,23}<0.8$, $P>0.4$) and was dropped from all models. Both selection history and total number of revolutions were significant predictors of bout speed (selection history, $F_{1,6}=23.6$, $P=0.003$; total revolutions, $F_{1,24}=25.6$, $P=0.0001$) and interbout pause (selection history, $F_{1,6}=8.4$, $P=0.027$; total revolutions, $F_{1,24}=13.9$, $P=0.001$). The results for bout speed and interbout pause indicate that, for a given total number of revolutions, selection-line females have higher bout speeds and shorter interbout pauses than control-line females. Neither selection history ($F_{1,6}<0.9$, $P>0.38$) nor total number of revolutions ($F_{1,24}<3.4$, $P>0.08$) was a significant predictor of either bout duration or bout frequency, suggesting that these models were over-specified by the inclusion of both the main effect of selection history and total revolutions as a covariate. Wheel freeness never had a significant effect in any of the second set of ANCOVA models (all $F_{1,24}<2.5$, $P>0.12$).

The characteristics of wheel bouts in the videotaped mice, as determined from the computer-recorded data over 24 h of day 6, were strikingly different from those of the videotaped running bouts. The duration of individual wheel bouts (the number of consecutive active 1-min intervals from computer-recorded data) ranged from 1 to 461 among selection mice and from 1 to 168 among control mice. The mean duration of
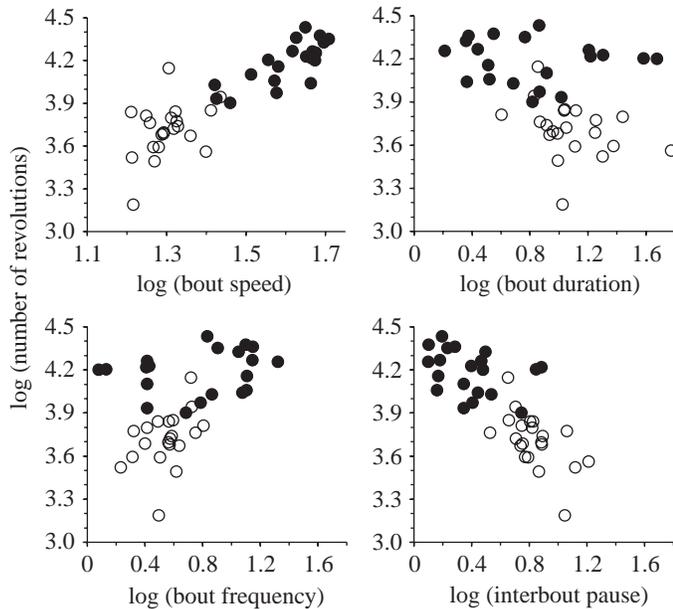
Fig. 5. Relationships between characteristics of wheel-running as measured during videotape microanalysis and the total number of revolutions over day 6 as recorded by an automated system. These data reflect the original selection criterion. Filled circles, selection-line mice; open circles, control mice. Note that the abscissa for bout speed is different from the others, reflecting its narrower range of variation on the logarithmic scale. Bout speed is measured in revs min^{-1}, bout duration in s, bout frequency in min^{-1} and interbout pause in s.

wheel bouts was 130 times longer in the selection group (21.7±3.14 min, N=20) and 50 times longer in controls (14.4±3.1 min, N=21) (least-square means ± S.E.M.) than the duration of videotaped running bouts, and did not significantly differ between selection-history groups ($F_{1,6}$=2.73, P=0.15). The maximum duration of a computer-recorded wheel bout was higher in selection mice than in controls (selection mice, 113±12 min, N=20; control mice, 61±10 min, N=21; $F_{1,6}$=20.5, P=0.004; least-square means ± S.E.M.). The total number of wheel bouts over day 6 ranged from 8 to 57 in selection mice and from 14 to 58 in control mice, with no statistically significant difference between groups (selection mice, 24.6±4.4 wheel bouts day^{-1}, N=20; control mice, 32.4±4.2 wheel bouts day^{-1}, N=21; $F_{1,6}$=2.0, P=0.20; least-square means ± S.E.M.).

Body mass and age

Selection-line females were significantly smaller than controls at the beginning and end of the 6-day exposure to wheels (Table 1), but the absolute change in mass during exposure was not significantly different between groups ($F_{1,6}$=1.0, P=0.35). Age during wheel exposure (range 48–65 days old) was also not significantly different between groups (selection mice, 56.7±0.96 days, N=20; control mice, 57.6±1.22 days, N=21; $F_{1,6}$=0.51, P=0.5; least-square means ± S.E.M.).

Discussion

Instantaneous running speeds

Although we have previously reported an increase in average wheel-running speed (as measured by the automated system) as a response to selection (Koteja and Garland, 2001; Koteja et al., 1999; Rhodes et al., 2000; Swallow et al., 1998b), this study provides the first direct measurements of instantaneous running speeds on the wheels. This result is important because in many studies, including ours, wheel-running activity is measured by an automated counting system, which may not adequately describe the actual speeds and patterns of wheel-running (Eikelboom, 2001; Koteja and Garland, 2001). The resolution of automated systems is limited in two main ways: data collected in bins (e.g. over 1 min or 24 h) do not measure instantaneous running speed and, in many types of wheel, revolutions can accrue during brief pauses in actual running, which may occur during coasting or short exits. A video-based system is the only method readily available that circumvents these problems.

The instantaneous speeds recorded for the mice from selection lines (2.7 km h^{-1}), although substantially higher than for mice from control lines (Table 1), are still considerably lower than the forced maximal sprint running speeds that we have recorded previously for mice from the base population. Dohm et al. (1996) reported an average maximal sprint speed of 5.1 km h^{-1} (range 3.4–8.1 km h^{-1}, N=200) for female Hsd:ICR mice and reported maximal speeds of 5.0 km h^{-1} for a smaller sample of 19 female Hsd:ICR mice (Dohm et al., 1994). Mice from the selected lines do not, therefore, appear to be running at maximal sprint speed on the wheels.

Videotape analysis demonstrated that computer-recorded data may not accurately reflect mean speeds during running and generally overestimate the duration of continuous running. For control animals, the average computer-recorded speed during the 5-min videotape observation period (total number of revolutions divided by 5×1-min intervals) was significantly lower than the speeds measured by direct observation during actual running (see Table 1). In selection animals, actual bout-running speeds obtained from direct observation did not differ significantly from mean computer-recorded speeds over the same 5-min period. This difference between control and selection-line mice is attributable to the fact that mice from control lines, even during their peak or near-peak 5-min periods of running, exhibit pauses that are long enough to allow significant deceleration of the wheel [for a discussion of differential effect of bin size on high and low runners, see Eikelboom (2001)].

The mean bout duration for all animals, however, was much shorter than the 1-min bins used to collect wheel-running data automatically. Although every wheel (including those with animals from control lines) rotated continuously over the 5-min observation period, no mouse ran continuously for more than 135 s, and mean bout lengths were considerably shorter than 1 min. Thus, the duration of a continuous running bout based on computer-recorded data (sequential active 1-min intervals) would have greatly exaggerated bout duration in every animal. For example, computer-recorded data for the

selection mouse in Fig. 2 (no. 26068) indicated that her wheel registered 60 consecutive active 1-min intervals during videotaping, although her longest running bout recorded by direct observation over 5 min was only 4 s. Estimates of bout duration derived from computer-recorded data over 24 h also differed by an order of magnitude or more from those measured by video observation during peak activity.

Effect of selection for high voluntary wheel-running on intermittency

Selective breeding based on total distance (number of revolutions) during voluntary wheel-running resulted in correlated increases in running speed and bout frequency and decreases in the duration of bouts and interbout pauses. All mice ran intermittently over the observation period, including those from the control lines, suggesting that intermittency during spontaneous wheel-running is characteristic of Hsd:ICR mice and may also be characteristic of other rodent species (De Kock and Rohn, 1971; Premack and Schaeffer, 1963; Rodnick et al., 1989). However, mice from selection lines ran shorter and more frequent bouts – hence, more intermittently – than controls. Thus, selection for voluntary exercise has altered the behavioral pattern of wheel-running, in addition to causing an increase in running speed. Because an increase in intermittency occurred in all four replicate selection lines, the correlated response in running pattern was clearly the result of our selection protocol. In contrast, in a study of a single population of rats, individuals demonstrating relatively high voluntary wheel-running activity had significantly longer bouts, but not significantly faster bouts, than those demonstrating low voluntary wheel-running activity (Rodnick et al., 1989). In addition, for two of the running characteristics (bout speed and interbout pause), selection has fundamentally altered the relationship with the total number of revolutions accrued over day 6: when adjusted for a similar total number of revolutions, selection-line females still run faster bouts and take shorter pauses than do control-line females, at least over an observation period during near-peak wheel-running activity.

Functional significance of increased intermittency

The functional significance of the correlated increase in intermittency could be related to increased distance endurance at running speeds that are higher than those of the control-line mice. Intermittent exercise may increase endurance for heavy work (see Introduction) by stimulating lactate removal and preventing blood lactate accumulation (Billat et al., 2000; Saltin et al., 1976) or by increasing the efficiency of oxidative enzymes (Timmons et al., 1998). Pauses could also allow for resaturation of myoglobin and hemoglobin levels and for resynthesis of glycogen and high-energy phosphate stores (Gaesser and Brooks, 1984; Piiper and Spiller, 1970), thereby increasing distance endurance when running effort is high.

During the 5-min periods of videotaping, selection-line females ran at a mean bout speed of 40.8 revs min^{-1}, which translates to a linear speed of 2.7 km h^{-1}. In older male mice from our selection lines, maximal oxygen consumption is elicited at
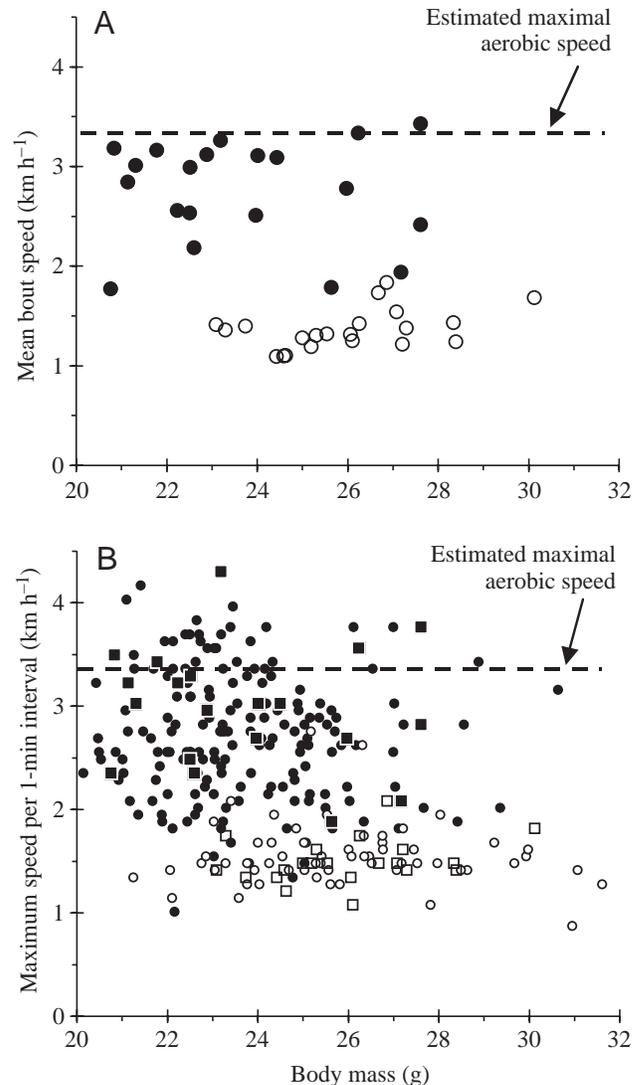

Fig. 6. Wheel-running speeds are greater in selection-line females (filled symbols) than in control females (open symbols) when measured by direct observation during videotaping (A; mean bout speed, $N=41$) or when estimated for all females of generation 23 using computer-recorded data (B; maximum speed in any 1-min interval, $N=268$). In B, squares designate videotaped animals. In some selection-line females, mean bout speed and maximum speed in any 1-min interval are near or above the predicted maximal aerobic speed [predicted for a 28 g animal from fig. 6 of Koteja et al. (1999)] on a running wheel (dashed line).

an average treadmill speed of 2.1 km h^{-1} (Swallow et al., 1998b), and maximal aerobic speed on the treadmill in female mice of another laboratory strain is reported to be 2.0 km h^{-1} (Baker and Gleeson, 1999). However, these speeds are likely to underestimate the maximal aerobic speed during voluntary exercise because psychological stress may be a component of forced exercise, elevating the rate of oxygen consumption at every speed and thus reducing the speed at which maximal oxygen consumption is reached. Indeed, an estimate derived from various studies of the cost of locomotion and maximal oxygen consumption in house mice predicts a maximal aerobic

speed (MAS) of 3.36 km h^{-1} (Koteja et al., 1999). Although the intensity of wheel-running relative to maximal oxygen consumption has not been measured in our mice, it is apparent that speeds during bouts of voluntary wheel-running can approach this estimated maximal aerobic speed (Fig. 6). In two laboratory studies of ectotherms [ghost crab, Weinstein and Full (1998); gecko, Weinstein and Full (1999)], distance endurance was improved during an intermittent regimen (compared with continuous locomotion at the same average speeds) in which bout speeds exceeded maximal aerobic speeds, although speeds lower than the MAS were not applied in either study.

Reducing the energetic cost of activity may be another function of increased intermittency in the selection-line mice. All mice ran intermittently on wheels, which may be more costly than continuous running. For example, when excess post-exercise oxygen consumption (EPOC) is included, the cost per unit distance of a single, brief, relatively high-intensity exercise bout of treadmill running is much higher than the cost during sustained locomotion (Baker and Gleeson, 1998). The high cost associated with brief locomotor behavior patterns such as intermittent wheel-running can be reduced, however, by increasing the frequency of locomotor bouts (Edwards and Gleeson, 2001). Mice running frequent, brief treadmill bouts (13 bouts of 15 s duration, at a speed during running of 1.8 km h^{-1}, with 15 s pause) had a cost of activity (in ml O$_2$ g^{-1} km^{-1}) comparable with that for continuous running at a similar average speed (Edwards and Gleeson, 2001). In fact, the cost of wheel-running in females, as estimated from food consumption (0.43 kJ km^{-1}) (see Koteja et al., 1999), was calculated to be approximately half the incremental cost of locomotion (0.87 kJ km^{-1}) (see Taylor et al., 1982), as measured during sustained treadmill running, suggesting that the high-speed intermittent wheel-running is not as energetically expensive as one might expect from treadmill studies of brief locomotor bouts [as measured by, for example, Edwards and Gleeson (2001)].

An alternative, non-exclusive hypothesis is that mice also use intermittent running, specifically the duration of interbout pauses, to control the speed of the wheel. The mice cannot actively decelerate the wheel, so they must instead allow the passive resistance of the wheel to slow it. The running wheels decelerate at an average of approximately 40 revs min^{-2} at high speeds (I. Girard, unpublished observations), such that the speed can be reduced by approximately 10 % over a few seconds of coasting. Mice could use pauses to reduce the speed of the wheel to match a psychological or physiological 'preferred' speed. However, we believe it is unlikely that the functional significance of the overall increased intermittency is related to controlling wheel speed. Mice could control the speed of the wheel more simply by running at a constant speed rather than by alternating periods of acceleration and deceleration. In addition, only the duration of interbout pauses could contribute to passive control of wheel speed, yet other measures of intermittency, such as bout frequency and bout duration, have also responded to selection for increased distance. One might imagine that experiments involving manipulation of wheel freeness and/or setting limits to

maximal wheel speeds (by some mechanical device) might be able to tease apart the possible role of intermittency for controlling speed, but it is important to note that, in the present analyses, wheel freeness was never a statistically significant predictor of interbout pause duration, of bout frequency or of any other measured aspect of wheel-running.

Other suggested 'functions' of intermittency (for a review, see Pennisi, 2000), such as improved detection of prey or predators [as suggested by Gendron and Staddon (1983); McAdam and Kramer (1998)], do not seem relevant for our laboratory-stock mice. It also seems unlikely that the brief pauses observed in our mice would allow for a substantial decrease in the rate of oxygen consumption as occurred in the ghost crab (Weinstein and Full, 1998) and gecko (Weinstein and Full, 1999). Even if our selection-line mice running on wheels experienced a decline in oxygen consumption during pauses, the decrement over a few seconds of coasting is likely to be quite small.

In conclusion, our results demonstrate that selection for total distance during voluntary wheel-running has resulted in higher instantaneous running speeds, shorter running bouts, more frequent running bouts and shorter pauses between bouts. Thus, at its apparent limit (an approximately 2.6-fold increase in the total number of revolutions), the evolutionary response to selective breeding has involved all measured bout components of wheel-running (bout speed, duration, frequency, pause), rather than only one or two. This comprehensive change in wheel-running behavior suggests that increased intermittency may play an important role in facilitating the increased total daily running distance of selection females compared with controls. A profitable area for future studies might be to compare free-living species of animal that differ in total daily movement distance (Garland, 1983; Girard, 2001; Goszczynski, 1986). We predict that, all else being equal, species that move greater distances will also tend to move more intermittently.

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