Behaviour of house mice artificially selected for high levels of voluntary wheel running

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We have developed a novel model to study the correlated evolution of behavioural and morphophysi-ological traits in response to selection for increased locomotor activity. We used selective breeding to increase levels of voluntary wheel running in four replicate lines of laboratory house mice, *Mus domesticus*, with four random-bred lines maintained as controls. The experiment presented here tested for correlated behavioural responses in the wheel-cage complex, with wheels either free to rotate or locked (environmental factor). After 13 generations, mice from selected lines ran 2.2 times as many revolutions/day as controls on days 5 and 6 of initial exposure to wheels (10 826 versus 4890 revolutions/day, corresponding to 12.1 and 5.5 km/day, respectively). This increase was caused primarily by mice from selected lines running faster, not more minutes per day. Focal-animal observations confirmed that the increase in revolutions/day involved more actual running (or climbing in locked wheels), not an increase in coasting (or hanging). Not surprisingly, access to free versus locked wheels had several effects on behaviour, including total time spent in wheels, sniffing and biting. However, few behaviours showed statistically significant differences between the selected and control lines. Selection did not increase the total time spent in wheels (either free or locked), the frequency of nonlocomotor activities performed in the wheels, nor the amount of locomotor activity in cages attached to the wheels; as well, selection did not decrease the amount of time spent sleeping. Thus, wheel running is, at the genetic level, a largely independent axis of behaviour. Moreover, the genetic architecture of overall wheel running and its components seem conducive to increasing total distance moved without unduly increasing energy or time-related costs. The selection experiment also offers a new approach to study the proximate mechanisms of wheel-running behaviour itself. For example, frequencies of sniffing and wire biting were reduced in selected females but not males. This result suggests that motivation or function of wheel running may differ between the sexes.

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Locomotion is an important component of behaviour from a variety of perspectives, including ecology, ethology and evolutionary biology. At the level of indi-vidual variation within natural populations, locomotor performance abilities may affect such major components of Darwinian fitness as survivorship (e.g. Christian & Tracy 1981; Jayne & Bennett 1990). Among species, locomotor abilities measured in the laboratory correlate with aspects of movement (speed, frequency, distance)

observed in the field (e.g. Irschick & Losos 1998; Garland 1999). Costs of locomotion are incorporated, explicitly or implicitly, in various models of resource acquisition, territory defence or migratory behaviour (for reviews see: Schoener 1987; Alerstam 1991). Locomotion has also played a major role in macroevolution. For example, according to the aerobic capacity model, selection for high levels of aerobically supported locomotor activity was a key factor causing the evolution of vertebrate endothermy (Bennett & Ruben 1979; Hayes & Garland 1995).

A powerful tool for testing hypotheses about correlated evolution is an artificial selection experiment, which can be used to mimic evolutionary processes (Garland & Carter 1994; Gibbs 1999). For example, C. B. Lynch and colleagues created lines of mice divergently selected for nest-building behaviour and observed correlated

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responses in several behavioural and physiological traits (e.g. Lynch 1992, 1994; Bult et al. 1993; Bult & Lynch 1997).

We present results from an artificial selection experiment (Swallow et al. 1998a) designed to investigate the ways in which locomotion coadapts with other aspects of behaviour and physiology (Swallow et al. 1998b; Koteja et al. 1999). We used selective breeding to increase the levels of voluntary wheel running in four replicate lines of laboratory house mice, *Mus domesticus*, with four random-bred lines maintained as controls (Swallow et al. 1998a). The base population was Hsd:ICR mice, an out-bred stock with relatively high levels of genetic variation, comparable to those in wild populations of house mice (Hauschka & Mirand 1973; Rice & O'Brien 1980; Carter et al. 1999). Comparisons of the Hsd:ICR mice with wild house mice from a Wisconsin population revealed some quantitative differences in locomotor behaviour and metabolic rate, but few differences in lower-level mor-phophysiological traits related to locomotor abilities (e.g. thyroid hormone levels, muscle fibre-type compo-sition), which suggests that these laboratory mice are not physiologically 'degenerate' (Dohm et al. 1994; Richardson et al. 1994; Garland et al. 1995).

For several reasons, voluntary wheel running provides an appropriate model for the study of how locomotor activity coadapts with other behavioural and morpho-physiological traits. First, almost all species that have been offered wheels will actually run in them, including nondomesticated species (De Kock & Rohn 1971; Sherwin 1998). Second, interspecies differences in the amount of daily wheel running are large (e.g. approximately four-fold among 13 species of muroid rodents studied under identical conditions; Dewsbury 1980). Third, wheel-running activity usually occupies a considerable part of the 24-h time budget of a captive rodent (e.g. several hours), and the distances run typically are at least as great as, and often much higher than, distances travelled by free-living individuals (De Kock & Rohn 1971). Fourth, within species, interindividual variation in the amount of wheel running is large (coefficients of variation as high as 75%), repeatable (Friedman et al. 1992) and heritable in the narrow sense (Swallow et al. 1998a). Therefore, the amount of wheel running can be selected at the pheno-typic level and should respond genetically to such selec-tion. Finally, by use of automated recording devices, wheel running can be measured simultaneously in many individuals, which allows the large sample sizes required in any quantitative genetic study, including artificial selection experiments (Boake 1994; Lynch 1994; Falconer & Mackay 1996).

At generation 13, when the present study was con-ducted, mice from the selected lines were running, on average, more than twice as many wheel revolutions per day as mice from control lines (see Results). This differen-tial spans half of the range observed among 13 species of muroid rodents (Dewsbury 1980) and exceeds the differ-ence observed between wild and laboratory house mice raised under common conditions (Dohm et al. 1994), thus suggesting that correlated responses may have occurred. Indeed, at generation 10, when mice from

selected lines were running only 70% more than controls, we found that high-selected lines had evolved increased maximum aerobic capacity (measured during forced treadmill exercise), increased food consumption rate, and decreased body mass (Swallow 1998; Swallow et al. 1998b, 1999; Koteja et al. 1999). Thus, as we expected, the selection for voluntary running resulted in changes in physiological and morphological traits related to locomotor performance and energy metabolism. In the present study, we asked whether the selection for increased locomotor activity on the wheels resulted also in correlated responses in other aspects of behaviour. We report that few of the behaviours we scored show differences between the selected and control lines, thus indicating that voluntary wheel running is a largely independent axis of behaviour (see also Sherwin 1998).

The main question we address is whether the selected mice are more or less active than unselected controls when not in the wheels (i.e. when in the normal housing cages to which the wheels are attached) or when the wheels are prevented from rotating. An equivalent question concerning wild animals is as follows. Suppose that individuals of a species obtain a net evolutionary advantage (increased Darwinian fitness) by increasing the distance travelled outside of their immediate home area (perhaps because it causes them to encounter more resources). If variation in distance travelled were herit-able in the narrow sense, then the population mean would evolve towards higher activity levels. How would this evolutionary change affect overall time and activity budgets? Three alternative hypotheses can be proposed.

First, according to an intuitive 'principle of allocation' based on time and energy budget considerations, one might argue that increased activity away from the normal home area (which might be viewed as analogous to activity in wheels) should be accompanied by decreased activity in the home area (analogous to activity in cages attached to wheels), such that the overall level of locomotor activity would remain constant. Alternatively, activity in the home area might remain unchanged, such that overall activity level would increase. Finally, if genes and neurophysiological pathways controlling activity outside and inside the home area were related, then mice from the lines selected for high wheel running should also be more active in cages, thus greatly increasing the total amount of activity. Obviously, these three different scenarios would have very different consequences for the amount of time and/or energy that remained to be devoted to other behaviours (e.g. feeding, grooming, sleeping). Hence, further hypotheses concerning corre-lated responses in morphophysiological and behavioural traits depend on which of the three scenarios actually happened.

Another objective of this study was to gain a better insight into wheel-related behaviour itself; in particular, whether the selected and control lines differ in the frequency of nonlocomotor behaviours performed inside the wheels and in the frequency of coasting in wheels (hanging onto a wheel while it rotates; see De Kock & Rohn 1971; Sherwin 1998, page 18). We also sought to survey patterns of behaviour more generally in order to

test for possible correlated responses in behaviours other than wheel running. In comparisons of selected and control lines, the occurrence of correlated responses may provide important clues about proximate neurophysiological mechanisms underlying both the selected behaviour and others that show correlated responses (Wimer & Fuller 1966; DeFries & Hegmann 1970; Bult et al. 1993). Hence, they may provide new insight into the unresolved issues of the causality and function of wheel-running behaviour (Sherwin 1998). As voluntary wheel running seems also to be a suitable model to study some consequences of human exercise, a better understanding of the behaviour may have broader, health-related implications (Eikelboom 1999).

METHODS

Apparatus

Voluntary wheel running was measured on Wahman-type activity wheels (1.12-m circumference, stainless steel and Plexiglas construction, Lafayette Instruments, Lafayette, Indiana). Activity wheels were divided equally between two rooms. The wheels were attached to standard clear polycarbonate housing cages (27 × 17 × 12.5 cm, metal tops, wood shavings as bedding) via a 5.5-cm long stainless steel tube inserted into a hole (7.7-cm diameter) in the wall of the cage. This arrangement allowed the mice continuous, voluntary access to the wheel, and it also allowed a clear distinction between activities in and out of the wheels (contrary to a 'revolving cage' design; e.g. Rundquist 1933).

Our automated system recorded the number of wheel revolutions in 1-min intervals. Therefore, in addition to total number of wheel revolutions per day, we were also able to calculate the number of active intervals per day (1-min intervals with any wheel revolutions) and average running speed (revolutions per active interval). This allowed us to distinguish two possible ways of increasing running distance.

Subjects

We studied mice from generation 13 of an artificial selection experiment for high voluntary wheel-running behaviour (Swallow et al. 1998a). The original progenitors were outbred, genetically variable Hsd:ICR house mice purchased from Harlan Sprague Dawley (Hauschka & Mirand 1973; Rice & O'Brien 1980; Dohm et al. 1996; Carter et al. 1999). In each generation, 10 pairs of mice were used to propagate each of eight lines: four selected and four control. 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 until measurement of wheel running.

The selection criterion was the total number of revolutions run on days 5 and 6 of a 6-day test (Swallow et al. 1998a). In the selected lines, the highest-running male and female from each family were chosen as breeders. In the control lines, one male and one female from each family were chosen randomly with respect to wheel running.

Throughout the selection experiment and during this study, water and food (Harlan Teklad Laboratory Rodent Diet (W) 8604) were available ad libitum, photoperiod was set a constant 12:12 h light:dark cycle centred at 1400 hours (Central Standard Time), and room temperature was controlled at approximately 23°C.

In the present study, we used one male and one female, sampled randomly from each of the 10 families per line. As part of the routine selection protocol, we placed all 160 mice individually in cages with attached wheels at about 49 days of age (range 41–52 days) and monitored wheel running for a 6-day period. Immediately thereafter, we prevented every other wheel from rotating by use of a wire tie. Within each family, one individual (either a male or a female) was assigned to a free wheel and the other to a locked wheel. Therefore, each of the eight lines was represented by five males with free wheels, five females with free wheels, five males with locked wheels, and five females with locked wheels. One locked-wheel male from a selected line died of unknown causes during the early stages of the experiment.

The 159 mice were maintained for the next 7 weeks as described above. During this time, as part of a separate study, we measured body mass every seventh day; wheel revolutions were not recorded during that day and the following night. An exception was the 10-day block of behavioural observations, during which the animals were not disturbed by any handling. In addition, at the beginning of the second week and at the end of the experiment, the mice were injected intraperitoneally with 50 ml of deuterium-labelled water (D_2O), and blood samples were taken for estimation of body composition (see Swallow 1998). Wheel running was not recorded on the day of injections.

Behavioural Observations

Behaviour was observed for 10 consecutive days (6–15 March 1997), during the sixth and seventh week of wheel access, beginning after the mice had spent 37 days in cages with attached wheels. To allow observations at night, incandescent red lamps were turned on 24 h/day for several days before and during the observations. For each of two rooms, one day observer and one night observer watched the mice in the same room for the duration of the experiment. Each day, 16 mice were observed on two occasions: during the day between 1000 and 1200 hours, and during the night between 2100 and 2300 hours. As explained below, day and night observations were analysed separately; also, all statistical analyses included a blocking variable for room, which also controlled for possible differences among observers.

We constructed a focal-animal activity budget based on 15-min observation periods with instantaneous sampling (Altmann 1974; Martin & Bateson 1986). Every 10 s, the observer checked one or two of a list of 27 possible behaviours (see Table 1) to record what the mouse was doing at that instant (15 × 6=90 checks). A red light-emitting diode was used as a timer. Observers were blind

Table 1. Description of 27 observed behaviours, 12 categories of behaviours used in statistical analyses, and three variables describing the percentage of particular behaviours relative to other categories

Behaviour recorded	Location*	Category analysed	Description
Run or climb	W	Run	Run on free wheel or climb on locked wheel
Coast or hang	W	Coast	Coast on free wheel or hang on locked wheel
Sit in wheel	W	Wheel Other	Sit at bottom of wheel whether free or locked
In tube	W	Wheel Other	Mouse in tube connecting cage to wheel
Dig in shavings	C	Cage Locomotion	Using paws or mouth/head to dig in shavings
Circling in cage	C	Cage Locomotion	Running in circles or figure eights in cage
Climb in cage	C	Cage Locomotion	Climb on sides or lid of cage (not in wheel)
Walk in cage	C	Cage Locomotion	Any other movement in cage
Groom	B	Groom	Groom body with mouth or paws
Scratch with hind limb	B	Groom	Scratch body using hind limb
Nose through hole	B	Sniff	Poking nose through holes of cage lid or wheel
Sniff air	B	Sniff	Sniff air while sitting, walking or climbing
Sniff shavings	C	Sniff	
Bite	B	Bite	Gnaw on wire of wheel or cage
Sniff water bottle	C	Drink	
Drink from water bottle	C	Drink	
Sniff food hopper	C	Feed	
Feed at hopper	C	Feed	
Sit and chew	B	Feed	
Chew on faeces	B	Feed	Pull bolus from anus and chew
Sleep in ball position	C	Sleep	
Sleep	C	Sleep	In nest, no movement (not in a ball position)
Stretch body	C	Sleep	
Defecate	B		
Freeze	B		Cessation of movement
Stare at observer	B		Stare directly at observer
Stand on hind limbs	B		
Run+Coast		Revolutions	Activities associated with wheel revolutions
Revolutions+Wheel Other		Wheel Time	Total time on wheels
100×Coast/Revolutions		%Coast	Percentage of coasting
100×Wheel Other/(100−Revolutions)		%Wheel Other	Percentage of nonlocomotor activity in wheel
100×Cage Locomotion/(100−Wheel Time)		%Cage Locomotion	Percentage of locomotor activity in cage

*W: In wheels, C: in cages, B: both locations possible, but when behaviours occurred in the wheel, they were also associated with another wheel category.

with respect to selection history of the mice; they wore white laboratory coats and were not allowed to wear perfume, scented deodorant or cosmetics.

While gathering data, we attempted to separate all distinct behaviours. We compiled an extensive list of recorded behaviours (Table 1) based on preliminary observations of our mice and on published behavioural studies of house mice (e.g. van Abeelen 1963, 1966; Mackintosh 1981). Counts for each behaviour observed were totalled and recalculated into percentage scores (i.e. $Y=100$ (count/90)). For statistical analyses, we grouped the 27 behaviours into 12 categories and three ratio variables (Table 1).

Two categories of directly observed behaviour, running in the wheel (Run) and coasting in the wheel (Coast), contributed to the total number of wheel revolutions, the trait directly selected in our artificial selection experiment (Swallow et al. 1998a). Obviously, animals with locked wheels could not run or coast in the wheel. However, climbing (Climb) and hanging in the wheel (Hang) were observed in the locked-wheel mice and were treated as analogues to Run and Coast, respectively, because if the mouse were climbing in a free wheel, the wheel would start to rotate, and if the mouse were hanging in a

rotating wheel, the mouse would coast. We realize, however, that the analogy might be a misinterpretation, and so when testing for the effect of selection we also performed the analysis separately for mice with free and locked wheels.

The third category, wheel revolutions (Revolutions), was a sum of Run and Coast (see Table 1). A score in Revolutions is analogous to the estimate of the number of active intervals recorded with our automated system (see above), and was used to test for the effect of selection on duration of wheel-running activity. We calculated the percentage of coasting (%Coast=100 × Coast/ Revolutions) to evaluate the contribution of coasting to wheel revolutions and to check whether the selection affected the pattern of wheel-running activity.

Other activities in wheel (Wheel Other) included any activity in the wheel that did not cause wheel rotation (e.g. standing on hind limbs, grooming, or in the connection tube). We calculated the percentage of other activities in the wheel (%Wheel Other) after subtracting from the total observations all observations of running or coasting in the wheels, that is, %Wheel Other=100 × Wheel Other/(100 − Revolutions). This allowed us to test the hypothesis that selection for

increased wheel revolutions increases also a preference to spend more time on nonlocomotor activities in the wheels.

We combined time in the wheel or in the connecting tube (i.e. sum of Revolutions and Wheel Other) to calculate Wheel Time. The remaining time ($100 -$ Wheel Time) was spent in cages. All behaviours involving locomotor activity in cages but outside of the wheels (walking in cage, digging in shavings, climbing in cage and circling in cage) were combined into Cage Locomotion. We also calculated the percentage of Cage Locomotion, that is, %Cage Locomotion$=100 \times$ Cage Locomotion/ ($100 -$ Wheel Time), to test the hypothesis that mice from selected lines differed from controls in locomotor activity outside of the wheels.

The Sleep category included stretching because it was always associated with waking. Although, theoretically, mice might sleep in wheels, they were never observed to do so. A test for the effects of wheel access or selection on Sleep is equivalent to a test for a difference in the total time of any activity.

We used the above seven categories and three ratio variables to answer the questions posed in the Introduction. We analysed five other categories (Drink, Feed, Bite, Sniff, Groom) to explore other possible changes in behaviour. With the exception of Drink, the categories were not mutually exclusive from the previous seven categories. We decided to group the observed behaviours to reduce the number of statistical tests, and also because some of the behaviours were closely related. Sniffing the food hopper was associated with feeding, and sniffing the water bottle was associated with drinking; hence, we grouped these behaviours into Feed and Drink, respectively. Grooming, sniffing and biting occurred both in cages and wheels, and could occur simultaneously with other categories. For example, a mouse could hang in the wheel and simultaneously bite the wire (in such situations, the observer checked both categories, Hang and Bite).

Statistical Analyses

The experimental design did not allow comparisons between night and day (which was not of interest in this study) and, consequently, we performed all analyses separately for day and night observations. We also analysed the sexes separately because (1) sex differences in behaviour, including wheel running, are well established (e.g. see Swallow et al. 1998a; Koteja et al. 1999) and (2) males and females were from the same families. A model including family as another level in a nested ANOVA design might be estimated; however, as each family was represented by only one male and one female, hypothesis testing would rely on assumptions of no interaction between sex and family effects.

We used a cross-nested ANCOVA to test simultaneously the effects of selection (line type), variation among replicate lines within the selection and control groups, wheel type (free versus locked), and the interaction between the genetic (line type) and environmental (wheel type) factors. Dependent variables were the percentage scores for particular behavioural categories. To test statistical significance of the effects, we calculated the appropriate F values as follows (Sokal & Rohlf 1981, pp. 387–389; Henderson 1989): (1) effect of selection over variation among replicate lines; (2) effect of wheel type and line type $\times$ wheel type interaction over variation associated with wheel type $\times$ lines interaction term; (3) effect of replicate lines over the residual variance (equal to variation among families).

The model also included covariates of the sequential day of observation (1–10) and Z transformed day squared, time of observation, and Z transformed time squared. In addition, we entered a block variable for room number. Because observations began at the same time every day, time of observation was entered as an order number (1–8) of the observation within a given session. The covariates were included in the model to control statistically for temporal trends in behaviour across days and/or time of observation, which could include possible effects of observer presence. Squared components were included to account for nonlinear trends. The block variable, room number, accounted for possible differences between the rooms as well as between observers.

Preliminary analyses indicated that residuals of the dependent variables were not normally distributed (significantly skewed or kurtotic). Therefore, we performed all analyses of scored behaviours with rank-transformed data (we applied square-root transformation to the total number of wheel revolutions recorded by the automated system). All tests were two tailed and statistical significance was judged at $P<0.05$. All analyses were performed with SYSTAT 7.0 for Windows (SPSS 1997).

RESULTS

Overview

As expected, mice from selected lines ran more revolutions per day than did mice from control lines (Fig. 1a). During days 5 and 6 of the first week of wheel access (time of the regular artificial selection protocol), selected females ran 2.22 times more revolutions per day than controls (11 562 versus 5210 revolutions/day, respectively; $P<0.001$), and selected males ran 2.21 times more than control males (10 090 versus 4570 revolutions/day, respectively; $P=0.012$). These selected/control ratios are greater than reported for generation 10 (1.73 in females and 1.76 in males: Table II in Swallow et al. 1998a).

The increased wheel running in selected lines was caused primarily by an increase in the average speed of running (Fig. 1c) and to a lesser extent by an increase in the amount of time spent running (Fig. 1b). During days 5 and 6 of the first week, the ratios of selected to control lines for revolutions/minute were 1.83 in females (effect of selection: $P=0.001$) and 1.82 in males ($P=0.004$), while the ratios for number of minutes with any wheel revolutions were only 1.25 in females ($P=0.048$) and 1.23 in males ($P=0.151$).

The amount of wheel running also changed over the 8 weeks of wheel exposure. Figure 1a shows that total revolutions increased for the first 3 weeks and then
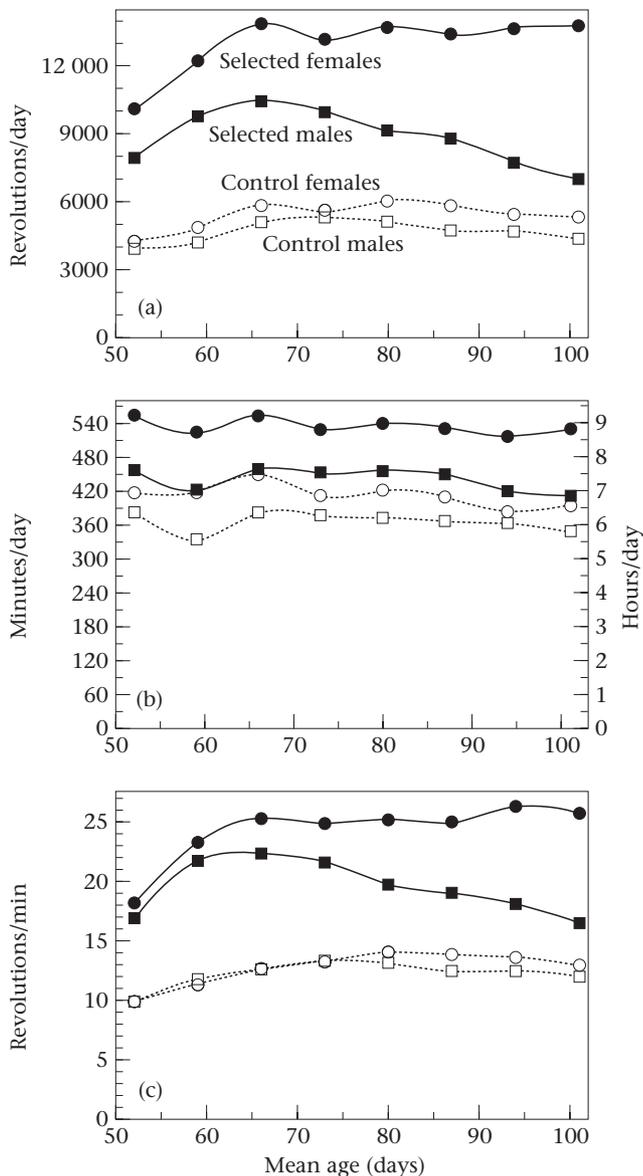
Figure 1. Computer-recorded wheel running of laboratory mice from lines selected for high activity and from control lines. Data points (raw weekly mean values of selection groups) are plotted at the midpoint of each consecutive week of the experiment. Vertical axes are comparable: in each panel, the maximum value is set as 5% higher than the maximum data point. Behavioural observations were made during weeks 6 and 7. The difference between selected and control lines in total revolutions per day (a) is caused mainly by a difference in running velocity (c), not the number of minutes during which any wheel revolutions occurred (b). Note that selected males showed a continuous decrease in running velocity and hence in total revolutions over the last 5 weeks: a similar pattern has sometimes been observed in other studies of rodents (references in Mather 1981; Sherwin 1998). The dip in minutes per day observed during week 2 for all animals may be attributable to the injections of D_2O as part of another study (see Methods, and Chapter V in Swallow 1998).

stabilized, except in selected males, which showed a continuous decrease over the next 5 weeks. The temporal pattern in total revolutions was caused mostly by changes
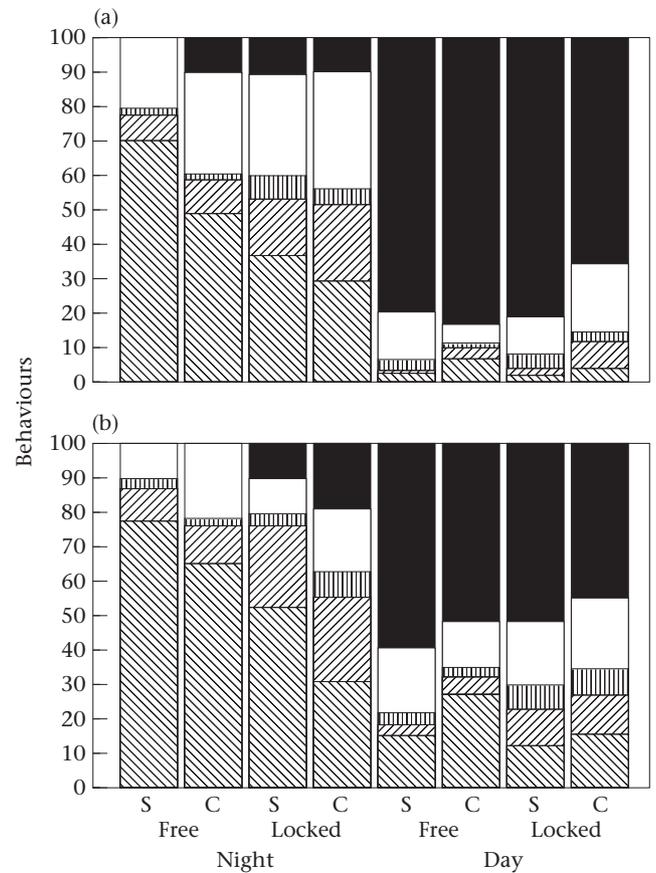

Figure 2. Frequencies of five mutually exclusive behavioural categories observed for (a) males and (b) females (see Table 1). Each vertical bar represents an average of 20 individuals (S: selected; C: control), each of which was observed once during the day and once at night. Each observation period lasted 15 min, with data recorded every 10 s. ■: Sleep; ▥: locomotor activity in cage (Cage Locomotion); ◩: activities associated with wheel revolutions (Revolutions); ▧: nonlocomotor wheel observations (Wheel Other); □: other cage activity (remaining time, equal to 100−Sleep−Cage Locomotion−Revolutions−Wheel Other).

in running speed (Fig. 1c). The number of minutes of wheel activity per day showed relatively little change over time.

During the 10 days when observations of behaviour were performed (weeks 6 and 7; Fig. 1), the number of wheel revolutions, the number of 1-min intervals with any revolutions, and mean running speed all differed significantly between selected and control females ($F_{1,6}=40.00$, $P=0.001$; $F_{1,6}=14.66$, $P=0.009$; $F_{1,6}=25.44$, $P=0.002$, respectively). In males, the difference was almost statistically significant for number of revolutions ($F_{1,6}=5.66$, $P=0.055$), not significant for number of active intervals ($F_{1,6}=2.38$, $P=0.173$), and significant for running speed ($F_{1,6}=6.13$, $P=0.048$).

We did not formally test for differences between sexes or between day and night observations; however, some differences in behaviour were clear (Figs 1, 2). For example, females were more active on the wheels than males, all mice were more active on the wheels at night and they mostly slept during the day (Fig. 2). These

Table 2. Summary statistics for the percentage scores of 12 categories and three ratio variables describing behaviour of mice from lines genetically selected for high wheel-running activity and from random-bred control lines, living in cages with wheels either locked or free to rotate

Variable*	Wheels locked				Wheels free				Significance of effects (P)†			
	Selected		Control		Selected		Control		Wheel type	Line type	Interaction	Room
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD				
Females (night)												
Wheel Time	76.2	30.0	55.4	38.9	86.8	18.7	76.2	21.9	0.007	0.067	0.894	0.168
Revolutions	52.6	24.5	30.8	26.4	77.4	19.6	65.3	20.7	0.001	0.003	0.711	0.881
Run	32.4	24.3	11.1	10.5	48.6	14.8	46.3	15.9	0.004	0.001	0.148	0.692
Coast	20.2	16.4	19.7	21.4	28.8	15.5	19.0	9.0	0.041	0.277	0.261	0.359
Wheel Other	23.6	16.3	24.6	22.9	9.4	11.3	10.9	7.9	0.003	0.755	0.203	0.181
Cage Locomotion	3.3	5.0	7.3	10.8	2.9	8.6	1.9	2.4	0.066	0.233	0.654	0.083
Sleep	9.9	30.4	18.8	35.2	0.0	0.0	0.0	0.0	0.002	0.067	0.082	0.943
Feed	5.9	13.5	7.9	18.1	6.6	9.9	13.9	16.5	0.030	0.224	0.177	0.124
Drink	1.1	1.0	1.2	1.7	0.8	1.4	1.2	1.6	0.091	0.744	0.170	0.057
Sniff	32.8	21.9	36.3	25.3	3.7	8.8	5.7	7.3	0.001	0.007	0.673	0.024
Bite	5.7	8.0	13.6	22.7	0.0	0.0	0.1	0.2	0.001	0.030	0.585	0.910
Groom	11.2	12.1	12.4	17.1	5.4	4.1	9.9	8.1	0.345	0.107	0.498	0.252
%Coast	41.5	29.8	54.1	27.1	35.0	16.4	28.7	10.4	0.022	0.170	0.115	0.048
%Wheel Other	57.8	31.8	43.6	38.1	55.5	34.3	42.5	32.2	0.682	0.241	0.731	0.139
%Cage Locomotion	31.1	35.4	27.4	29.4	22.2	28.6	14.5	23.9	0.291	0.854	0.690	0.028
Males (night)												
Wheel Time	53.0	29.8	51.1	31.9	77.3	19.8	58.8	34.6	0.006	0.266	0.124	0.488
Revolutions	36.5	24.3	29.1	21.8	69.8	19.6	49.2	33.4	0.000	0.123	0.103	0.013
Run	17.5	13.8	13.6	21.4	46.4	13.6	29.7	22.0	0.000	0.024	0.017	0.025
Coast	19.1	15.8	15.5	15.5	23.3	9.6	19.5	14.1	0.066	0.428	0.745	0.046
Wheel Other	16.4	11.3	22.1	20.7	7.5	5.9	9.6	13.1	0.043	0.801	0.842	0.230
Cage Locomotion	6.9	6.3	4.8	3.6	1.8	2.3	1.5	2.2	0.001	0.181	0.495	0.136
Sleep	11.2	26.6	10.2	28.4	0.0	0.0	10.1	25.5	0.185	0.793	0.229	0.027
Feed	13.0	19.4	22.4	30.9	16.7	17.3	20.3	24.9	0.607	0.299	0.687	0.105
Drink	0.9	1.1	1.4	1.3	1.4	1.5	1.3	1.3	0.461	0.429	0.417	0.603
Sniff	32.3	18.0	34.3	25.6	4.8	7.1	6.7	17.2	0.000	0.892	0.316	0.001
Bite	9.3	13.4	11.8	15.8	0.2	0.5	0.7	3.2	0.003	0.801	0.772	0.266
Groom	14.1	14.2	8.2	7.1	4.9	3.7	9.3	12.7	0.127	0.763	0.124	0.003
%Coast	47.2	25.1	48.4	22.3	33.2	8.2	41.5	14.9	0.007	0.236	0.778	0.518
%Wheel Other	32.0	24.0	34.2	27.8	37.3	32.3	28.6	27.1	0.711	0.719	0.692	0.698
%Cage Locomotion	27.2	28.7	17.0	15.3	9.7	9.7	7.8	13.2	0.013	0.117	0.862	0.416

*See Table 1 for definitions of behavioural categories.
†Significance of effects of wheel type (locked versus free) and line type (selected versus control) was tested with cross-nested ANCOVA (see Methods) on ranked data. The effect of replicate lines was never statistically significant and so is not shown. For the behaviour categories associated with running in the wheels, the effect of line type was also tested separately for locked- and free-wheel groups (see Results).

differences were expected and not of main interest to this study. Instead, we focus on the effects of line type (selected versus control) and of wheel type (free versus locked), analysed separately for males and females and for day and night.

Because each individual was observed only once during the night and once during the day, we do not have an estimate of repeatability of the observations across individuals. However, the effects of line type and wheel type were not tested over the variation among individuals, but over the variation among lines and the line × wheel type interaction, respectively. Therefore, a more important problem is whether differences among lines are consistent. To some extent, we can address this by comparing the results obtained in the two rooms (blocks), which could be treated as replications of the entire experiment. Although the room variable appeared significant for some variables (e.g. more sniffing observed in one of the rooms;

Table 2), the pattern of differences between selected versus control and free- versus locked-wheel groups was always the same in both rooms.

The large number of traits analysed, multiplied by four group categories (day–night, sex, line type and wheel type), creates a large number of possible comparisons. To minimize the problem of uncertain probability levels in multiple tests (e.g. Rice 1990), we focus on the comparisons that were planned to answer the questions presented in the Introduction. Table 2 provides extensive results for all the behaviour categories analysed, together with significance levels for the effects of wheel and line type. However, the significance values are not adjusted for the number of comparisons made, nor for interdependence among the scores of particular behaviours. Therefore, our results should be treated as exploratory analyses, rather than confirmatory hypothesis testing (e.g. Martin & Bateson 1986).

Activity During the Night

At night, total time spent in wheels (category Wheel Time; Table 1) was significantly greater with free wheels than with locked wheels (Table 2). However, mice from selected lines did not spend significantly more time in wheels than did controls. We next consider components of the total time spent in wheels.

As explained in Table 1, Revolutions included running or climbing (Run) and coasting or hanging (Coast). At night, the effect of wheel type was clear in both sexes: the mice were more active on free wheels than on locked wheels (Revolutions: females: $F_{1,6}=52.52$, $P<0.001$; males: $F_{1,6}=90.67$, $P<0.001$; Table 2). Mice from selected lines had higher Revolution scores than mice from control lines, but the difference was statistically significant only in females (females: $F_{1,6}=23.23$, $P=0.003$; males: $F_{1,6}=3.21$, $P=0.123$). The interaction between line type and wheel type was not significant for either sex (Table 2), which suggested that mice from selected lines tended to be more active on the wheels even when the wheels were locked. However, when the wheel-type groups were analysed separately, the line-type difference was significant only in the free-wheel group, in both sexes (females: $F_{1,6}=11.59$, $P=0.014$; males: $F_{1,6}=7.16$, $P=0.037$). With locked wheels, females from selected lines engaged in climbing more often than did controls (Run: females: $F_{1,6}=6.48$, $P=0.044$; males: $F_{1,6}=0.45$, $P=0.526$).

Coasting comprised 29–42% of observations of mice active in rotating wheels (%Coast=100 × Coast/Revolutions; Table 2). In females, %Coast did not differ significantly between selected and control mice. In males, %Coast was actually significantly higher in control mice (free-wheel group: $F_{1,6}=14.77$, $P=0.009$). Thus, we found no evidence that selection for high wheel-running activity (total revolutions per day) resulted in an increased tendency for coasting.

We also found no evidence that the artificial selection changed an individual's preferred location within the cage–wheel complex. Individuals from selected and control lines did not differ in the time spent in wheels on activities not associated with wheel revolutions, measured either as a total score (Wheel Other) or as the percentage of observations left after accounting for time spent on locomotor activity in wheels (%Wheel Other; Table 2).

Locomotor activity in cages (Cage Locomotion) did not differ significantly between selected and control lines (Table 2). This result indicates that the selection for high wheel-running activity was very specific and did not substantially change the amount of nonwheel locomotor activity. On the other hand, access to free wheels decreased locomotor activity in cages, at least in males (Table 2). The results were similar when locomotor activity was expressed as a percentage of time remaining after subtracting the total time spent in wheels (%Cage Locomotion).

With free wheels, no females and only a few males from control lines were observed sleeping (Fig. 2, Table 2). With locked wheels, females from control lines tended to sleep more than controls, but the difference was not statistically significant. The only significant effect on Feed was that females with free wheels ate more (Table 2). Drink, a relatively uncommon behaviour, showed no statistically significant effects.

Sniff, Bite and Groom (Table 1) were observed both in cages and in wheels. The amount of grooming was not significantly affected by either wheel type or line type (Table 2). In both sexes, Sniff and Bite were significantly more common in the locked-wheel group compared with the free-wheel group. Sniff and Bite were also more common in control females than in selected ones, but the effect of selection was not significant in males (Table 2).

Activity During the Day

During the day, the mice mainly slept (Fig. 2). The observed amount of sleeping could actually be lower than normal, because our presence may have tended to awaken animals. Indeed, in females we observed a positive trend of amount of sleeping across time of day ($F_{1,59}=12.64$, $P=0.001$) and across consecutive days of observations ($F_{1,59}=9.07$, $P=0.004$), and corresponding negative trends for activity on the wheels (time of day: $F_{1,59}=30.30$, $P<0.001$; day of observation: $F_{1,59}=9.86$, $P=0.003$). The trend across time of day may be a result of the females being awakened when observers entered the room, part of a normal daily rhythm of activity, or both. The trend across days of observations suggests that mice were becoming accustomed to the observation protocol. In males, the scores for sleeping were higher than in females, and the amount of sleeping did not change significantly across time of day or across days of observation.

During the day, wheel activity and the counts of sleeping, feeding and drinking were not significantly affected by either wheel type or line type (Fig. 2). In females, the counts in categories Sniff, Bite and Groom were significantly higher in the locked-wheel group than in the free-wheel group (Sniff: $F_{1,6}=13.96$, $P=0.010$; Bite: $F_{1,6}=47.72$, $P=0.001$; Groom: $F_{1,6}=13.41$, $P=0.011$). Sniffing was also more frequent in the females from control lines than in those from selected lines ($F_{1,6}=30.93$, $P=0.001$). In males, none of the behavioural categories showed significant effects of either wheel type or line type.

DISCUSSION

Overview

The idea that behavioural and morphophysiological traits should evolve together in response to selection is noncontroversial, yet difficult to test. Many approaches are possible, including comparative studies of differences among species. Artificial selection offers an experimental approach that can directly reveal underlining genetic correlations between a behaviour under selection and other traits that are genetically related. Moreover, in a two-way experiment as presented here, the effects of genetic differences between the selected and control lines

can be studied simultaneously with the effects of differences in environment (in the present study, access to free versus locked wheels), and the interactions between the genetic and the environmental factors can be revealed.

Not surprisingly, we found that access to a wheel that was either free to rotate or locked (a difference in environment) had several effects on the behaviour of house mice (Fig. 2, Table 2). However, the effects of genetic selection for high wheel-running activity were generally restricted to locomotor activity in wheels, with frequencies of other behaviours remaining mostly unchanged. In other words, the effect of our phenotypic selection protocol has been rather specific for running in wheels (or attempting to run in locked wheels). This suggests that the genes and neurophysiological pathways controlling voluntary wheel-running behaviour are largely independent of those controlling other behaviours (at least of those studied), and that wheel running can evolve independently of other behaviours. We will consider the broader implications of our selection experiment from two perspectives: as a model to study coadaptation of behavioural and morphophysiological traits, and as a tool to study the phenomenon of wheel-running behaviour itself.

The Artificial Selection Experiment as an Evolutionary Model

In the Introduction, we outlined three possible scenarios of evolutionary response to selection for increased daily running distance. The results of our experiment support the second hypothesis that wheel running is genetically an independent trait. Selection for increased wheel running did not produce mice that were, generally, more or less active. Specifically, the selected and control lines did not differ in the amount of locomotor activity within the attached cages or in time spent sleeping (Fig. 2, Table 2). Thus, in quantitative genetic terms, wheel running is genetically uncorrelated with other forms of locomotor activity. Therefore, whatever its biological function and proximate causes, wheel running should be able to evolve relatively independently of other locomotor activities. Consistent with this prediction, DeFries et al. (1970) found that mice bidirectionally selected for open-field behaviour did not differ significantly in exercise wheels. Moreover, Dewsbury (1980) found that voluntary wheel running was not significantly correlated with open-field behaviour among 13 species of muroid rodents.

Note, however, that the 'principle of allocation' prediction is supported by our comparison across wheel-access groups: mice with free wheels had lower locomotor activity in cages (Cage Locomotion) than did individuals with locked wheels, especially for males (Table 2). Lachmansingh & Rollo (1994) also found a negative correlation between locomotor activity of laboratory house mice in cages and in wheels. Thus, the effect of our genetic selection was not as it would be predicted from correlations observed at the purely phenotype level. This result shows the usefulness of selection experiments in testing hypotheses concerning evolutionary processes

(see also Garland & Carter 1994; Lynch 1994; Hayes & Garland 1995; Sandnabba 1996; Gibbs 1999).

Mice from the selected lines increased the distance travelled primarily by increasing their average running speed rather than their time spent running in wheels (Fig. 1). In females at night, the observed amount of time spent running or coasting on the wheels (Revolutions; Table 2) was significantly higher in the selected mice than in controls, but the selected/control ratio was only 1.18 (which is similar to the ratio of the number of intervals with running recorded by the automated system; see Fig. 1). In males, the ratio was 1.41, but the difference was not statistically significant. In quantitative genetic terms, this result indicates that the additive genetic covariance between total distance travelled and running speed is higher than that between distance travelled and duration of the activity. This result is also consistent with the predictions of an optimization model based on consideration of the energy costs of locomotion: increasing speed is the less expensive way of increasing distance run (Koteja et al. 1999, and literature cited therein). Moreover, in the wild, increasing running speed rather than time active might avoid additional exposure to predators. Note also that the selection did not increase the time spent on nonlocomotor activities in the wheels (outside the home area; Table 2). Thus, the genetic architecture of overall wheel running and its components seem conducive to increasing total distance moved without unduly increasing energy or time-related costs, and hence might be considered 'adaptive' (e.g. Lynch 1992).

Coasting (hanging onto a wheel while it rotates; see De Kock & Rohn 1971; Sherwin 1998, page 18) comprised more than 30% of the observations of mice on wheels that were rotating at the time of observation (Table 2). Similarly, Drickamer & Evans (1996) found that, depending on sex and age, 30–50% of wheel rotations involved mice hanging onto running wheels (based on video-camera observations over 24-h periods). An increased proportion of coasting in selected lines could be viewed as corresponding to developing a cheaper way of travelling, such as gliding, sailing or snowboarding, which use specific properties of the physical environment to obtain 'free' rides. Although such an evolutionary path has occurred during both biological (e.g. many birds glide) and cultural (e.g. human beings use gliders, skis, etc.) evolution, it did not occur in our selected lines: the percentage of coasting (%Coast) did not increase significantly (Table 2; in males it even decreased). One reason that this evolutionary response has not occurred may be that the energetic cost of wheel running is not a very large percentage of the overall energy budget (Koteja et al. 1999) nor were mice ever food restricted during the selection experiment.

The present and previous studies indicate that our artificial selection protocol has caused changes in the selected lines, as compared with control lines, that reproduce some of the characteristics of wild house mice, as compared with laboratory mice. Specifically, both our selected lines and wild mice show increased total wheel running per day, which is caused mainly by running faster rather than for more minutes per day, they had

higher maximal aerobic capacity during forced treadmill exercise and a smaller body mass (Swallow et al. 1998a, b, 1999; Koteja et al. 1999). In a comparison of mice from the base population with wild house mice from a Wisconsin population (Dohm et al. 1994, unpublished data), we found that wild females ran approximately 68% more revolutions/day than laboratory females (only females were tested), and we hypothesized that a 1.7-fold differential might correspond to a limit that would be reached in our selection experiment. This hypothesis can now be rejected: at generation 13, females from selected lines ran 2.2–2.4 times more (depending on time of observation) than those from control lines (Fig. 1). Thus, it is possible to select laboratory strains of house mice to show activity levels that exceed those of their wild counterparts. Similarly, Lynch (1992, 1994) selected laboratory mice for high thermoregulatory nest building and exceeded levels shown by wild mice.

Artificial Selection as a Novel Approach to Study Wheel-running Behaviour

Sherwin (1998), with some desperation, reviewed conflicting results of numerous experiments that attempted to identify the causes or functions of wheel-running behaviour. He also pointed out that 'the frequent disparities between results may well be due to the relative ease and inexpense of recording this activity. These have led to a plethora of investigations . . . [which] have begged the question of why the activity occurs at all.' Our ongoing artificial selection experiment offers a novel approach because it is the first to select directly on voluntary wheel-running behaviour and then test for correlated behavioural responses. Rundquist (1933) selected for high and low total activity in rats housed in revolving cages, which cannot be treated as equivalent to selection for voluntary activity in attached wheels (see Sherwin 1998). Dunnington et al. (1981a) also selected on voluntary wheel running in ICR mice, for five generations, but did not study correlated responses in behaviour (Dunnington et al. 1981b).

Correlated responses to the selection for total wheel revolutions (i.e. consistent differences between selected and control lines: Henderson 1989; Gromko 1995) would suggest the presence of genetic correlations (Boake 1994; Garland & Carter 1994; Falconer & Mackay 1996). Genetic correlations are caused mainly by pleiotropy, that is, one gene affecting more than one aspect of the phenotype. In turn, pleiotropy suggests the presence of shared biochemical or physiological pathways.

Most importantly, as discussed above, selection for increased wheel running did not produce mice that were more active in the attached cages, even when the wheels were prevented from rotating (Table 2). This result is consistent with other lines of evidence suggesting that wheel-running behaviour is not an indicator of general activity level, and must have a specific motivational mechanism (Sherwin 1998, and references therein). Another important observation is that the effect of genetic selection on other behaviours differs from that expected from correlations observed at the phenotypic

level (Lachmansingh & Rollo 1994; see above). The difference between the response to external (environmental) and internal (genetic) factors may explain part of the conflicting results obtained in previous studies of wheel-running behaviour (Sherwin 1998).

When discussing motivation for voluntary wheel running, Sherwin (1998) argued that 'controlling bouts of wheel running, but not necessarily the speed, is perceived [by animals] to be important'. If that were true, and if among-individual variation in wheel running resulted from genetically based differences in motivation levels, then we might expect that the selection for high wheel-running activity would have resulted primarily in increased time spent running rather than velocity. Instead, we found that selection for increased total revolutions was accomplished mainly by mice running faster, not for more minutes per day (Fig. 1).

Selection for increased wheel revolutions did not cause an increase in the frequency with which other behaviours were performed inside the wheels (Table 2). In other words, 'preferred microhabitat' was unchanged: we did not simply produce mice that 'liked' to enter and spend time in wheels. Thus, the motivation for wheel running was not associated, at the genetic level, with a preference for performing other activities in the wheels. On the other hand, the selected mice tended to be more active on the wheels even when the wheels were prevented from rotating ('locked' wheel type; Table 2). This result indicates that the motivation for wheel running may be not associated with sensory perception involving the sight or sound of a rotating wheel.

Both Sniff and Bite were affected by wheel type, with animals from the locked-wheel group scoring higher than the free-wheel group. This result might be an observational artefact. In the locked-wheel group, both activities were often observed in mice hanging in the wheel. In the free-wheel group, mice could Sniff and Bite while coasting, but this would have been difficult to observe, thus leading to an underestimation of the frequency of these behaviours. However, a significant difference between the selection groups, observed only in females, could not be explained in this way. Both with free and locked wheels, females from selected lines had lower occurrences of biting (gnawing on wires) and sniffing.

Mather (1981) suggested that wheel running is a redirected form of exploratory behaviour. Others have treated wheel running as a stereotypic behaviour (e.g. Richards 1966). Although Sherwin (1998) argues against both interpretations, our results may be seen as providing some support for both of these hypotheses. If sniffing is a form of exploratory behaviour and biting wires is a stereotypical behaviour (Richards 1966), then the behaviour of selected females in our study may reflect a correlated response in the way exploration is performed and in the form of stereotypy. On the other hand, neither sniffing nor biting differed between selection groups in males. This result suggests that the functions or proximate causes of wheel running may differ between sexes, as has been suggested by several other workers (e.g. Perrigo & Bronson 1985; Sherwin 1998, and references therein).

Acknowledgments

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