

Maternal-care behavior and life-history traits in house mice (*Mus domesticus*) artificially selected for high voluntary wheel-running activity

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Abstract

To test the hypothesis that selective breeding for high voluntary wheel running negatively affects maternal performance in house mice, we observed maternal behavior and compared litter size and mass, in replicate lines of selected ($N=4$) and control ($N=4$) mice from generations 20 and 21 of an artificial selection experiment. At generation 21, selected-line females ran 2.8-times more revolutions per day than females from random-bred control lines, when tested at approximately 6 weeks of age as part of the normal selection protocol. After giving birth, dams from selected and control lines exhibited similar frequencies of maternal behaviors and also spent similar amounts of time in general locomotor activity at litter ages of both 9 and 16 days. Dams from selected lines also performed equally well as controls in repeated pup-retrieval trials. At first parturition, selected-line dams averaged 2.4 g smaller in body mass as compared with dams from the control lines; however, neither litter size nor litter mass at birth (generation 20) or at weaning (generation 21) differed significantly between selected and control lines. We conclude that, at least under the husbandry conditions employed, maternal behavior and reproductive output at first parturition are genetically independent of wheel-running behavior. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Reproduction in mammals is characterized by high maternal investment in young (Clutton-Brock, 1991; Thompson, 1992). In small rodents with altricial pups, successful reproduction requires both high-energy expenditures during pregnancy and lactation, and substantial time investments in such maternal behaviors as nursing, licking, and warming the pups (Hill, 1992). Although increasing energetic and behavioral investments in young is generally expected to be effective in increasing reproductive fitness, maternal care must be balanced against other demands, such as locomotor expenditures associated with foraging. Indeed, reproductive effort can be constrained by proximate trade-offs between locomotion and maternal care, as in house mice required to run long distances to obtain food (Perrigo, 1987). It has also been suggested that the positive relation between locomotor activity and reproductive performance played an important role in the evolution of endothermy in mammals (Farmer, 2000; Koteja, 2000). Since small mammals probably evolved under conditions in which food intake depended strongly upon locomotor effort (Perrigo, 1987), increased locomotor activity may have been a critical component of enhanced parental care.

The interaction between locomotion and maternal care in both proximate and ultimate contexts can be explored through the application of artificial selection. Our on-going experiment has used selective breeding to increase voluntary wheel-running behavior of laboratory house mice (*Mus domesticus*) (Swallow et al., 1998a). Twenty-one generations of within-family selection for high voluntary wheel-running activity produced four replicate lines in which females ran, on average, 180% more revolutions per day than females in the four replicate control lines (see Section 3); these levels are also substantially greater than those exhibited by wild house mice tested in our laboratory (Dohm et al., 1994). Here, we test for correlated responses in maternal behavior and reproductive performance.

Selection for high voluntary locomotor activity has the potential to affect reproductive output in

our selection experiment in several ways. First, a trade-off between maternal investment in locomotor activity and production of offspring might exist if selected lines show increased general cage activity. As noted above, lactation is energetically expensive (Millar, 1978, 1979; König and Markl, 1987; Hammond and Diamond, 1997) and can be constrained by energy expenditure during activity (Perrigo, 1987). Dams also face temporal demands and must limit time spent away from their pups (Hill, 1972), particularly when young pups are not yet able to maintain body temperature. If dams from our selected lines have increased cage activity during lactation, when wheels are not provided, then energy- and time-related costs of activity might be expected to reduce pup survivorship and mass at weaning (Perrigo, 1987). Indeed, data from a 2-day trial of adult females in photo-beam activity cages suggest that mice from selection lines are more active even when wheels are not provided (Rhodes et al., 2001), although we lack information about the behavior of dams in familiar home cages.

Second, selection for high locomotor activity, which has resulted in changes in some components of exercise physiology (Swallow et al., 1998b; Garland et al., 2000; Houle-Leroy et al., 2000; Dumke et al., 2001), may also have altered physiological capacities for reproduction. For example, body mass is the major morphological predictor of litter mass and offspring mass in laboratory mice (Johnson et al., 2001), and mice from our selected lines are smaller in body mass (Swallow et al., 1999). Since large female house mice tend to have large litters (Falconer, 1960), it would be predicted that mice from our selected lines might have smaller litters. In addition, mice from our selected lines are leaner (lower % fat) than controls (Swallow et al., 1999), and ovulation rate in mice is reduced by low body fat stores (Barnett and Dickson, 1984; Hastings et al., 1991), which would also suggest decreased fertility and litter size in our selected lines.

Third, females from our selection lines exhibit higher plasma corticosterone levels than controls when housed without wheels, suggesting that selection has resulted in an upregulation of the hypothalamic–pituitary–adrenal axis (Girard and

Garland, 2000). Maternal stress during pregnancy can cause a number of abnormalities in offspring, including alterations in sexual differentiation and sexual behavior (Herrenkohl, 1983, 1986; Holson et al., 1995), and brain and vertebral anomalies (Miller and Chernoff, 1995). It is believed that fetal abnormalities result from stress-induced secretions of the HPA axis, including adrenocorticotrophic hormone (ACTH), glucocorticoids, and adrenal androgens (Carlberg et al., 1996). Thus, selection could also result in impaired reproductive output through effects on the HPA axis.

Fourth, selection for high wheel running may have caused correlated changes in other behaviors, including maternal behavior, possibly because locomotor and maternal behaviors are centrally or peripherally linked. We already have evidence of correlated changes in non-locomotor behaviors: thermoregulatory nest-building is reduced in our selected lines (Carter et al., 2000). Conversely, in an earlier selection experiment, lines selected for low nest-building demonstrated increased wheel-running as compared with control lines (Bult et al., 1993; Bult and Lynch, 1996). Thermoregulatory nest-building is genetically correlated with maternal nest-building (Lynch, 1981), which affects offspring survival in the cold (Lynch and Possidente, 1978). Maternal nesting is also related to prolactin (Saito et al., 1983; Orpen et al., 1987), a hormone that is important for the onset of other types of maternal care in rodents (Siegel and Rosenblatt, 1980; Bridges, 1994; Bridges and Mann, 1994; Alston-Mills et al., 1999). Dopamine is the major inhibitor of prolactin secretion in the adenohypophysis (Brown, 1994), and pharmacological studies in our lines suggest that selection for wheel-running is associated with impaired dopaminergic function (Rhodes et al., 2001). In the *hubb/hubb* mutant mouse, low milk production and poor maternal behavior was associated with an increase in the dopamine metabolite, dihydroxyphenylacetic acid (DOPAC) (Alston-Mills et al., 1999). If selection has affected prolactin secretion by altering dopaminergic function, then changes in maternal-care behavior in the selected lines are expected, and such changes would likely have negative effects on pup survivorship and body mass at weaning.

In this study, we examined maternal-care behavior and characteristics of litters at birth and at weaning in lines of mice selected for high voluntary wheel-running behavior. We hypothesized that lines selected for high wheel running would exhibit lower frequencies of maternal care, smaller litter sizes at birth, and smaller litter sizes and lower pup mass at weaning.

2. Materials and methods

2.1. Selection experiment and wheel running

The study animals were adult female mice from an ongoing selection experiment for voluntary wheel running (Swallow et al., 1998a). Beginning from a base population of Hsd:ICR house mice, four replicate lines of mice were subjected to within-family selection for high voluntary wheel running, and four control lines were randomly bred. The trait of selection is the average revolutions per day on days 5 and 6 of a 6-day trial. At the time of selection, mice were approximately 42–56 days old. Further details of the selection and maintenance protocols are given in Swallow et al. (1998a), which reports results through the first ten generations. The present study animals were from generations 20 and 21. Mice were maintained on a 12-h light:12-h dark cycle with ad libitum access to water and food (Teklad Rodent Diet W 8604).

As part of the routine selection protocol, mice were tested for wheel-running activity for a 6-day period at about 48 days of age (range 41–53 days). In the selection 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 to be breeders. Mice chosen to breed ($n = 112$ pairs per generation) were paired at 65–70 days of age. Males were removed 15 days after first pairing. Beginning on the 18th day after pairing, females were checked every 8 h for new births and the birth litter size was recorded. Pups were weaned at 21 days of age, at which time they were individually weighed, sexed, and toe-clipped. Dam mass at weaning was also recorded.

Several dams from generation 20 suffered from abdominal bloat of unknown origin during the second and third weeks of lactation (with symptoms as described in Rollman et al., 1998), a condition possibly related to the relatively low fat content of the diet. Dams scored as being in poor condition by our veterinarian were excluded from analyses of maternal behavior, leaving 40 control and 40 selected dams for inclusion in analyses. Since symptoms did not appear until the second week of lactation, no dams were excluded from analyses of litter characteristics at birth. Subsequently, following the recommendation of our veterinarian, generation 21 dams were supplemented with 2 g of Jif[®] peanut butter daily during the second and third weeks of lactation. No clinical symptoms appeared in generation 21 dams and all were scored in good condition, although a single dam died during rearing from unknown causes.

2.2. Maternal-care observations

Behavioral observations were conducted on 43 control and 42 selected dams from generation 20. Observations were made during the light and dark periods when the litters were 9 and 16 days old (Day 9, Night 9, Day 16, Night 16). Diurnal observations were made 1–3 h after lights on and nocturnal observations were made 1–3 h after lights off under red light. The frequency of maternal care was assessed by a scan sampling technique (Martin and Bateson, 1986) in which each dam was briefly and repeatedly watched during each observation period. Dams were randomly assigned an observation order at the beginning of each observation period. A single observer watched each dam for 10 s as timed by a small flashing LED, and the dam's instantaneous behavior at the end of 10 s was recorded. The 10 s of observation prior to recording provided context in which to distinguish closely related behaviors, such as nest-building and digging. Observed instantaneous behaviors were categorized as one or more of 13 behavior types. Maternal-care behaviors included nursing, resting with pups, nest-building, grooming pups, and carrying pups (Hennessy et al., 1980; Cierpial et al., 1987; Co-

hen-Salmon, 1987; Benus and Rondigs, 1996). Non-maternal behaviors included resting alone, grooming self, feeding, drinking, digging, climbing, walking or running, jumping, and inactivity (alert but motionless) (e.g. see Mackintosh, 1981; Koteja et al., 1999a). A dam could be scored as exhibiting two or more behaviors simultaneously, with the exception of resting with pups, nursing, resting alone, and inactive, which were defined to be mutually exclusive. Dams were scanned sequentially at 10 s intervals, with 5 min between scans of the same animal. Each dam was observed 24 times during a 2-h observation period.

The score for each behavior was determined as the number of times the behavior was recorded in 24 scans. A frequency for general maternal care was assessed as the number of scans in which any maternal-care behavior (as listed above) was observed. A frequency for locomotor activity was determined as the number of scans in which any non-maternal, locomotor-type behaviors occurred: digging, climbing, walking or running, or jumping. Statistical analyses were conducted on scores for individual behaviors, such as nursing and feeding, and on the frequency of the general behaviors of maternal care and locomotor activity. Dam age at birth, litter size at observation, dam condition, and observation day were covariates or cofactors in statistical models.

2.3. Pup-retrieval trials

Pup-retrieval trials were conducted on 42 control and 40 selected dams of generation 21. On the day of birth, dams and pups were placed in clean cages with pine shavings and a 10 × 10 cm piece of paper towel for nesting material. One trial was conducted each day for 4 days when the litters were 3–6 days old. At the start of each trial, the dam was removed from her home box and placed in a clean holding box. The pups were removed from the nest by latex-gloved hand and placed in a pile at the opposite corner of the cage. The distance between the nest and the new location of the pups was measured with a ruler. Timing was initiated when the dam was reintroduced to the home cage at the location of the empty nest. The latency to retrieve each pup was recorded, as well

as the number of times the dam approached and then moved away from the displaced litter without retrieving a pup. A ‘move-away’ was scored each time the dam came within 1 cm of a displaced pup and then moved away by a distance greater than her body length (excluding tail). The maximum time allowed for completion of the trial was 600 s. A note was recorded if the dam began to build a new nest at the location of the displaced pups. Pups not returned to the nest at the end of the trial were replaced by the observers. Trials were conducted at a time range approximately midway through the light cycle.

2.4. Litter characteristics

For both generations, the frequency of infertile pairings and the incidence of dam and pup mortality were recorded. In generation 20, number of pups at birth (alive and dead), live litter mass, and dam mass were recorded within 8 h of birth. In generation 21, litter size, litter mass, pup mass, sex ratio, and dam mass were recorded at weaning.

2.5. Statistical analyses

The effects of selection on maternal-care behavior, pup retrieval performance in trial 1, and litter size were tested by nested analysis of variance (ANOVA) or nested analysis of covariance (ANCOVA) with the SAS[®] (1996) General Linear Models procedure (PROC GLM). To test statistical significance of the effects of selection, the appropriate *F*-value was calculated as the variance (mean square) associated with the effect of selection over the variation among lines (Sokal and Rohlf, 1995). Maternal-care behavior scores were analyzed by ANCOVA with dam age, litter size at observation, dam condition, and observation day as covariates. Results from pup-retrieval trials were examined for the first trial and for trials 2–4 separately. Data from trial 1 were considered separately, as the novelty of the task may have influenced dam performance. Trial 1 data were analyzed by ANCOVA, excluding data from two dams (one control, one selected) who built new nests around the displaced pups rather

than retrieving them. Results from trials 2 to 4 were analyzed with a repeated-measures ANCOVA using SAS[®] PROC MIXED with random line effects and a fixed effect of linetype. In all analyses of pup-retrieval performance, litter size, observer, test day, and time of day were used as covariates or cofactors. In preliminary analyses, the distance between the nest and location of displaced pups did not have a significant effect on retrieval times, so this measure was not included as a covariate in final analyses. Data from dams who did not complete retrieval within the allotted time were assigned the maximum value of 600 s for latency to retrieve all pups, and were included in all analyses. Data for move-aways were rank transformed prior to analysis. Litter characteristics at birth and at weaning were analyzed both without dam mass and including dam mass as a covariate. In preliminary analyses, dam age did not have a significant effect on litter characteristics at birth or weaning and so it was not included as an additional covariate.

Considering values for individual dams, the relationships between wheel running and maternal behavior, and between wheel running and litter characteristics, were examined by Pearson product–moment correlation of residuals. Residual wheel running (average revolutions per day on days 5 and 6) at 6 weeks of age was obtained from an ANCOVA (SAS[®] PROC GLM) with line nested within linetype, and age, day, wheel resistance, and average body mass during wheel access as covariates or cofactors. Residual frequency of maternal care and locomotion, and residual litter characteristics at birth and weaning, were obtained from the models described above, with dam mass included as a covariate in models of litter characteristics. The differences in correlations between selected and control lines were tested by ANCOVA.

For some of the traits measured, we expected differences between selected and control lines in a particular direction (see Section 1), which would suggest one-tailed statistical inference. For other traits, however, the direction of a probable difference was unclear. Therefore, for simplicity, we report two-tailed *P* values throughout.

3. Results

3.1. Wheel running

At generation 21, female mice from the selected lines were running on average 14289 ± 4155 S.D. revolutions per day, or $2.8 \times$ the revolutions run by control females (5150 ± 1522 revolutions per day) (mean of days 5 and 6 of the routine 6-day wheel exposure—see Swallow et al., 1998a). As was true in earlier generations (Swallow et al., 1998a,b; Koteja et al., 1999b; Houle-Leroy et al., 2000; Rhodes et al., 2000; Girard et al., 2001), this increase in wheel-running in the selected lines was caused primarily by an increase in the average speed of running with a marginal increase in the time spent running (number of 1-min intervals with any revolutions). Average speed was $2.4 \times$ higher in the selected lines (females = 26.5 ± 7.13 mean rpm) than in controls (females = 10.9 ± 2.62 mean rpm), but mice in selected lines only spent $1.14 \times$ more intervals running (females = 542 ± 88.9 1-min intervals per day) than controls (females = 473 ± 100 intervals per day).

Characteristics of wheel-running in generation 20 were similar, although linetype differences were smaller than in generation 21. In generation 20, selected females ran $2.5 \times$ more revolutions per day, $2.2 \times$ faster, and $1.15 \times$ longer than control females.

3.2. Maternal behavior and pup retrieval

Dams from selected and control lines generally demonstrated similar patterns of behavior as scored during the day and night observation periods when pups were 9 and 16 days old (see Fig. 1). Aspects of maternal care were the most frequently observed behaviors, occurring in up to an average of 18 of 24 scans. Nursing constituted the largest portion of maternal care for both selected and control lines. The frequency of maternal care was not significantly different between selected and control dams in any period ($F(1,6) < 5.52$, two-tailed $P > 0.05$), but selected-line dams demonstrated a significantly lower frequency of the nursing on Day 16 than did controls ($F(1,6) = 9.84$, $P = 0.02$).

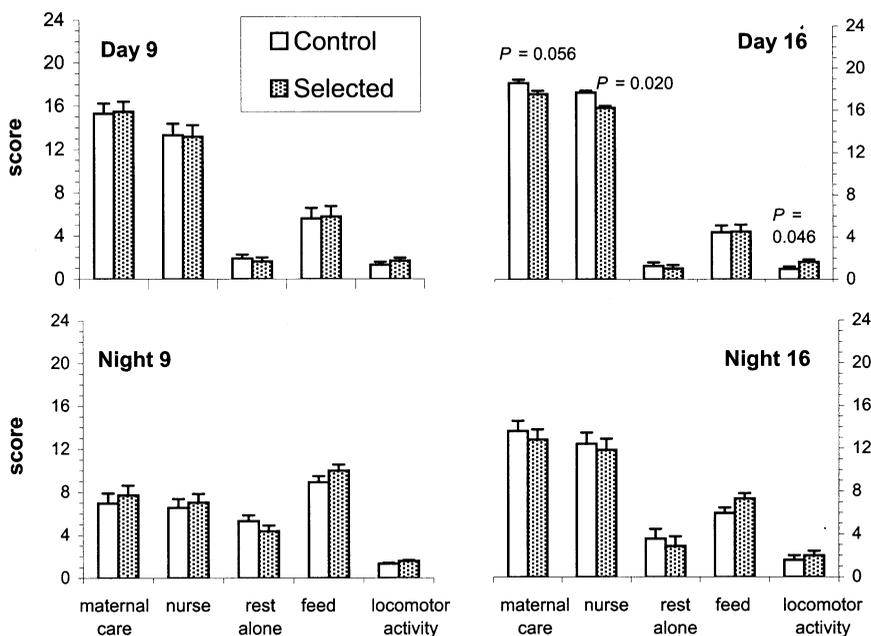


Fig. 1. Behavior in control and selected dams (generation 20) during diurnal and nocturnal observation periods when litters were 9 and 16 days old. Where P values are not shown, $P > 0.10$.

General locomotor activity, feeding, and resting alone accounted for most of the non-maternal-care behavior of dams. The score for locomotor-activity behaviors (including digging, climbing, walking or running, and jumping) was quite low in all periods, with locomotor activity occurring on average in less than 12% of all scan observations. The frequency of locomotor-activity behaviors was not significantly different between selected- and control-line dams during Day 9, Day 16, and Night 16. Selected dams had marginally higher locomotor activity than control dams during Day 16 ($F(1,6) = 6.58$, $P = 0.046$), a difference possibly related to the lower frequency of nursing in selected dams during the same period. Dams from both selected and control lines fed and rested alone more at night than during the day, but no significant linetype differences in these two behaviors existed in any period.

At the level of individual variation (residuals from nested ANCOVA models), there were no significant correlations between wheel running at the time of selection and frequency of dam locomotion in cages with pups during any period in either selected or control lines (Pearson's $r < 0.3$, $P > 0.10$). The frequency of maternal care was never significantly correlated with wheel running in selected lines ($r < 0.2$, $P > 0.25$), but maternal care during Day 9 and 16 was negatively correlated with wheel running in control lines ($r_{\text{Day } 9} = -0.431$, $P = 0.009$, $n = 35$; $r_{\text{Day } 16} = -0.412$, $P = 0.014$, $n = 35$). The frequency of maternal care was negatively correlated with the frequency of locomotion in selected lines at Day 9 ($r = -0.353$, $P = 0.032$, $n = 37$), and in control lines at Day 16 ($r = -0.381$, $P = 0.022$, $n = 35$) and Night 16 ($r = -0.364$, $P = 0.029$, $n = 35$).

Dams from selected and control lines did not differ in performance during four pup-retrieval trials. Retrieval latencies and number of move-aways decreased between trial 1 and subsequent trials, but performance measures were not significantly different between selected and control lines in either the initial trial, or in a repeated-measures analysis of trials 2–4. No linetype differences were observed in the latency to retrieve all pups (trial 1: $F(1,6) = 0.04$, $P = 0.85$; trials 2–4: $F(1,6) = 0.16$, $P = 0.70$), in the latency to retrieve

the first pup (trial 1: $F(1,6) = 1.4$, $P = 0.29$; trials 2–4: $F(1,6) = 0.04$, $P = 0.87$), or in the average time to retrieve all pups excluding retrieval of the first pup (trial 1: $F(1,6) < 0.001$, $P > 0.99$; trials 2–4: $F(1,6) = 0.28$, $P = 0.62$). Selected-line dams tended to have higher numbers of move-aways in the first trial, but no significant linetype differences were found (trial 1: $F(1,6) = 3.2$, $P = 0.12$; trials 2–4: $F(1,6) = 0.17$, $P = 0.69$). The number of dams not completing a trial ranged from 11 (trial 1) to 4 (trial 2). During the first trial, more selected-line dams ($n = 8$) did not complete retrieval than control-line dams ($n = 3$), but this difference was not significant ($\chi^2 < 2.3$, $df = 1$, $P > 0.13$).

3.3. Litter characteristics

Of the 56 control-line females paired in generation 20, one died early in pairing and an additional three were never visibly pregnant and did not give birth (5.3% infertile matings). Of the 56 selected-line females paired in generation 20, none died during pairing but two were never visibly pregnant (3.6% infertile matings). No dam and few pup mortalities occurred at birth. Litter size ranged from 5 to 15 live pups in the control lines and from 4 to 17 live pups in the selected lines. As can be seen in Fig. 2, litter size, total litter mass, and mean pup mass were not significantly different between linetypes (Table 1). Dams from selected lines gave birth at a similar age but were significantly smaller at the birth than dams from control lines (Table 1, see also Fig. 2). Dam mass at birth was strongly positively correlated with litter size ($r = 0.380$, $df = 104$, $P < 0.0001$) and with total live litter mass ($r = 0.520$, $df = 104$, $P < 0.0001$), but not with mean pup mass (see Fig. 2). Since selected dams were smaller at birth but had litter sizes similar to those of controls, selected dams actually gave birth to relatively larger live litters for their body mass, although this trend was not statistically significant (Table 1, least-squares means).

Of the 56 control-line females paired in Generation 21, two (3.6% infertile matings) did not become pregnant and one died during rearing. Of the 56 selected-line females paired, one (1.8%

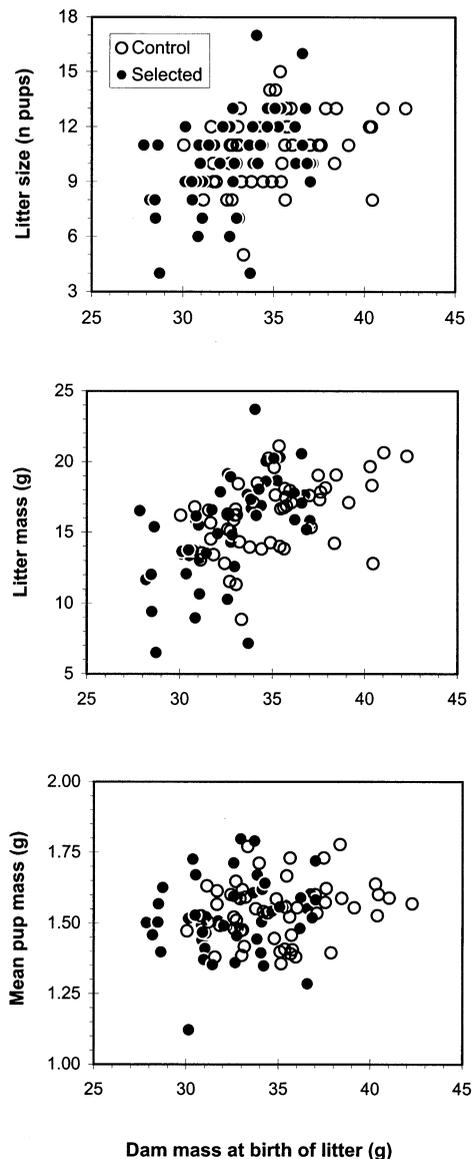


Fig. 2. Litter size, live litter mass, and mean live pup mass at birth in first parturition litters of control and selected dams (generation 20).

infertile matings) did not become pregnant and one died during rearing. Two control litters died shortly after birth, yielding 52 control litters at weaning. Six litters from selected-line dams died shortly after birth, yielding 48 that survived to weaning. Dam age and dam mass at weaning were not significantly different between selected and

control mice (Table 2), although dams from selected lines tended to be smaller. As can be seen in Fig. 3, weaning litter size, total litter mass, and mean pup mass did not differ significantly between the control and selected linetypes. Dams from selected lines tended to wean larger litters when effects of dam mass were included in analyses, but mass-adjusted litter characteristics were still not significantly different between linetypes (Table 2, least-squares means). Mean sex ratios approximated 1:1 for litters of both linetypes.

3.4. Correlation of wheel running with maternal behavior and life-history traits: individual variation

Within selected lines, a significant positive correlation existed between wheel running in females tested at 6 weeks of age and their subsequent litter mass ($r = 0.286$, $P = 0.042$, $n = 51$; see Fig. 4A) and litter size ($r = 0.329$, $P = 0.018$; see Fig. 4B) at parturition about 9 weeks later. In control dams, wheel running was not significantly correlated with litter mass or litter size at birth ($-0.15 < r < -0.13$, $P > 0.28$). The relationships between wheel running and litter mass and size within selected-line dams were significantly different from the relationships within control-line dams (ANCOVA: litter mass $F(1,95) = 4.73$, $P = 0.032$; litter size $F(1,95) = 5.01$, $P = 0.028$). At weaning, these trends were reversed. Dams from control lines demonstrated a positive correlation between weaning litter mass ($r = 0.400$, $P = 0.003$, $n = 52$; see Fig. 4C) and size ($r = 0.237$, $P = 0.090$; see Fig. 4D), but the correlations within selected lines were not significant ($-0.13 < r \leq -0.08$, $P > 0.35$, $n = 47$). The relationship between wheel running and litter mass at weaning was significantly different between selected and control lines ($F(1,95) = 6.94$, $P = 0.010$); the relationship between wheel running and litter size at weaning was marginally not significantly different between selected and control lines ($F(1,95) = 3.67$, $P = 0.058$).

At the level of individual variation within selected lines, females (generation 20) that exhibited higher levels of voluntary wheel running at the time of selection (age 42–56 days) subsequently

had higher-than-average litter size (Fig. 4B) and mass (Fig. 4A) at birth. This pattern was not apparent in females from the control lines. For females from generation 21, higher levels of wheel running were associated with higher-than-average litter mass at weaning (Fig. 4C) in control-line but not in selected-line animals.

4. Discussion

Despite our expectations (see Section 1), no differences in maternal behavior or reproductive output existed between four lines of mice selected for high wheel running as compared with their four random-bred control lines. Dams from the selected lines invested similar time rearing pups, performed equally well in retrieval trials, and, when housed without wheels, did not demonstrate increased locomotion as compared with controls. Reproductive output measured as litter characteristics at birth and at weaning were also not affected by selection for voluntary exercise. The litter characteristics at weaning (Table 2) were also consistent with those of Hsd:ICR mice maintained at the Harlan Sprague–Dawley breeding facility in Indianapolis, IN (Dennis Renner, Pers. Comm., 3 February 1999), with litter sizes of Hsd:ICR mice bred previously in our laboratory (Hayes et al., 1992), and to the base population for the present experiment before selection had begun (unpublished data).

The finding that dams selected for high voluntary wheel running did not have increased locomotion in home cages supports results from an earlier generation. Behavioral studies in non-reproductive animals (both females and males were studied) of generation 13 showed that individuals from selected lines did not have increased locomotor activity in cages attached to wheels, whether the wheels were free to rotate or locked with a wire tie (Koteja et al., 1999a). Indeed, wheel-running appears to be relatively independent of (genetically uncorrelated with) other locomotor activities (see review in Sherwin, 1998; Koteja et al., 1999a; Bronikowski et al., 2001). For example, our selected and control lines do not differ in traditional measures of open-field activity (Bronikowski et al., 2001), which is consistent with previous studies reporting that (1) mice bi-directionally selected for open-field activity did not differ in wheel-running (DeFries et al., 1970) and (2) voluntary wheel-running was not significantly correlated with open-field behavior among 13 species of rodents (Dewsbury et al., 1980).

Since all of our dams spent little time, and presumably expended little energy, in locomotion, it is perhaps unsurprising that we found no evidence of reduced maternal care or reproductive output in our selected lines. Although we did find some evidence for a trade-off between locomotion and maternal care at the level of individual variation (residuals from nested ANCOVA models), the overall performance of dams from selected

Table 1
Characteristics of dams and their litters at birth in control and selected lines of mice at generation 20

	Control (<i>n</i> = 52)				Selected (<i>n</i> = 54)				<i>P</i> ^b	<i>P</i> ^c	<i>P</i> ^M
	Mean	S.D.	LSM ^a	S.E. ^a	Mean	S.D.	LSM ^a	S.E. ^a			
Dam age (days)	91.3	2.07	–	–	91.3	2.63	–	–	0.312	–	–
Dam mass (g)	35.1	2.85	–	–	32.7	2.54	–	–	0.029	–	–
Live pups (<i>n</i>)	10.6	2.05	10.3	0.248	10.5	2.50	10.6	0.242	0.375	0.482	<0.001 (+)
Litter mass (g)	16.23	2.58	15.83	3.08	15.39	3.38	16.34	3.05	0.288	0.291	<0.001 (+)
Pup mass (g)	1.54	0.105	1.53	0.017	1.52	0.121	1.53	0.017	0.496	0.934	0.076 (+)

^a Least-squares means (LSM) and standard errors (S.E.) adjusted for dam mass at birth.

^b For litter traits: two-tailed significance of the linetype effect in a nested ANOVA without dam body mass as a covariate.

^c For litter traits: two-tailed significance of the linetype effect in a nested ANCOVA including dam body mass as a covariate.

^M For litter traits: two-tailed significance and sign (positive or negative relationship) of the effect of dam body mass.

Table 2
 Characteristics of dams and their litters at weaning in control and selected lines of mice at generation 21

	Control (<i>n</i> = 51)				Selected (<i>n</i> = 47)				<i>P</i> ^b	<i>P</i> ^c	<i>P</i> ^M
	Mean	S.D.	LSM ^a	S.E. ^a	Mean	S.D.	LSM ^a	S.E. ^a			
Dam age (days)	117.6	3.31	–	–	116.7	2.44	–	–	0.412	–	–
Dam mass (g)	37.8	3.55	–	–	35.2	3.35	–	–	0.263	–	–
Live pups (<i>n</i>)	10.4	2.22	9.72	3.63	10.4	2.39	10.9	2.96	0.368	0.168	<0.001 (+)
Litter mass (g)	103.1	18.15	95.81	3.26	98.33	21.22	100.3	2.17	0.738	0.296	0.035 (+)
Pup mass (g)	10.24	2.11	10.40	1.06	9.71	1.80	9.40	1.04	0.268	0.210	0.019 (–)
Females (%)	49.63	17.24	52.48	4.05	48.82	15.20	49.73	2.69	0.467	0.593	0.384 (+)

^a Least-squares means (LSM) and standard errors (S.E.) adjusted for dam mass at birth.

^b For litter traits: two-tailed significance of the linetype effect in a nested ANOVA without dam body mass as a covariate.

^c For litter traits: two-tailed significance of the linetype effect in a nested ANCOVA including dam body mass as a covariate.

^M For litter traits: two-tailed significance and sign (positive or negative relationship) of the effect of dam body mass.

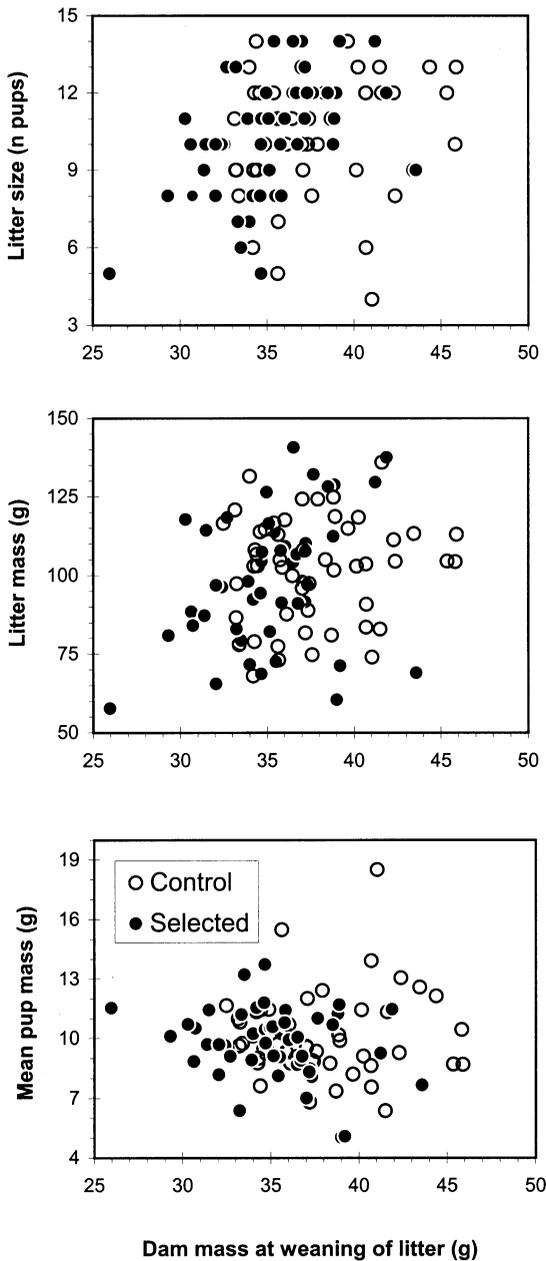


Fig. 3. Litter size, litter mass, and mean pup mass at weaning at 21 days in first parturition litters of control and selected dams (generation 21).

lines was not different from that of control-line dams. These results suggest that wheel running and maternal behaviors are not genetically correlated. This means that the evolution of wheel

running should not be constrained by maternal care behavior, and that in the absence of other genetic correlations wheel running may be able to evolve to its selection limit. In addition, it suggests that in these mice, thermoregulatory nesting behavior is probably not correlated with maternal care behavior, as thermoregulatory nesting has responded in a negatively correlated way to selection for wheel running (Carter et al., 2000).

The lack of a correlated response to selection may be specific to the conditions of this study. The ICR strain was originally selected for high fecundity and high weaning success (the ' + selected' line shown in Figure 2 of Hauschka and Mirand, 1973; E. Mirand, in litt. 17 May 2000), and the progenitors of our mice have undergone continued selection for high reproductive output at both Charles River Labs (Pat Mirley, Pers. Comm., 13 December 1999) and Harlan Sprague–Dawley (Lynette Guindon, Pers. Comm., 16 December 1999). In our experiment, selection for high maternal care continues in conjunction with selection for high wheel running, because pups from neglectful moms usually die, as we rarely attempt to foster pups to other dams. The consequence of this continual selection may be a low level of additive genetic variance for traits that affect reproductive output, which could also reduce the level of additive genetic covariance with wheel running.

Dams in this study had no access to wheels, and food was provided ad libitum. These are the normal conditions for our selection experiment (see Swallow et al., 1998a). Effects of selection on maternal care and/or substantial trade-offs may be evident only when dams are housed with access to running wheels, such that they can exhibit the specific phenotype for which they have been selectively bred. Wheel access may be necessary for the maintenance or expression of hypothesized differences in dopaminergic function (see Section 1). Therefore, future studies are planned in which dams will be housed with access to wheels and/or with restricted food availability during pregnancy and lactation. Under such conditions, we hypothesize that dams from selected lines will indeed exhibit reduced reproductive performance.

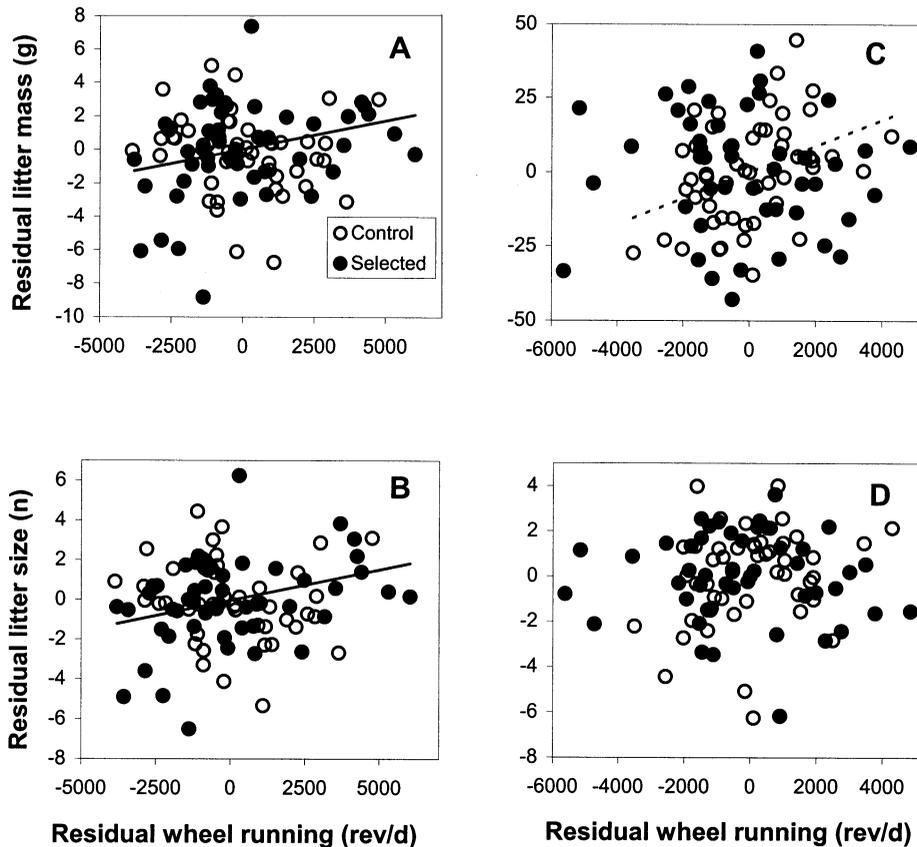


Fig. 4. Correlations between voluntary wheel running in females (generation 20) and their subsequent litter mass (A) and litter size (B) at birth, and between voluntary wheel running in females (generation 21) and their subsequent litter mass (C) and litter size (D) at weaning, in control and selected lines of mice. Values are residuals from models accounting for variation among replicate lines within linetype and correlations with female body mass. Significant correlations ($|r| > 0.25$, $P < 0.05$) are indicated for mice from control (dashed line) and selected (solid lines) lines: selected, mass at birth $r = 0.286$, $P = 0.042$; selected, size at birth $r = 0.329$, $P = 0.018$; control, mass at weaning $r = 0.400$, $P = 0.003$. Significance levels are for two-tailed tests.

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