Locomotor play behavior evolves by random genetic drift but not as a correlated response to selective breeding for high voluntary wheel-running behavior

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ABSTRACT

Locomotor play is vigorous and seemingly purposeless behavior, commonly observed in young mammals. It can be costly in terms of energy expenditure, increased injury risk, and predator exposure. The main hypothesized benefit of locomotor play is enhancement of neuromuscular development, with effects persisting into adulthood. We hypothesized that levels of locomotor play would have evolved as a correlated response to artificial selection for increased voluntary exercise behavior. We studied mice from 4 replicate lines bred for voluntary wheel running (High Runner or HR) at 6-8 weeks of age and four non-selected Control (C) lines. Mice were weaned at 21 days of age and play behavior was observed for generations 20 (22-24 days old), 68 (22-23 days old), and 93 (15 days old). We quantified locomotor play as (1) rapid, horizontally directed jerk-run sequences and (2) vertical "bouncing." We used focal sampling to continuously record behavior in cages containing 4-6 individuals during the first 2-3 hours of the dark cycle. Observations were significantly repeatable between observers and days. A two-way, mixed-model simultaneously tested effects of linetype (HR vs. C), sex, and their interaction. Contrary to our hypothesis, HR and C lines did not differ in any generation, nor did we find sex differences. However, differences among the replicate HR lines and among the replicate C lines were detected, and may be attributed to the effects of random genetic drift (and possibly founder effects). Thus, play behavior did evolve in this selection experiment, but not as a correlated response to selection for voluntary exercise.

Key words: Artificial selection, Genetic drift, Locomotor play, Motor performance, Play, Voluntary exercise

1. Introduction

Broadly, "play" is defined by three distinct sets of behavior patterns: object exploration, social play (e.g., play fighting), and locomotor play (Marks et al., 2017). Why animals play is not well understood. Play requires energy and may entail other costs, such as diversion of energy away from growth, increased chance of injury, and increased exposure to predators (Berghänel et al., 2015; Caro, 1988; Miller and Byers, 1991). On the positive side, play has been hypothesized to serve as “practice” or “training” for associated adult behaviors (Brownlee, 1954; Byers, 1977; Caro, 1988; Spinka et al., 2001).

A leading hypothesis for the functional significance of locomotor play is that it aids in muscular and neural development of young individuals, with benefits that may persist into adulthood (Brownlee, 1954; Burghardt, 2014; Byers, 1998, 1984; Byers and Walker, 1995, 1995; Fagen, 1976). Locomotor play is generally defined as vigorous, apparently purposeless locomotor behavior, most commonly performed by young animals (Byers and Bekoff, 1981). Examples of locomotor play include running around, leaping vertically in the air, twisting the body, and/or kicking out the hindfeet (e.g., see Burghardt, 2014; Byers, 1984; Poole and Fish, 1975). Such behaviors are distinguished from avoidance of predators by their non-threatening context, loose body tone, and sensory non-focus or inattentiveness (Fagen, 1974).

Locomotor play is common in many mammals, including primates, Carnivora, ungulates, and even rodents (Burghardt, 2014; Nunes et al., 2004, 1999; Nunes and Monroy Montemayor, 2023). In rodents under laboratory conditions, locomotor play has been defined to include very rapid, horizontally directed jerk-run sequences (Wolff, 1981) and rapid "bouncing" in a vertical direction (Walker and Byers, 1991). Walker and Byers (1991) considered these movements to be locomotor play for four reasons: 1) they do not appear to have an obvious purpose; 2) they are mainly seen during the developmental period (~14-30 days after birth); 3) often a whole litter engaged in them for 10-15 min, consistent with the typical occurrence of play periods in other mammals; and 4) previous descriptions of play behavior in mice (Poole and Fish, 1975; Wolff, 1981) were similar to what they observed.

If locomotor play aids in locomotor development, then species of animals that are highly active might be hypothesized to show more locomotor play during development than would animals that are relatively inactive. This kind of comparative (interspecific) hypothesis is difficult to test in a rigorous fashion because it requires detailed, quantitative observations of multiple species of wild animals. However, one can also test whether locomotor play is associated with high activity by comparing individuals within a single species. Here again, young individuals that show high levels of locomotor play would be predicted to exhibit high activity and motor performance as adults. Motor performance includes multiple components, such as vigor (the ability to perform energetically expensive acts repeatedly) and skill (the successful performance of challenging motor acts) (Byers and Walker, 1995).

We evaluated locomotor play, as defined by Walker and Byers (1991), in the context of an artificial selection experiment for high activity behavior. We compared mice from four replicate High Runner (HR) lines that had been selectively bred for high voluntary wheel running with those from four non-selected Control (C) lines (Swallow et al., 1998b). The artificial selection experiment began with a base population of the outbred Hsd:ICR strain (Swallow et al., 1998a). All four of the HR lines reached apparent selection limits by about generation 17-27, depending on sex and replicate line, at which point they ran approximately three-fold more revolutions/day than the C lines (Careau et al., 2013), which led to substantially increased energy expenditure and food consumption (Copes et al., 2018; Rezende et al., 2009). Mice from the HR lines also have higher endurance and higher maximal aerobic capacity (VO_2max) as measured during forced exercise (Cadney et al., 2021; Meek et al., 2009; Rezende et al., 2009; Schwartz et al., 2023; Singleton and Garland Jr., 2019; Swallow et al., 1998b). Importantly, the elevated running by HR mice appears immediately after weaning (Copes et al., 2018; e.g., see Kelly et al., 2006; Meek et al., 2010; Swallow et al., 1998b). Therefore, we hypothesized that young mice from the HR lines would also have elevated levels of locomotor play behavior, which may have evolved because it increases their motor coordination and performance abilities later in life, i.e., at the time of adult wheel testing. If so, then the energetic cost of play (Berghänel et al., 2015) might be one cause of their lower growth rates and adult body size (Copes et al., 2018; Khan, 2023; Malisch et al., 2007).

2. Materials and methods

All procedures were approved by the Institutional Animal Care and Use Committees at the respective institutions (see below).

2.1. Animal model

Mice (*Mus domesticus*) used in this study were from 4 replicate High Runner (HR) lines that have been selectively bred for voluntary wheel-running behavior, and were compared with four non-selected Control (C) lines (Careau et al., 2013; Rhodes et al., 2005; Swallow et al., 2009, 1998a). Mice were sampled from generations 20, 68, and 93 (Table 1). Mice from generation 20 were evaluated at the University of Wisconsin, Madison, those from generation 68 at Ohio Wesleyan University, and those from generation 94 at the University of California, Riverside. In each generation, mice were maintained at a constant temperature and humidity, food and water were provided ad libitum, and the light-dark cycle was 12:12.

2.2. Behavioral observations

Observations for generation 20 occurred when mice were 22-24 days old, which is within the age range when play was commonly observed in the five inbred strains and crosses of mice studied by Walker and Byers (1991). Each of the 80 cages (N = 42 HR and 38 C) contained four same-sex sets of full siblings (N = 320 total mice). Locomotor play was observed during the first two hours of the dark cycle (under illumination by red light), which is the normally active phase for mice (Eckel-Mahan and Sassone-Corsi, 2015), including those studied here (Malisch et al., 2009). During a given sampling period, we observed a single cage at a time (focal sampling: Martin and Bateson, 2007) for 12 minutes and utilized continuous recording (Martin and Bateson, 2007). Two behaviors were recorded as previously described by Walker and Byers (1991): (1) very rapid, horizontally directed jerk-run sequences; (2) rapid 'bouncing' in a vertical direction. These movements are discrete and do not last more than a second, so they were counted as opposed to measuring their duration. To increase the reliability of our observations, all cages were scored simultaneously by two trained observers. Their counts were compared to establish repeatability (inter-observer reliability) and then averaged for analyses. Note that because the unit of observation was a cage, as opposed to individual mice, each cage yielded two numbers, the total occurrences of behaviors (1) horizontal and (2) vertical play.

Procedures for generation 68 generally followed those for generation 20, but with a total sample size of 97 cages (N = 388 total mice). To produce these mice, 176 mice were shipped to Ohio Wesleyan University from University of California, Riverside, including retired female breeders from generation 67 and their male offspring (generation 68), which were then paired to produce mice for behavioral observations (disallowing pairing of offspring with their mothers). Here, we refer to these mice as generation 68 for simplicity. Mice were weaned at 21 days of age and then cages containing 2-4 individuals, mostly full siblings, of the same sex were observed on the second and third nights after weaning (i.e., 22 and 23 days of age), during the first ~4 hours of the dark cycle, by two observers on each day (with the exception of 18 cases that had only a single observer on one of the two days). Their counts were compared to establish repeatability (inter-observer reliability) and then averaged for analyses. Of the 97 total cages observed, most or all of the mice were sleeping on one of the two days in a total of 21 cages, so these were excluded from analyses, leaving 76 cages.

To capture an earlier developmental window, mice from generation 93 were observed when litters were 15 days old, an age when play behavior is relatively uncommon in laboratory mice (Walker and Byers, 1991). Recordings under red light illumination were made with a webcam (Logitech c34) during the first 3 hours of the dark cycle. Immediately prior to recording, pups in excess of six were rapidly removed and placed into a holding cage. Sexes of the mice observed were mixed and were not recorded. The total sample size was 81 cages, with eight having fewer than six mice observed because litter size was smaller than six (3 cages with 5 mice, 4 cages with 4, and 1 cage with 3) (N= 474 total mice). Total litter size ranged from 3-14 (mean = 9.9, mode = 12, median = 10). Therefore, the number of mice in the cage observed (range = 3-6) was used as a covariate in statistical analyses (see below). The two behaviors as described above were scored from the recordings on a per-cage basis by a single observer.

2.3. Statistical analyses

Statistical analyses were performed with SPSS (version 20, SPSS Inc., Chicago, IL, USA) and SAS 9.1 (SAS Institute Inc., Cary, NC, USA). Analyses were performed separately by generation, and each considered behaviors (1) and (2) (see above) separately, as well as their sum. For each generation, a two-way, mixed-model was used to simultaneously test the effects of linetype (HR vs. C mice), sex (only for generations 20 & 68), and their interaction in SAS Procedure Mixed with REML estimation and Type III tests of fixed effects. Replicate line was a random effect nested within linetype, and the sex-by-line interaction was included as an additional nested effect, such that the main effects and their interaction were tested relative to the among-line variance with 1 and 6 d.f. For generation 20, time of day and age were used as covariates. For generation 68, the number of mice in the cage observed was used as a covariate; however, analyses were based on the averages of four values across days 2 and 3, and so time of day was not used as a covariate. For generation 93, the number of mice in the cage observed and the total litter size (e.g., see Parra-Vargas et al., 2023) were used as covariates. Play behavior tended to be positively skewed and was transformed as needed to improve normality of residuals for statistical models. Outliers were removed when the standardized residual exceeded ~3 and/or the most extreme point was >1 SD from the next point. Statistical significance was taken as $P \leq 0.05$.

3. Results

3.1. 22-24-day old mice at generation 20

Body mass at weaning (21 days of age) tended to be smaller in females ($P = 0.0611$), with no significant effect of linetype and no sex-by-linetype interaction (Table 2).

Both components of play behavior and total play were strongly positively skewed but transforming by raising to the 0.4 power normalized the distributions (e.g., see Figure 1). For the transformed values, observations of horizontal movements were statistically consistent between the two observers (N = 80, Pearson's $r = 0.941$, $P < 0.0001$), as were observations of vertical movements ($r = 0.987$, $P < 0.0001$) and of total play (Figure 1: $r = 0.987$, $P < 0.0001$).

Neither horizontal play nor total play showed any statistical effect of linetype, sex or their interaction (Table 2). We considered the frequency of vertical play to be too low for comparing groups (only 20 of 80 cages showed any vertical play movements) if treated as a continuous-valued trait, but analyses with SAS Procedure GLIMMIX, treating it as a 0-1 variable (no play or some play), indicated no significant effects (results not shown).

Comparison of the 4 Control lines indicated that females were smaller than males, with no statistical differences among lines and no sex-by-line interaction (Table 2). Neither horizontal play nor total play was affected by sex, line or their interaction (Table 2, Figure 2).

Comparison of body mass for the 4 HR lines did not indicate a sex difference but did demonstrate significant differences among the replicate lines (Table 2). Results for play behavior were similar for total and horizontal (Table 2). Play varied among lines (P = 0.0411 for total [Figure 2], P = 0.0662 for horizontal) and was affected by a sex-by-line interaction (P = 0.0697 for total, P = 0.0197 for horizontal), as well as decreasing with age.

3.2. 22-23-day old mice at generation 68

Weaning body mass was significantly smaller in females than males (P = 0.0186), with no significant effect of linetype and no sex-by-linetype interaction (Table 3). The statistical significance of the sex effect disappeared (P = 0.3167) when mean values per cage were analyzed (Table 3).

Scores for total play were repeatable between the two observers on both day 2 (N = 74, r = 0.912, P << 0.0001) and day 3 (N = 63, r = 0.921, P << 0.0001) after weaning.

Total, horizontal, and vertical play were positively related to the number of mice per cage, as would be expected, but were unaffected by linetype, sex or their interaction (Table 3).

Total play did not vary among lines for either C or HR lines, with no effect of sex and no sex-by-line interaction (Table 3). In C lines, the number of mice in the cage was a positive predictor of total play (P = 0.0048), but not in HR mice, apparently because fewer cages had less than four mice. Results were similar for horizontal and for vertical play (Table 3).

3.3. 15-day old mice at generation 93

Linetype did not affect total, horizontal, or vertical play, but all three measures were positively correlated with the number of mice in the cage (Table 4), as would be expected. We also tested for interactions between linetype and the three covariates (number of mice in cage, litter size, time of day), with the interaction terms included individually or in combinations of two or all three. For total play, the linetype-by-litter size interaction was significant when it was entered alone (P = 0.0865) or in combination with the linetype-by-time interaction (P = 0.0840). Results were similar for horizontal play (P = 0.0772 and P = 0.0749, respectively). For vertical play, none of the interaction terms was ever significant.

All three components of play differed significantly among the 4 Control lines (Fig. 3) and were negatively related to litter size (Table 4). All three components of play also differed significantly among the 4 HR lines (Fig. 3), but were not related to litter size (Table 4).

4. Discussion

As reviewed in the Introduction, one hypothesis for the function of locomotor play behavior is that it helps neuromuscular development of young animals. Accordingly, we hypothesized that lines of mice selectively bred for high voluntary wheel-running behavior for 20-93 generations would also have evolved higher levels of juvenile play behavior, as a developmental component increasing the physical ability for wheel running in adults. Differences among the replicate HR lines were detected by generation 20, and may be attributed to the effects of random genetic drift (and possibly founder effects). These results show that play behavior was genetically variable within the original founding populations (cf. Walker and Byers, 1991) and hence could evolve. However, contrary to our hypothesis, mice from the selectively bred High Runner lines did not show more play behavior than those from the non-selected Control lines in any of the three generations studied.

One obvious possibility for these "negative" results is that locomotor play does not actually improve adult locomotor abilities, and so was not favored to increase in the HR lines. Importantly, we know of no experimental studies -- in any animal -- in which the level of play was manipulated experimentally to test for effects on adult motor abilities. Importantly, the HR mice locomote differently in wheels as compared with C mice, e.g., greater intermittency of running (Girard et al., 2001), in addition to running faster and greater daily distances. In addition, the elevated wheel running of HR mice is apparent even right after weaning (e.g., Copes et al., 2018; Kelly et al., 2006; Meek et al., 2010; Swallow et al., 1998b), which is when we measured play behavior in generations 20 and 68. Thus, if play behavior were related to the ability to engage in aerobic exercise (or the motivation for such exercise), then we would have expected play behavior to be higher in the HR lines just after weaning.

Another possibility is that some other correlated response has disallowed or diminished an increase in juvenile play. For example, previous research has shown that HR mice have increased circulating corticosterone levels (Garland Jr. et al., 2016) along with alterations in both dopamine (Rhodes et al., 2005) and endocannabinoid (Schmill et al., 2022; Thompson et al., 2017) signaling, any of which might affect play behavior (e.g see Lundberg et al., 2017; Vanderschuren et al., 2016; Veenit et al., 2013). In addition, mice from the HR lines tend to have larger periaqueductal gray (Schmill et al., 2023), and a recent study found that play and tickling responses map to this brain region in laboratory rats (Gloveli et al., 2023).

Interestingly, the amount of play was significantly negatively correlated with litter size at weaning in the analysis of the four Control lines at generation 93, but not for the four HR lines (Table 4). As noted recently by Parra-Vargas (2023), litter size can influence many physiological traits. One mechanism for such influences has to do with energetics, as pups reared in small litters have more access to milk, which affects their growth rate and body size at weaning, and potentially also later in life. Previously, in a study of the base population used to start the HR mouse selection experiment, we reported that body mass and litter size at weaning are negatively correlated r = -0.418 in their Table 3 (Hayes et al., 1992). In the present study, for the mice from generation 93, mean body mass and litter size at weaning were also negatively correlated (for all mice, N = 111, r = -0.585, P << 0.0001; for C mice, N = 62, r = -0.671, P << 0.0001; for HR mice, N = 49, r = -0.541, P << 0.0001). In principle, behavioral traits might also be affected by such energetic trade-offs, e.g., if brain development was hindered or otherwise altered in mice from large versus small litters. Further study will be required to elucidate the mechanism that causes this negative correlation in the C lines, and also why such a relationship is absent in the HR lines.

Another potential future direction would be the study of social play in the HR lines of mice. Although we did not attempt to score social play in the present study, incidentally we observed that it was rare under the testing conditions used. Also of interest would be play fighting, given that among species of rodents, locomotor play and play fighting are negatively correlated (Pellis and Iwaniuk, 2004).

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Declaration of Interests

The authors declare no competing or financial interests.

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Tables

Table 1
Mice sampled from three generations of the High Runner selection experiment.

Generation	Age	Sexes	Total Cages	Observations
Generation 20	22-24 days old	Separated (4 to each cage)	80 cages (N = 320 mice)	1 day, 2 observers
Generation 68	22-23 days old	Separated (4 to a cage)	97 cages (N = 388 mice)	2 days, 2 observers
Generation 94	15 days old	Mixed (6 to a cages)	81 cages (N = 474 mice)	1 day, 1 observer (video)

Table 2

Body mass at weaning (mean value for all mice in a cage) and play behavior of mice sampled from generation 20 of the High Runner (HR) selection experiment. Eighty cages were observed for play behavior. First part of table show comparisons of the four replicate HR lines with the four replicate non-selected C lines. Females tended to be smaller than males at weaning. Second part of the table shows comparisons of the four C lines with each other: females were significantly smaller than males. Third part of the table shows comparisons of the four HR lines with each other. The replicate HR lines differed significantly for body mass at weaning as well as total play. For analyses of play behavior, age (range = 22-24 days) and time of observation (range = 8:06-10:05 PM) were used a covariates. P-values < 0.05 are in **bold**.

Generation 20			Linetype			Sex			Sex*Linetype			Age			Time		
Trait	N	trans-form	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Body Mass (g)	79	none	1,6	0.04	0.8474	1,6	5.29	0.0611	1,6	1.68	0.2430						
Total Play	80	^0.4	1,6	1.16	0.3225	1,6	0.60	0.4665	1,6	0.52	0.4962	1,62	2.7011	0.1053	1,62	0.9021	0.3459
Horizontal	80	^0.4	1,6	2.07	0.2003	1,6	1.84	0.2238	1,6	0.94	0.3699	1,62	3.3364	0.0726	1,62	2.5543	0.1151

C Lines			Line			Sex			Sex*Line			Age			Time		
Trait	N	trans-form	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Body Mass (g)	37	none	3,29	1.05	0.3836	1,29	4.30	**0.0472**	3,29	0.10	0.9570						
Total Play	38	^0.4	3,28	0.75	0.5313	1,28	0.80	0.3781	3,28	0.37	0.7740	1,28	0.0157	0.9012	1,28	0.0685	0.7955
Horizontal	38	^0.4	3,28	0.26	0.8565	1,28	2.41	0.1319	3,28	0.46	0.7105	1,28	0.0573	0.8126	1,28	0.0071	0.9335

HR Lines			Line			Sex			Sex*Line			Age			Time		
Trait	N	trans-form	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Body Mass (g)	42	none	3,34	4.22	**0.0122**	1,34	0.56	0.4616	3,34	0.23	0.8783						
Total Play	42	^0.4	3,32	3.08	**0.0411**	1,32	0.01	0.9239	3,32	2.59	0.0697	1,32	8.8066	**0.0056**	1,32	1.6239	0.2117
Horizontal	42	^0.4	3,32	2.64	0.0662	1,32	0.07	0.7950	3,32	3.79	**0.0197**	1,32	7.8446	**0.0086**	1,32	3.6490	0.0651

Table 3
Body mass at weaning and play behavior of mice sampled from generation 68 of the High Runner (HR) selection experiment. Seventy six cages were observed for play behavior. First part of table show comparisons of the four replicate HR lines with the four replicate non-selected C lines. Females were significantly smaller than males at weaning. Second part of the table shows comparisons of the four C lines with each other: females were significantly smaller than males and the replicate lines differed in mass. Third part of the table shows comparisons of the four HR lines with each other: females were significantly smaller than males and the replicate lines differed in mass, with a sex*line interaction. P-values < 0.05 are in **bold**.

Generation 68			Linetype			Sex			Sex*Linetype			N Mice		
Trait	N	trans-form	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Body Mass (g)	353	none	1,6	1.04	0.3467	1,6	10.24	**0.0186**	1,6	0.37	0.5677			
Body Mass (g)	97	log10	1,6	0.87	0.3871	1,6	1.19	0.3167	1,6	0.77	0.4127			
Total	76	^0.6	1,6	0.25	0.6350	1,6	0.03	0.8672	1,6	0.69	0.4366	1,59	14.44	**0.0003**
Horizontal	76	^0.6	1,6	0.14	0.7238	1,6	0.04	0.8432	1,6	0.72	0.4286	1,59	11.70	**0.0011**
Vertical	76	^0.6	1,6	0.90	0.3797	1,6	0.04	0.8518	1,6	0.12	0.7410	1,59	15.05	**0.0003**

C Lines			Line			Sex			Sex*Line			N Mice		
Trait	N	trans-form	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Body Mass (g)	163	none	3,22	7.08	**0.0017**	1,133	4.43	**0.0372**	3,133	1.27	0.2863			
Body Mass (g)	47	log10	3,39	10.62	**<0.0001**	1,39	0.03	0.8596	3,39	0.06	0.9825			
Total	37	^0.6	3,28	1.16	0.3413	1,28	0.05	0.8307	3,28	1.50	0.2356	1,28	9.41	**0.0047**
Horizontal	37	^0.6	3,28	1.37	0.2730	1,28	0.03	0.8536	3,28	1.85	0.1604	1,28	8.03	**0.0084**
Vertical	37	^0.6	3,28	2.14	0.1182	1,28	0.06	0.8108	3,28	0.20	0.8978	1,28	11.55	**0.0021**

HR Lines			Line			Sex			Sex*Line			N Mice		
Trait	N	trans-form	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Body Mass (g)	190	none	3,25	7.57	**0.0009**	1,157	16.34	**0.0001**	3,157	2.81	**0.0413**			
Body Mass (g)	50	log10	3,42	17.23	**<0.0001**	1,42	2.56	0.1171	3,42	0.45	0.7163			
Total	39	^0.6	3,30	0.36	0.7824	1,30	0.39	0.5380	3,30	0.73	0.5412	1,30	0.02	0.8790
Horizontal	39	^0.6	3,30	0.31	0.8212	1,30	0.45	0.5078	3,30	0.89	0.4558	1,30	0.01	0.9156
Vertical	39	^0.6	3,30	0.59	0.6235	1,30	0.16	0.6891	3,30	0.09	0.9652	1,30	1.68	0.2045

Table 4
Play behavior of mice sampled from generation 93 of the High Runner selection experiment. Eight one cages were observed for play behavior. Slope indicates value for partial regression coefficient of the covariate. P-values < 0.05 are in **bold**.

Generation 93			Linetype				Time of Day				Number Observed				Litter Size			
Variable	N	trans-form	df	*F*	*P*	*Slope*	df	*F*	*P*	*Slope*	df	*F*	*P*	*Slope*	df	*F*	*P*	*Slope*
Total	81	^0.3	1,6	1.07	0.3419	-	1,70	3.47	0.0667	+	1,70	14.29	**0.0003**	+	1,70	3.27	0.0749	-
Horizontal	81	^0.4	1,6	0.46	0.5240	-	1,70	1.87	0.1759	+	1,70	8.72	**0.0043**	+	1,70	1.57	0.2145	-
Vertical	81	^0.5	1,6	1.32	0.2948	-	1,70	5.13	**0.0267**	+	1,70	13.12	**0.0005**	+	1,70	3.60	0.0621	-

C Lines			Line			Time of Day				Number Observed				Litter Size			
Variable	N	trans-form	df	*F*	*P*	df	*F*	*P*	*Slope*	df	*F*	*P*	*Slope*	df	*F*	*P*	*Slope*
Total	40	none	3,33	3.29	**0.0325**	1,33	0.20	0.6599	+	1,33	0.63	0.4317	+	1,33	5.36	**0.0270**	-
Horizontal	38	^0.4	3,31	6.82	**0.0012**	1,31	2.23	0.1453	-	1,31	8.64	**0.0062**	+	1,31	19.62	**0.0001**	-
Vertical	37	^0.7	3,30	9.21	**0.0002**	1,30	0.55	0.4657	-	1,30	6.83	**0.0139**	+	1,30	19.83	**0.0001**	-

HR Lines			Line			Time of Day				Number Observed				Litter Size			
Variable	N	trans-form	df	F	P	df	F	P	Slope	df	F	P	Slope	df	F	P	Slope
Total	41	^0.3	3,34	5.43	**0.0037**	1,34	3.17	0.0840	+	1,34	8.30	**0.0068**	+	1,34	0.06	0.8144	-
Horizontal	40	^0.4	3,33	13.30	**0.0000**	1,33	6.31	**0.0170**	+	1,33	9.63	**0.0039**	+	1,33	0.40	0.5327	+
Vertical	41	^0.5	3,34	5.00	**0.0056**	1,34	4.10	0.0507	+	1,34	8.51	**0.0062**	+	1,34	0.20	0.6613	-

Figures

Figure 1. Frequency distribution and inter-observer consistency of total play behavior for 80 cages of mice from generation 20 (22-24 days of age). Raw data (A, C) were positively skewed, but transformation by raising to the 0.4 power (B, D) yielded an approximately normal (or bivariate normal) distribution. Inter-observer consistency was high for the transformed values (D: Pearson's r = 0.987, P < 0.0001).

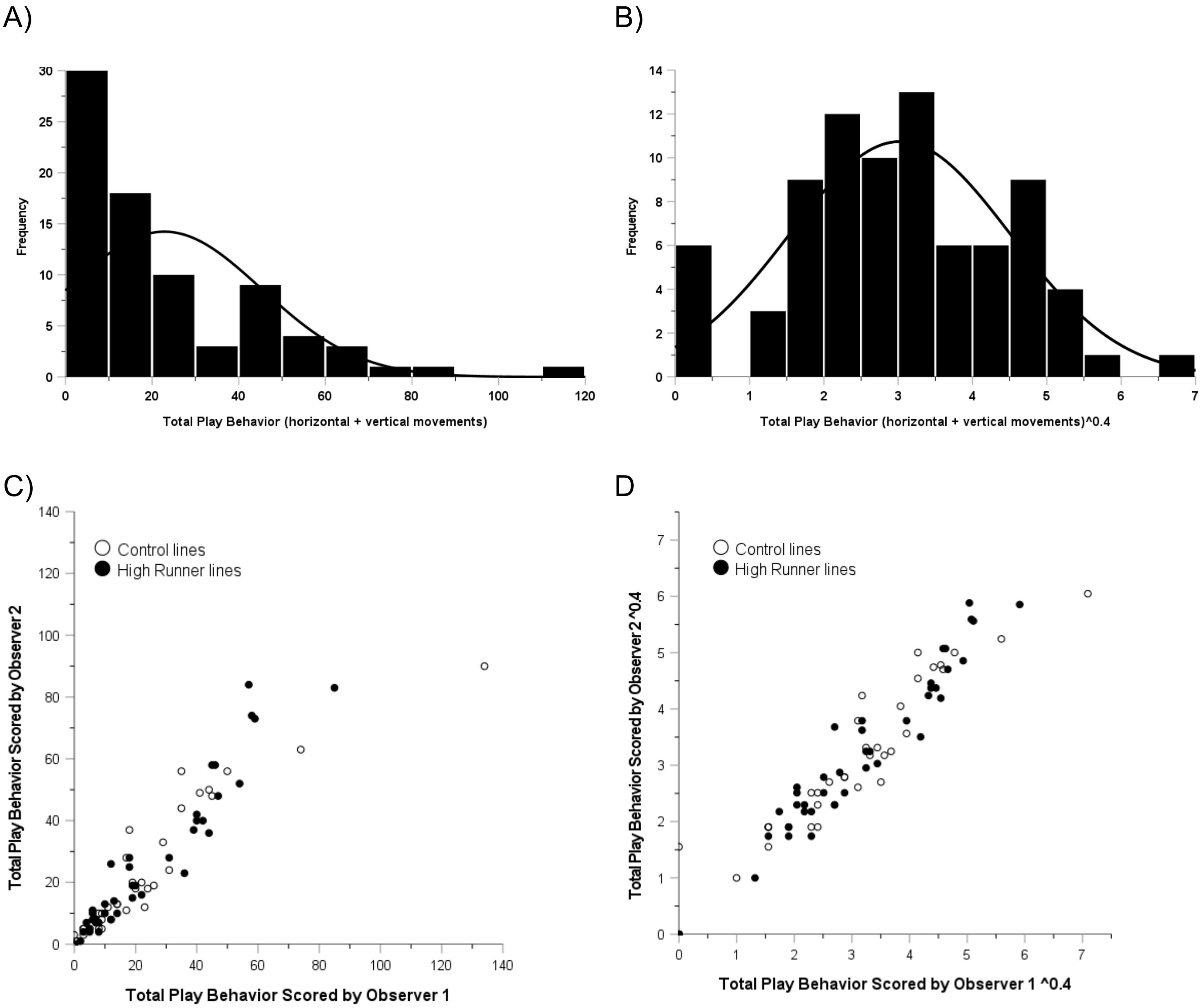

Figure 2. Variation among replicate High Runner and Control lines for play behavior measured at generation 20. Play behavior did not significantly differ among the four replicate C lines, but did differ among the HR lines, where a sex-by-line interaction was also apparent (Table 2).

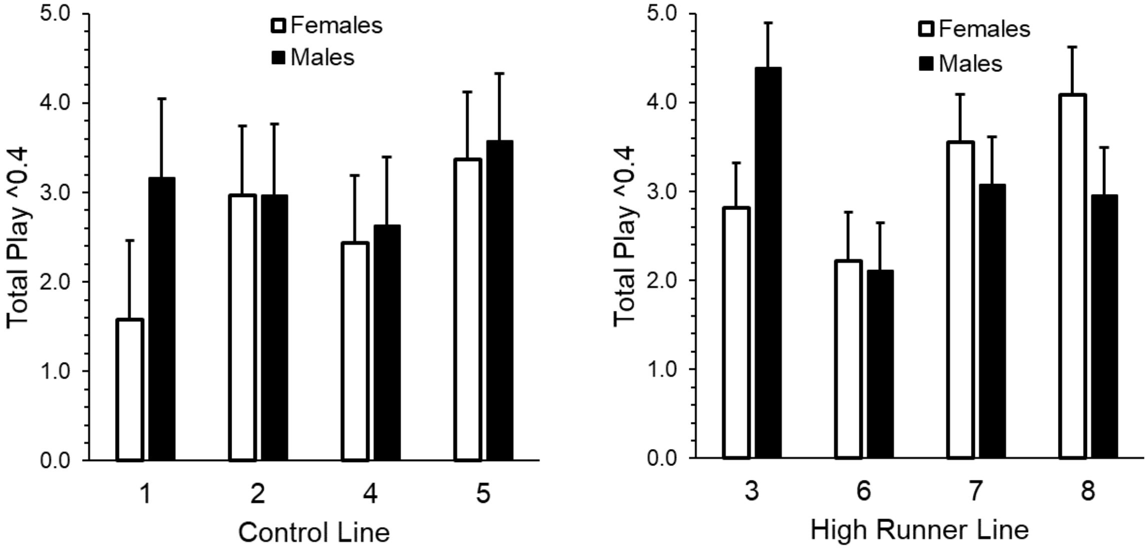

Figure 3. Variation among replicate High Runner and Control lines for play behavior measured at generation 93. Play behavior varied significantly both among the four replicate C lines and among the HR lines (Table 3). For the C lines, play behavior was negatively related to their litter size at weaning (see Discussion).