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ORIGINAL RESEARCH

I Smell a Mouse: Indirect Genetic Effects on Voluntary Wheel-Running Distance, Duration and Speed

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Abstract

Indirect genetic effects (IGEs; the heritable influence of one organism on a conspecific) can affect the evolutionary dynamics of complex traits, including behavior. Voluntary wheel running is an important model system in quantitative genetic studies of behavior, but the possibility of IGEs on wheel running and its components (time spent running and average running speed) has not been examined. Here, we analyze a dataset from a replicated selection experiment on wheel running (11,420 control and 26,575 selected mice measured over 78 generations) in which the standard measurement protocol allowed for the possibility of IGEs occurring through odors because mice were provided with clean cages attached to a clean wheel or a wheel previously occupied by another mouse for 6 days. Overall, mice ran less on previously occupied wheels than on clean wheels, and they ran significantly less when following a male than a female. Significant interactions indicated that the reduction in running was more pronounced for females than males and for mice from selected lines than control mice. Pedigree-based "animal model" analyses revealed significant IGEs for running distance (the trait under selection), with effect sizes considerably higher for the initial/exploratory phase (i.e., first two of six test days). Our results demonstrate that IGEs can occur in mice interacting through scent only, possibly because they attempt to avoid conspecifics.

Keywords Artificial selection · Exercise · Experimental evolution · Heritability · Physical activity

Introduction

Indirect genetic effects (IGEs) are "genetically-based environmental influences and are generated whenever the phenotype of one [conspecific] individual acts as an environment for another" ["conspecific" added] (Moore et al. 1997). In other words, IGEs are environmental influences on the phenotype of an organism (e.g., its growth, physical activity) resulting from the expression of genes in another individual (Dingemanse and Araya-Ajoy 2015). Maternal genetic effects are a well-known special case of IGEs. Aside from these, IGEs are easy to comprehend in the context of aggressive interactions between conspecifics because the behavior of a focal individual will likely depend on the behavior of the individual with whom it interacts (Wilson et al. 2009).

When present, IGEs confound the strict separation of genetic and environmental effects on the phenotype (Wolf et al. 1998), can be a significant part of a trait's genetic architecture, and hence can influence its evolutionary dynamics (Wolf 2003). In effect, IGEs result in part of the environment of an organism being heritable, and thus that part of the environment is itself capable of responding to selection. Some IGEs are especially important in mammals because of the strong influence of maternal effects, including heritable maternal effects, on many traits (Mousseau and Fox 1998; McAdam et al. 2014).

IGEs were originally considered in the context of parents and offspring (e.g., maternal effects), or other relatives,

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but can also occur for unrelated individuals where social interactions of conspecifics provide an important source of environmental variation that can affect the expression and evolution of behavior (Dingemanse and Araya-Ajoy 2015). Numerous behavioral traits have been shown to have IGEs as a component of their heritability, including aggression and dominance in mammals (Camerlink et al. 2015; Wilson et al. 2009, 2010, 2011; Sartori and Mantovani 2013) and breeding and laying dates in birds (Germain et al. 2016). Moreover, IGEs can either facilitate or constrain behavioral evolution, depending on whether IGEs are, respectively, positively or negatively correlated with direct genetic effects. For example, aggression in deer mice (*Peromyscus maniculatus*) is not only influenced by both direct and indirect genetic effects, but the strong positive correlation between direct and indirect genetic effects implies that selection for high aggressiveness in these mice will create a social environment that elicits aggressiveness even more (Wilson et al. 2009). Overall, past studies have clearly shown the importance of quantifying IGEs—and their correlation with direct genetic effects—to more fully understand the genetic architecture of behavior.

Ever since Stewart (1898) first measured activity in rats using rotating drum cages, wheel running by rodents, measured in various ways, has provided an example of a complex, polygenic trait influenced by both performance factors (e.g., endurance, maximal aerobic capacity) and motivation (Swallow et al. 2009). Wheel running is widely used in studies of exercise, motivation, and reward systems (Novak et al. 2012), and is genetically correlated with some behaviors (e.g., nest building: Carter et al. 2000), but not others (e.g., open-field behavior: Careau et al. 2012). Although sometimes viewed as a pathological or stereotypical behavior of captive rodents, Mather (1981) argued that wheel-running behavior reflects "exploratory migration" related to "the search for potential resources" (or the need to "monitor" resources; Perrigo and Bronson 1985; but see Careau et al. 2012). Importantly, wild house mice run voluntarily on wheels when given access in the field (Meijer and Robbers 2014). In any case, innate differences in performance capacity or motivation for physical activity may have important implications for foraging, patrolling a territory or dispersal (see Feder et al. 2010). Although some work has examined how interactions between mice may affect voluntary wheel running (e.g., Drickamer and Evans 1996), heritable indirect effects on wheel-running distance, duration, and speed have not been considered.

In this study, we tested whether individual variation in voluntary wheel running can be affected by indirect genetic effects using a large dataset from a long-term artificial selection experiment on voluntary wheel running in house mice (*Mus musculus*) (Swallow et al. 1998, 2009; Wallace and Garland 2016; Garland et al. 2016). Because mice were wheel-tested in batches (see "Methods"), a mouse's wheel running could be affected by the previous mouse measured in the same wheel enclosure in a prior batch within a given generation. Using the data for the first 78 generations of the experiment, we examined how wheel running of focal mice was influenced by attributes of the preceding mouse, specifically sex and selection treatment (i.e., whether the mouse was from one of the four non-selected control lines or from one of the four lines bred for high wheel running). We used a type of mixed model known as an "animal model" (Henderson 1973; Lynch and Walsh 1998; Wilson et al. 2010) to integrate pedigree and phenotypic information and estimate such key parameters as direct additive genetic variance, indirect additive genetic variance, and their covariance (or correlation). Finally, we replicated our analyses for time spent running (duration) and average running speed, the two components of distance run as measured in the current experiment.

Methods

Selection experiment

The experiment, ongoing since 1993 (Swallow et al. 1998), used eight lines of mice bred from 224 outbred Hsd:ICR mice, divided into four non-selected control lines and four lines selected for high wheel running (HR, for "high-runners"). Wheel running was measured over 6 days, and the selected trait was the average number of wheel revolutions on the fifth and sixth days. Over the first 15–20 generations, wheel running in HR mice increased ~2.5–3.0-fold, before reaching plateaus with no further increase in wheel running despite continued directional selection and significant additive genetic variance that persisted for a substantial number of generations past the selection limits (Careau et al. 2013, 2015). The selected trait (distance run) is a direct product of average running speed and duration. Although both components of running distance increased as correlated responses to selection on distance run, running speed has evolved more than duration (Garland et al. 2011).

Measurement protocol

Ten families per generation are maintained in each line and housed in same-sex groups of four per cage, except during breeding and wheel running. At 6–9 weeks of age, mice are individually introduced into clean cages (with ad lib food and water) attached to a stainless-steel, Wahman-type activity wheel (circumference = 112 cm, diameter = 35.7 cm, and width = 10 cm; Lafayette Instruments, Lafayette, IN); the cages are divided between two rooms.

Computerized photocell counters record revolutions at 1-min intervals and the number of wheel revolutions (distance) is measured daily over 6 days for each mouse. Time spent running (duration) is also extracted by counting the number of 1-min intervals with at least one revolution. Finally, average running speed is calculated as distance run per day divided by number of active intervals (Swallow et al. 1998). In the four HR lines, breeders are chosen based on the average number of revolutions run on the fifth and sixth days.

Approximately 600 individuals are measured per generation in three batches of up to 200 individuals on successive weeks. A fourth measurement batch was conducted in a total of 22 generations. Between each measurement batch, clean cages are provided with fresh wood shavings, food hoppers, and water bottles. A clean tray with fresh wood shavings is also provided underneath the wheel. Therefore, each mouse is introduced to a clean cage for the 6 days of wheel testing. However, for logistical reasons, the wheels themselves are not cleaned between measurement batches within a generation. Thus, it is possible that wheel-running behavior of mice in later batches could be affected by the previous mouse, by urine, feces, or other chemical cues remaining on the wheel surfaces.

Data and pedigree

We used the same data and pedigree for wheel-running distance on the fifth and sixth days as compiled and checked up to generation 31 in Careau et al. (2013), with the first 4 days added in Careau et al. (2015). Here, we added data on all 6 days (1–6) for generations 35–78 and performed the same data checking and cleaning as in Careau et al. (2013). Briefly, we excluded individuals for which wheel-running distance on a given day was missing, zero, or abnormal compared to wheel running on the other days (e.g., caused by wheel problems that were detected and corrected during the first 4 days). We also deleted all data from generations 0–20 for mice from the selected lines because this is the time frame over which IGEs may have been evolving to become different between the control and selected lines (i.e., before selection limits were reached: see Careau et al. 2013). This resulted in a sample of 11,420 control and 26,575 selected individuals with wheel running measured on all 6 days over 78 generations. Note that wheel-running data are absent for generations 32–35 as the colony was moved from University of Wisconsin-Madison to University of California, Riverside. During these generations, selection was not applied in the HR lines. Mice were assigned to their wheel in a semi-random fashion, such that the previous and focal mice were of the same line in only 9% (2781) of cases. Of the 29,762 mice measured, 40% (11,869) had clean wheels (the third and fourth batches often had slightly fewer mice measured, and some wheels were unoccupied in the first batches, in which case there was no previous mouse attributed to the observations made on these wheels in the subsequent batch).

Statistical analysis

The distance run on each day (1–6) was standardized to z-scores (mean = 0, SD = 1) separately in control and HR lines within each generation. This standardization makes the estimates of variance and regression coefficients directly comparable among days, between selection treatments, and among generations. Thus, generation and selection treatment need not be included as fixed effects in models (except when fitted through an interaction, see below). For each mouse, the sex of the previous mouse in the same wheel was determined as a three-level factor: male, female, or a clean wheel (clean wheels occurred only for mice in the first batch of each generation). Similarly, the selection treatment (or linetype) of the previous mouse was recorded as control, selected, or a clean wheel.

We used ASReml-R (version 3.0; Butler et al. 2007) to analyze wheel-running distance, time spent running, and average running speed with a type of mixed model known as an "animal model" (Henderson 1973; Lynch and Walsh 1998; Wilson et al. 2010). The animal model allows integrating pedigree and phenotypic information to partition the phenotypic variation and estimate direct additive genetic variance, indirect additive genetic variance, and their covariance (or correlation). Separate models were run for each day, because the 6 days of wheel exposure represent somewhat different environments for the mice (e.g., novelty on day 1) and hence result in somewhat different behaviors with potentially varying genetic underpinnings (e.g., see QTL results of Kelly et al. 2010). An alternative approach would be to include data on all 6 days and model temporal changes in IGEs using random regression (i.e., analyze the slope and intercept of regressions fitted to the data for each individual). However, pooling the data in this manner made the models difficult to run due to the size of the dataset, even when assuming a simple linear function for changes in IGEs over time.

For a focal mouse i in a cage previously occupied by a mouse j, the model fitted was:

$$y_{ki} = \mu + \text{ fixed effects } + ce_{\text{F}i} + ce_{\text{P}j} + a_{\text{F}i} + a_{\text{P}j} + \text{Cor}\left(a_{\text{F}}, a_{\text{P}}\right) + e$$

where y_{ki} is either the wheel-running distance, time spent running, or average running speed of focal mouse i on day k, μ the mean trait value, fixed effects are described below, and e the residual error (see also Fig. 1). The random effects included were $ce_{\text{F}i}$, the identity of the focal mouse's dam (i.e., common environment); $ce_{\text{P}j}$, the identity of the previous mouse's dam (i.e., common indirect environment); $a_{\text{F}i}$, the additive genetic contribution of the focal mouse (i.e., direct

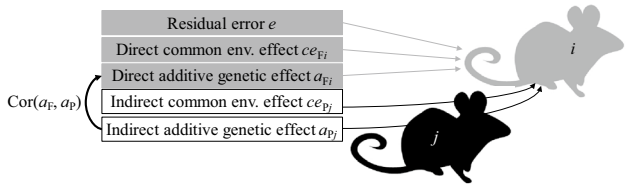

Fig. 1 Diagram of the quantitative genetic model fitted to the data (cf. Fig. 1 in Wilson et al. 2009). Wheel-running distance, duration, or speed by the focal individual i on day k (y_{ki}) is affected by: (1) the common environment of the focal mouse (ce_{Fi}), (2) the common environment of the previous mouse j (ce_{Pj}), (3) direct genetic effects (a_{Fi}, which are assumed to be entirely additive in nature: see "Methods"), (4) indirect genetic effects of the previous mouse (a_{Pj}), and (5) a correlation between direct and indirect genetic effects (Cor(a_F, a_P))

additive genetic effects); a_{Pj}, the additive genetic contribution of the previous mouse (i.e., indirect additive genetic effects); and Cor(a_F, a_P), the genetic correlation between direct and indirect effects. All random effects (ce_{Fi}, ce_{Pj}, a_{Fi}, a_{Pj}, and e) and Cor(a_F, a_P) were fitted separately for control and selected lines. Variance components extracted from this heterogenous model provide separate estimates for additive genetic variance (σ^2_{AF}), indirect genetic variance (σ^2_{AP}), and their correlation ($r_{AF,\,AP}$) in control and selected lines. We used the proLik() function from the nadiv R package (Wolak 2012) to estimate the 95% confidence intervals (CI) for all indirect genetic variance (σ^2_{AP}) components and all correlation estimates between the direct and indirect genetic effects ($r_{AF,\,AP}$).

All models included the same set of "standard" fixed effects: batch and room in which measurement took place, line, sex, age, inbreeding coefficient of the focal mouse, and an interaction between selection treatment and sex. These fixed effects are the same as in previous publications of the first 31 generations of this dataset (Careau et al. 2013, 2015), and are therefore not reported in the results. The animal models also included fixed effects of the sex and selection treatment of the previous mouse. As one goal was to test if these effects differed in control versus selected lines, we included all four possible two-way interactions between selection treatment and sex of the focal and previous mice [i.e., interactions between (1) sexes of the focal and previous mice, (2) selection treatment of the focal and previous mice, (3) sex of the focal mouse and selection treatment of the previous mouse, and (4) selection treatment of the focal mouse and sex of the previous mouse]. The 95% CI for the fixed-effect estimates were calculated as ± 1.96 times their standard error.

We note that neither dominance nor epistatic genetic variance is accounted for in the present models, and so these types of genetic effects are assumed to be negligible (see Careau et al. 2013). Alternatively, if dominance and epistatic variance are not negligible, then we assume these sources of variance would end up in the common environment or error term, not in our estimates of direct or indirect genetic variance. This assumption, however, may not hold when inbreeding occurs (as in the present selection experiment) because additive and dominance genetic effects may covary (Wolak and Keller 2014). Several studies have reported evidence for both dominance and/or epistasis in wheel running (e.g., Leamy et al. 2008; Nehrenberg et al. 2009; Kelly et al. 2010, 2011), and so it would be important for future studies to explore this issue.

Results

Running distance

Overall, mice generally ran less distance (the trait under selection) on previously occupied wheels than on clean wheels (Figs. 2a, S1, S2). Moreover, among mice that ran on a previously occupied wheel, individuals ran significantly less when the previous mouse was male than when it was female on days 2 and 4–6 (Table 1a; Fig. 2a). Selection treatment of the previous mouse did not significantly influence running distance (Table 1b; see also Fig. S2). The interaction between the sexes of the focal and previous mice was significant on all days except days 1 and 3 (Table 1c). Females were more strongly negatively influenced by the previous mouse being male than were males (Fig. 2a, compare circles vs. triangles). The interaction between the sex of the previous mouse and selection treatment of the focal mouse was significant on all days (Table 1e). HR mice were more strongly negatively influenced by the previous mouse being male than were control mice (Fig. 2a, compare blue vs. red symbols).

Estimates for the indirect genetic variance term (σ^2_{AP}) were considerably lower than for the direct additive genetic variance (Table 2a). Interestingly, σ^2_{AP} was noticeably higher for days 1–2 than days 3–6 for both control and selected lines (Table 2a; Fig. 3a). The correlation between direct and indirect genetic variance ($r_{AF,\,AP}$) was not significantly different from 0 on any day (Table 2a; Fig. 3b). Estimates of the other components of phenotypic variance (common environment, direct additive genetic, and residual variance) are shown in Table S1.

Running duration and speed

The sex and selection treatment of the previous mouse did not significantly influence time spent running by the focal mouse (Fig. 2b; Table 1a, b). The interaction between the sexes of the previous and focal mice was only significant on days 1 and 3 (Table 1c). All of the significant interactions detected for running distance (see previous

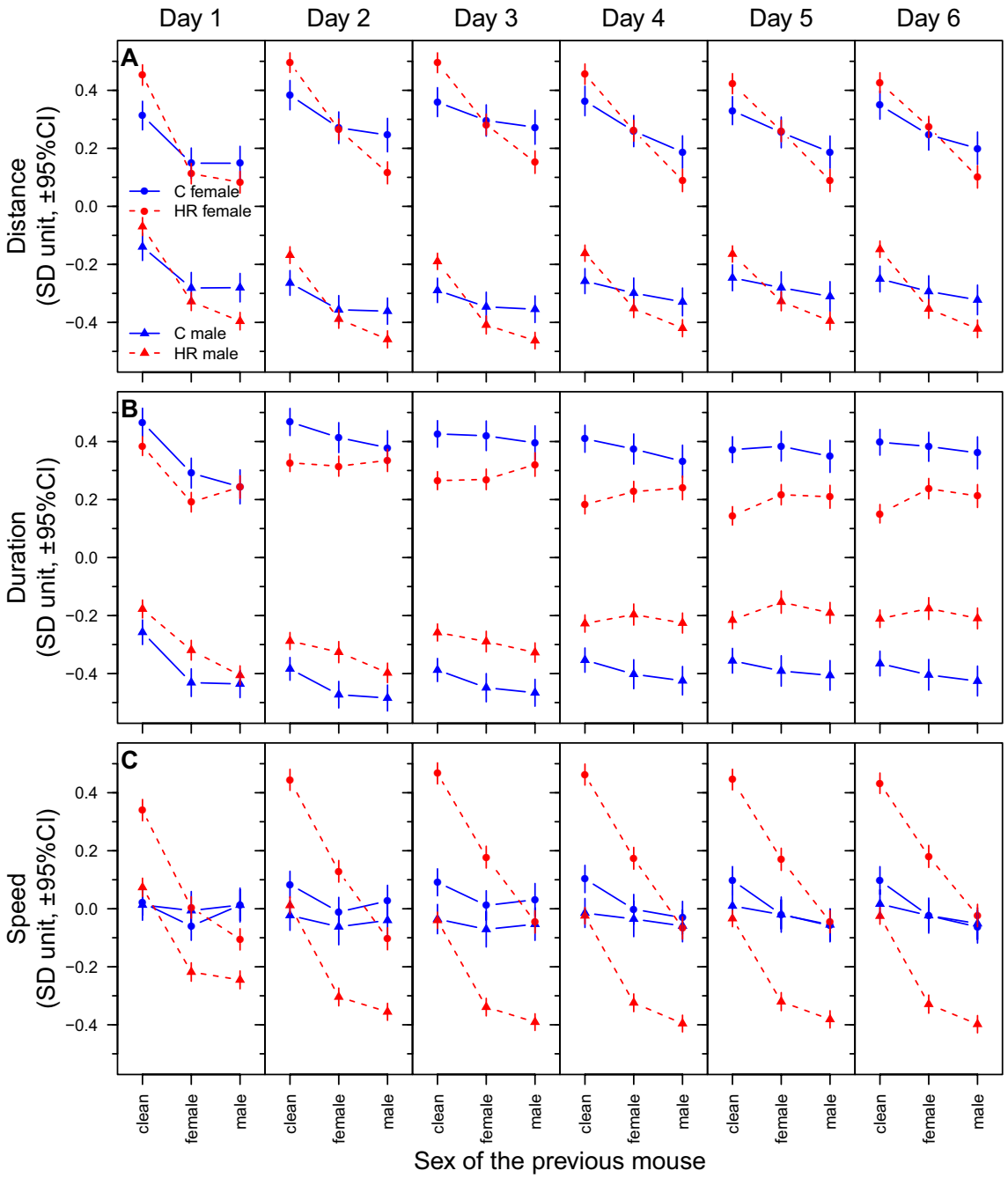

Fig. 2 Voluntary wheel-running **a** distance, **b** duration, and **c** speed in focal male (triangles) and female (circles) mice from control (C, blue) or high-runner lines (HR, red) as a function of the sex of the mouse previously occupying the same wheel (clean wheel, female, or male). Wheel-running behavior was measured over six consecutive days (each shown in a separate panel). Symbols denote the average and the 95% CI of distance (number of wheel revolutions), duration (number of 1-min intervals with at least one revolution), and speed (revolutions/duration) after standardization to mean of 0 and a variance of 1 for each generation and separately for control and high-runner lines (i.e., means are shown in units of standard deviation, SD, see Fig. S1 for the same figure on the raw scale). (Color figure online)

section) were also present for average running speed, with even stronger effect sizes (Table 1). Mice ran at a slower speed on previously occupied wheels than clean wheels (Figs. 2c, S1, S2), and the effect was more pronounced in females than males (Fig. 2c; dots vs. triangles), and in HR than control mice (Fig. 2c, red vs. blue symbols).

For running duration, estimates for indirect genetic variance (σ^2_{AP}) were considerably higher for days 1–2

Table 1 Estimates (in units of standard deviations) and 95% CI associated with the effects of (a) sex and (b) selection treatment (linetype) of the previous mouse on wheel-running distance, duration, and speed of the focal mouse. Also included are all four possible interactions between the sex and linetype of the focal and previous mice (c–f). Estimates whose 95% CI do not overlap with zero are indicated in bold

	Distance			Duration			Speed		
	Estimate	95% CI		Estimate	95% CI		Estimate	95% CI	
		Lower	Upper		Lower	Upper		Lower	Upper
(a) Effect of the sex of the previous mouse (female over male)									
Day 1	−0.026	−0.076	0.023	−0.028	−0.075	0.019	−0.022	−0.073	0.030
Day 2	**0.051**	**0.002**	**0.100**	0.013	−0.032	0.059	0.038	−0.014	0.090
Day 3	0.042	−0.007	0.091	−0.019	−0.065	0.026	**0.056**	**0.004**	**0.108**
Day 4	**0.105**	**0.055**	**0.154**	0.035	−0.011	0.080	**0.101**	**0.049**	**0.153**
Day 5	**0.094**	**0.044**	**0.143**	0.018	−0.027	0.063	**0.107**	**0.055**	**0.159**
Day 6	**0.081**	**0.031**	**0.130**	0.017	−0.028	0.062	**0.093**	**0.041**	**0.145**
(b) Effect of the linetype of the previous mouse (selected over control)									
Day 1	0.061	−0.018	0.139	0.075	−0.006	0.157	0.026	−0.032	0.084
Day 2	0.046	−0.047	0.140	0.055	−0.058	0.167	0.020	−0.041	0.081
Day 3	0.014	−0.041	0.069	0.006	−0.045	0.058	0.012	−0.046	0.071
Day 4	0.030	−0.035	0.096	0.022	−0.045	0.090	0.013	−0.045	0.071
Day 5	0.020	−0.037	0.076	0.016	−0.044	0.075	0.029	−0.032	0.091
Day 6	−0.011	−0.093	0.071	−0.041	−0.148	0.066	0.031	−0.031	0.093
(c) Interaction between the sex of the focal and previous mouse									
Day 1	0.032	−0.021	0.084	**0.082**	**0.031**	**0.134**	−0.051	−0.104	0.003
Day 2	**−0.056**	**−0.108**	**−0.003**	0.047	−0.003	0.098	**−0.127**	**−0.180**	**−0.074**
Day 3	−0.049	−0.101	0.004	**0.066**	**0.015**	**0.116**	**−0.132**	**−0.186**	**−0.079**
Day 4	**−0.083**	**−0.136**	**−0.030**	0.018	−0.032	0.069	**−0.135**	**−0.189**	**−0.082**
Day 5	**−0.091**	**−0.144**	**−0.038**	0.005	−0.046	0.055	**−0.128**	**−0.181**	**−0.075**
Day 6	**−0.081**	**−0.135**	**−0.028**	0.005	−0.046	0.055	**−0.112**	**−0.165**	**−0.059**
(d) Interaction between the linetype of the focal and previous mouse									
Day 1	−0.023	−0.126	0.079	−0.038	−0.139	0.063	0.000	−0.082	0.082
Day 2	−0.022	−0.140	0.096	−0.024	−0.151	0.103	0.000	−0.087	0.087
Day 3	0.014	−0.052	0.081	0.026	−0.034	0.087	0.003	−0.074	0.080
Day 4	0.006	−0.071	0.083	0.021	−0.054	0.096	0.022	−0.055	0.099
Day 5	0.028	−0.039	0.094	0.021	−0.047	0.088	0.013	−0.061	0.088
Day 6	0.026	−0.064	0.116	0.063	−0.048	0.175	−0.011	−0.091	0.069
(e) Interaction between the sex of the previous mouse and linetype of the focal mouse									
Day 1	**0.075**	**0.021**	**0.128**	0.017	−0.034	0.068	**0.130**	**0.075**	**0.185**
Day 2	**0.098**	**0.046**	**0.151**	−0.004	−0.054	0.046	**0.181**	**0.126**	**0.236**
Day 3	**0.081**	**0.028**	**0.134**	−0.022	−0.073	0.028	**0.159**	**0.104**	**0.215**
Day 4	**0.065**	**0.012**	**0.118**	−0.036	−0.086	0.014	**0.135**	**0.080**	**0.190**
Day 5	**0.076**	**0.022**	**0.129**	−0.005	−0.055	0.045	**0.108**	**0.053**	**0.163**
Day 6	**0.086**	**0.033**	**0.140**	0.004	−0.046	0.054	**0.113**	**0.058**	**0.168**
(f) Interaction between the linetype of the previous mouse and sex of the focal mouse									
Day 1	0.020	−0.037	0.077	0.005	−0.050	0.061	0.035	−0.024	0.093
Day 2	0.017	−0.040	0.074	0.005	−0.050	0.059	0.032	−0.026	0.090
Day 3	0.013	−0.044	0.070	0.019	−0.036	0.073	0.014	−0.044	0.072
Day 4	0.020	−0.037	0.077	0.026	−0.028	0.081	0.009	−0.049	0.067
Day 5	0.025	−0.033	0.083	0.029	−0.025	0.084	0.008	−0.050	0.065
Day 6	0.042	−0.016	0.100	0.041	−0.014	0.095	0.021	−0.037	0.078

than days 3–6, and relatively similar to σ^2_{AP} estimates for running distance (Table 2b; Fig. 3c). By contrast, σ^2_{AP} were much smaller for average running speed (Table 2c; Fig. 3e). In the selected lines, the correlation between direct and indirect genetic variance ($r_{AF,\,AP}$) was significant and positive for running duration on days 1–2 (Table 3b; Fig. 3d). The $r_{AF,\,AP}$ was not significant on any day for average running speed (Table 3c; Fig. 3f). Estimates of the other components of phenotypic variance (common environment, direct additive genetic, and residual variance) are

Table 2 Direct genetic variance ($\sigma^2_{AF} \pm$ SE) and indirect genetic variance ($\sigma^2_{AP} \pm$ SE) in voluntary wheel-running (a) distance, (b) duration, and (c) speed, quantified over six consecutive days in a total of 11,420 control and 26,575 selected mice. Also shown are the 95% CI for σ^2_{AP} calculated using profile likelihoods. Note that the estimates are much larger for running duration than speed, and much larger on days 1–2 than 3–6 (see also Fig. 3). NAs indicate that the variance component estimate was fixed at the boundary of the parameter space (i.e., 0). See Table 3 for the correlation between direct and indirect genetic variance, Table 1 for significance of fixed effects, and Tables S1, S2, S3 for all other variance components included in these models

	Control mice						Selected mice					
	σ^2_{AF}	SE	σ^2_{AP}	SE	95% CI		σ^2_{AF}	SE	σ^2_{AP}	SE	95% CI	
					Lower	Upper					Lower	Upper
(a) Distance												
Day 1	0.2580	0.0259	0.0032	0.0030	0.0004	0.0113	0.0996	0.0198	0.0022	0.0015	0.0008	0.0051
Day 2	0.2289	0.0248	0.0070	0.0049	0.0008	0.0179	0.1098	0.0207	0.0027	0.0019	0.0010	0.0065
Day 3	0.2041	0.0235	0.0000	NA	NA	NA	0.0982	0.0200	0.0004	0.0006	0.0000	0.0019
Day 4	0.2182	0.0242	0.0011	0.0018	0.0000	0.0069	0.0997	0.0199	0.0005	0.0007	0.0000	0.0019
Day 5	0.2179	0.0238	0.0001	0.0011	0.0000	0.0043	0.0904	0.0185	0.0002	0.0004	0.0000	0.0011
Day 6	0.2233	0.0243	0.0040	0.0031	0.0008	0.0104	0.0881	0.0181	0.0003	0.0006	0.0000	0.0017
(b) Duration												
Day 1	0.2039	0.0218	0.0045	0.0036	0.0009	0.0145	0.0849	0.0188	0.0017	0.0014	0.0004	0.0048
Day 2	0.1574	0.0191	0.0162	0.0078	0.0054	0.0307	0.0838	0.0185	0.0017	0.0015	0.0002	0.0057
Day 3	0.1167	0.0166	0.0000	NA	NA	NA	0.0894	0.0187	0.0000	0.0004	0.0000	0.0007
Day 4	0.1715	0.0195	0.0020	0.0024	0.0000	0.0119	0.0833	0.0179	0.0000	NA	NA	NA
Day 5	0.1779	0.0195	0.0009	0.0015	0.0000	0.0079	0.0862	0.0176	0.0002	0.0006	0.0000	0.0016
Day 6	0.1666	0.0189	0.0149	0.0070	0.0059	0.0277	0.0792	0.0165	0.0003	0.0007	0.0000	0.0019
(c) Speed												
Day 1	0.3644	0.0316	0.0000	0.0005	0.0000	0.0019	0.1618	0.0238	0.0016	0.0013	0.0004	0.0042
Day 2	0.3595	0.0320	0.0003	0.0011	0.0000	0.0033	0.1667	0.0240	0.0019	0.0014	0.0006	0.0046
Day 3	0.3553	0.0320	0.0001	0.0005	0.0000	0.0023	0.1584	0.0231	0.0011	0.0010	0.0002	0.0032
Day 4	0.3264	0.0310	0.0003	0.0009	0.0000	0.0034	0.1805	0.0236	0.0011	0.0010	0.0002	0.0031
Day 5	0.3222	0.0307	0.0006	0.0011	0.0000	0.0034	0.1778	0.0223	0.0006	0.0007	0.0000	0.0024
Day 6	0.3259	0.0309	0.0006	0.0013	0.0000	0.0037	0.1726	0.0219	0.0011	0.0010	0.0002	0.0033

shown in Table S2 for running duration and Table S3 for average running speed.

Discussion

We tested for the presence of IGEs on voluntary wheel-running behavior that might occur when mice are tested in wheel enclosures that were previously occupied by a conspecific. It is worth emphasizing that these IGEs are doubly indirect because (1) they come from other individuals (as in the definition of IGEs) and (2) no direct physical interaction with those individuals ever occurs—only odors from urine and feces are encountered by the focal animal. This is different from the commonly-considered case of conspecific aggressive interactions (e.g., Wilson et al. 2009). In the present study system, it is presumed that the effects detected occurred through variation in the odors (amount and/or composition) left in wheels.

Overall, we found that mice generally ran less on previously occupied wheels than on clean wheels. Interestingly, the sex of the previous mouse affected the running distance and speed of the subsequent mouse on most days, with relatively little effect on running duration (Fig. 2). We also found that the sex effect on running distance (and speed) were larger in mice from the selectively bred HR lines than control lines (Figs. 2, S1), implying that the sensitivity to conspecific odors has *increased* as a correlated response to selection on wheel running. Simultaneously, we detected significant indirect additive genetic variance for wheel-running distance, with considerably higher estimates for the first two of six test days. In contrast to the sex effects, the IGE variance components were relatively strong for time spent running but very small for average running speed. Hence, the wheel-running distance of house mice in this study system can be affected in two independent ways by the mouse that previously occupied its wheel enclosure: (1) through sex-specific, non-heritable variation in odors that affect

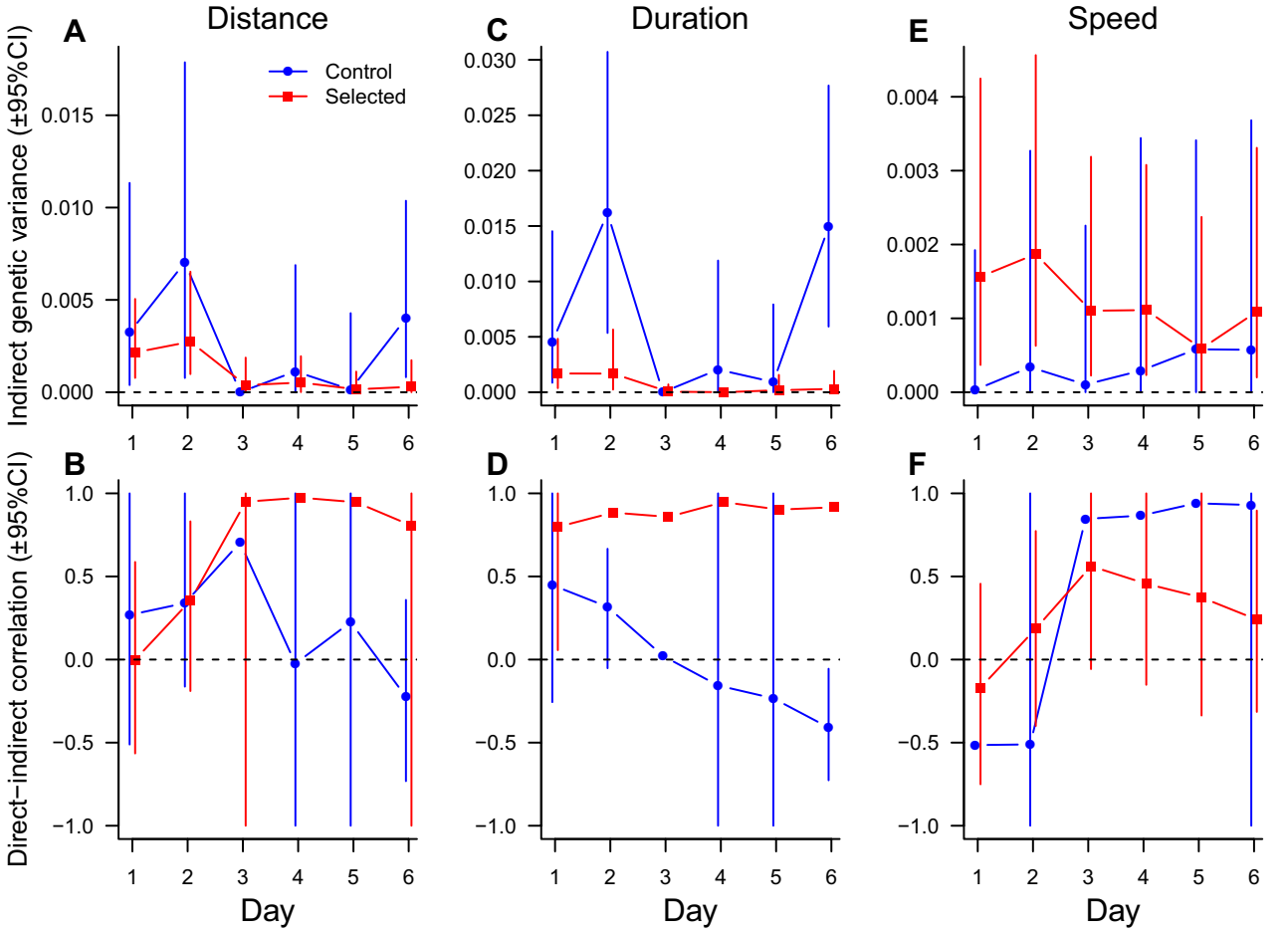

Fig. 3 Indirect genetic variance ($\sigma^2_{AP} \pm 95\%$ CI; top row) and its correlation with direct genetic variance ($r_{AF,AP} \pm 95\%$ CI; bottom row) in voluntary wheel-running behavior, including **a, b** running distance, **c, d** running duration, and **e, f** average running speed across six consecutive days. Variance components and correlations were modelled separately in control (red circles) and selected (blue squares) mice. All traits were standardized to a mean of 0 and a variance of 1 for each generation and separately for control and selected mice, such that the estimates are comparable across days and traits. (Color figure online)

mostly running speed, and (2) through heritable variation in the odors left in wheels that affect mostly running duration.

Drickamer and Evans (1996) found that the presence of urine chemosignals generally increased wheel running in wild-derived house mice, whereas mice in the present experiment ran less when there had been a previous mouse occupying the wheel than in clean wheels. In Drickamer and Evans (1996), the samples of urine were placed in the attached home cage and the wheels were clean, whereas in the present experiment, cages were clean and wheels were not. Drickamer and Evans (1996) suggest that at least some of the increase in wheel running can be explained due to avoidance of another mouse. In the selection experiment, in order for a mouse to avoid the scent it must avoid the wheel, whereas in Drickamer and Evans (1996), a mouse avoiding the scent would avoid the cage, and thus probably spend more time in the wheel. Similarly, Vargas-Pérez et al. (2009) reported that male Balb/c mice adjust wheel-running behavior to avoid potential conflict with other individuals: subordinate mice ran less in the presence of a dominant mouse than on their own, but only when provided with a single wheel; when provided with two wheels, subordinate mice ran more than their dominant cage-mate. Hence, our results for wheel-running distance concur with those of Drickamer and Evans (1996) and Vargas-Pérez et al. (2009) and are consistent with the idea that access to and amount of wheel running are modulated by social interactions, specifically that mice might avoid a wheel that has been previously used. However, analysis of the underlying components is more complicated, because one would expect that avoidance effects might manifest themselves more through wheel-running duration than speed. Indeed, the sex of the previous mouse only significantly influenced speed, whereas most of the IGEs detected occurred through duration.

Given that odors from other mice can affect wheel-running behavior, and more specifically can suppress wheel

Table 3 Correlation between direct and indirect additive genetic variance ($r_{AF,\,AP} \pm$ SE) in voluntary wheel-running (a) distance, (b) duration, and (c) speed, quantified over six consecutive days in a total of 11,420 control and 26,575 selected mice. Also shown are the 95% CI for σ^2_{AP} calculated using profile likelihoods. NAs indicate that the correlation estimate was fixed at a boundary of the parameter space (i.e., −1 or 1)

	Control mice				Selected mice			
	$r_{AF,\,AP}$	SE	95% CI		$r_{AF,\,AP}$	SE	95% CI	
			Lower	Upper			Lower	Upper
(a) Distance								
Day 1	0.2683	0.5130	−0.5112	1.0000	−0.0008	0.4765	−0.5649	0.5872
Day 2	0.3396	0.3556	−0.1630	1.0000	0.3544	0.4223	−0.1896	0.8317
Day 3	0.7039	NA	NA	NA	0.9497	0.8168	−1.0000	1.0000
Day 4	−0.0247	0.6853	−1.0000	1.0000	0.9743	NA	NA	NA
Day 5	0.2249	1.9885	−1.0000	1.0000	0.9464	NA	NA	NA
Day 6	−0.2241	0.4274	−0.7318	0.3590	0.8060	0.9704	−1.0000	1.0000
(b) Duration								
Day 1	0.4481	0.4509	−0.2562	1.0000	0.7982	0.4593	0.0566	1.0000
Day 2	0.3172	0.2601	−0.0521	0.6666	0.8843	NA	NA	NA
Day 3	0.0225	NA	NA	NA	0.8585	NA	NA	NA
Day 4	−0.1562	0.5198	−1.0000	1.0000	0.9477	NA	NA	NA
Day 5	−0.2334	0.7446	−1.0000	1.0000	0.9024	NA	NA	NA
Day 6	−0.4091	0.2498	−0.7266	−0.0554	0.9163	NA	NA	NA
(c) Speed								
Day 1	−0.5146	NA	NA	NA	−0.1695	0.4828	−0.7507	0.4561
Day 2	−0.5108	1.5852	−1.0000	1.0000	0.1856	0.4690	−0.3995	0.7735
Day 3	0.8463	NA	NA	NA	0.5621	0.4889	−0.0573	1.0000
Day 4	0.8663	NA	NA	NA	0.4556	0.4816	−0.1519	1.0000
Day 5	0.9402	NA	NA	NA	0.3734	0.6144	−0.3367	1.0000
Day 6	0.9300	1.1884	−1.0000	1.0000	0.2406	0.4851	−0.3150	0.8965

running in the context of the present selection experiment, it is of considerable interest that a recent genome-wide single nucleotide polymorphism (SNP) association study detected SNPs related to vomeronasal organ genes that are significantly differentiated in frequency between HR and control lines at generation 61 (Xu and Garland 2017). One possible explanation is that sensitivity to conspecific scents via the vomeronasal organ is also a heritable trait that evolved as a correlated response to selection for high voluntary wheel-running behavior. Indeed, if mice that are less sensitive to the smell of conspecifics are less suppressed from entering the wheel on the initial day, then training effects could "snow ball" into major differences during the following days, thus increasing the likelihood of being selected based on wheel running on days 5 & 6. This is inconsistent, however, with the presence of a significant interaction between selection treatment of the focal mouse and sex of the previous mouse (Table 1e), which indicates that, compared to control mice, wheel running in selected mice is more strongly influenced by the sex of the previous mouse. One would expect the opposite (i.e., if alleles reducing sensitivity are favored in selected mice, it should translate to less inhibition of wheel running). More experiments are needed to verify if changes in vomeronasal organ allele frequencies detected in mice of this selection experiment are related to the inhibitory effects of the scents left by the previous mouse occupying the wheel enclosure.

Although we found evidence of IGEs on daily wheel-running distance, the variance components associated with IGEs were perhaps only large enough to be biologically relevant on days 1 and 2 (Fig. 3a). That IGEs would only be present on the first 2 days could be explained by the dissipation of scent signals of the previous mouse after 2 days; however, this is rendered less plausible by the fact that there is no such diminution in the effect of the sex of the previous mouse (Table 1a). Alternatively, the IGEs detected as variance component in running distance (Table 2) could simply be most important in the initial/exploratory/acclimatory phase of wheel running. A previous study on the first 31 generations of the current experiment found that wheel-running distance on different days are all positively genetically correlated, but the genetic correlations can be substantially smaller than 1, especially between days 1–2 versus 5–6 (Careau et al. 2015). Such differences in the underlying quantitative genetic architecture of wheel running across days were hypothesized to be related to anxiety or fear upon initial exposure to a novel environment (i.e., wheel attached to the home cage). The factors related to this initial exposure were proposed to be changes in housing (in same-sex group vs. alone) and/or exposure to the new environment (the wheel enclosure), to which we add chemosignals left by previous

mice occupying the wheel for the majority of individuals in the current experiment.

The magnitude of the indirect genetic variance was relatively small: it accounted for only ~ 1–2% of the total phenotypic variation (Tables S1, S2, S3). However, one should not conclude that IGEs are unimportant for voluntary wheel running in this system. Indeed, the indirect genetic variance components for running duration were up to ~ 10% of the direct additive genetic variance (Table 2). Moreover, it was possible to detect IGEs in this dataset, despite mice being able to interact only by scent (and possibly the physical presence of dried urine or feces in the wheels), which suggests that IGEs may be an important contributor to variation in physical activity when mice are able to interact more directly, as in wild populations. Future experimental work to elucidate the mechanism and importance of IGEs on activity in familiar versus novel environments could involve experimental manipulations with more possibility of interactions between individuals (e.g., housing mice with access to a common wheel) or applying urine to wheels.

Our findings eliminate IGEs as a potential explanation for the selection limits observed in HR lines (Careau et al. 2013; see also Careau et al. 2015). To constrain response to selection, the correlation between direct and indirect genetic variance ($r_{AF, AP}$) would have to be negative (e.g., see Wilson et al. 2011), so that selecting for high running distance would cause HR mice to increasingly suppress wheel running in the subsequent mice (including other HR mice). In the current study, however, the correlation was never statistically significant for running distance (Table 3), meaning there is no genetic correlation between the trait of running distance itself and the indirect genetic effects. This result is also supported by the absence of an effect of the selection treatment of the previous mouse on wheel running (Table 1b).

Our findings also provide new opportunities for the study of IGEs by showing that they are present in an existing, well-studied model system. The confirmation of IGEs on wheel running expands our knowledge of and suggests new avenues for research on IGEs occurring through sequence effects. Sequence effects may be particularly important in the wild, for example through scent left behind at a foraging site, or through succession of territory (i.e., through the previous owner of a territory) or nesting site. In conclusion, we suggest that examining IGEs occurring through sequential scent marking may be a promising line of research in behavioral genetics.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflicts of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Statement of human and animal rights This article does not contain any studies with human participants performed by any of the authors.

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Table S1. Variance component estimates (±se) extracted from univariate mixed models of voluntary wheel-running **distance** on six consecutive days, including direct common (maternal) environmental variance (σ^2_{CF}), indirect common (maternal) environmental variance (σ^2_{CP}), direct additive genetic variance (σ^2_{AF}), indirect additive genetic variance (σ^2_{AP}), correlation between σ^2_{AF} and σ^2_{AP} ($r_{AF, AP}$), and residual variance (σ^2_e), all fitted separately for control and selected mice. All traits were standardized to a mean of 0 and a variance of 1 for each generation, such that the estimates represent the proportion of total phenotypic variance.

Day	Component	Control mice		Selected mice	
		Estimate	SE	Estimate	SE
1	σ^2_{CF}	0.0858	0.0089	0.1037	0.0070
1	σ^2_{CP}	0.0000	NA	0.0000	NA
1	$r_{AF, AP}$	0.2683	0.5130	-0.0008	0.4765
1	σ^2_{AF}	0.2580	0.0259	0.0996	0.0198
1	σ^2_{AP}	0.0032	0.0030	0.0022	0.0015
1	σ^2_e	0.6319	0.0127	0.6915	0.0091
2	σ^2_C	0.0758	0.0085	0.1121	0.0075
2	σ^2_{CP}	0.0000	NA	0.0113	0.0063
2	$r_{AF, AP}$	0.3396	0.3556	0.3544	0.4223
2	σ^2_{AF}	0.2289	0.0248	0.1098	0.0207
2	σ^2_{AP}	0.0070	0.0049	0.0027	0.0019
2	σ^2_e	0.6250	0.0125	0.6587	0.0091
3	σ^2_C	0.0720	0.0087	0.1106	0.0075
3	σ^2_{CP}	0.0003	0.0092	0.0104	0.0061
3	$r_{AF, AP}$	0.7039	NA	0.9497	0.8168
3	σ^2_{AF}	0.2041	0.0235	0.0982	0.0200
3	σ^2_{AP}	0.0000	NA	0.0004	0.0006
3	σ^2_e	0.6422	0.0128	0.6764	0.0092
4	σ^2_C	0.0795	0.0085	0.1071	0.0075
4	σ^2_{CP}	0.0000	NA	0.0180	0.0065
4	$r_{AF, AP}$	-0.0247	0.6853	0.9743	NA
4	σ^2_{AF}	0.2182	0.0242	0.0997	0.0199
4	σ^2_{AP}	0.0011	0.0018	0.0005	0.0007
4	σ^2_e	0.6283	0.0123	0.6990	0.0094
5	σ^2_C	0.0746	0.0084	0.1100	0.0076
5	σ^2_{CP}	0.0000	NA	0.0231	0.0068
5	$r_{AF, AP}$	0.2249	1.9885	0.9464	NA
5	σ^2_{AF}	0.2179	0.0238	0.0904	0.0185
5	σ^2_{AP}	0.0001	0.0011	0.0002	0.0004
5	σ^2_e	0.6351	0.0124	0.7077	0.0093
6	σ^2_C	0.0715	0.0084	0.1064	0.0074
6	σ^2_{CP}	0.0000	NA	0.0119	0.0064
6	$r_{AF, AP}$	-0.2241	0.4274	0.8060	0.9704
6	σ^2_{AF}	0.2233	0.0243	0.0881	0.0181
6	σ^2_{AP}	0.0040	0.0031	0.0003	0.0006
6	σ^2_e	0.6361	0.0125	0.7149	0.0093

Table S2. Variance component estimates (±se) extracted from univariate mixed models of voluntary wheel-running **duration** on six consecutive days, including direct common (maternal) environmental variance (σ^2_{CF}), indirect common (maternal) environmental variance (σ^2_{CP}), direct additive genetic variance (σ^2_{AF}), indirect additive genetic variance (σ^2_{AP}), correlation between σ^2_{AF} and σ^2_{AP} ($r_{AF, AP}$), and residual variance (σ^2_e), all fitted separately for control and selected mice. All traits were standardized to a mean of 0 and a variance of 1 for each generation, such that the estimates represent the proportion of total phenotypic variance.

Day	Component	Control mice		Selected mice	
		Estimate	SE	Estimate	SE
1	σ^2_{CF}	0.0810	0.0080	0.1021	0.0071
1	σ^2_{CP}	0.0159	0.0087	0.0106	0.0064
1	$r_{AF, AP}$	0.4481	0.4509	0.7982	0.4593
1	σ^2_{AF}	0.2039	0.0218	0.0849	0.0188
1	σ^2_{AP}	0.0045	0.0036	0.0017	0.0014
1	σ^2_e	0.5343	0.0111	0.7057	0.0094
2	σ^2_C	0.0601	0.0071	0.0885	0.0068
2	σ^2_{CP}	0.0144	0.0084	0.0271	0.0069
2	$r_{AF, AP}$	0.3172	0.2601	0.8843	NA
2	σ^2_{AF}	0.1574	0.0191	0.0837	0.0185
2	σ^2_{AP}	0.0162	0.0078	0.0017	0.0015
2	σ^2_e	0.5130	0.0104	0.6844	0.0092
3	σ^2_C	0.0554	0.0070	0.0800	0.0066
3	σ^2_{CP}	0.0187	0.0080	0.0256	0.0068
3	$r_{AF, AP}$	0.0225	NA	0.8585	NA
3	σ^2_{AF}	0.1166	0.0166	0.0898	0.0188
3	σ^2_{AP}	0.0000	NA	0.0000	0.0004
3	σ^2_e	0.5341	0.0101	0.6950	0.0093
4	σ^2_C	0.0636	0.0071	0.0805	0.0066
4	σ^2_{CP}	0.0101	0.0078	0.0258	0.0068
4	$r_{AF, AP}$	-0.1562	0.5198	0.9477	NA
4	σ^2_{AF}	0.1715	0.0195	0.0833	0.0179
4	σ^2_{AP}	0.0020	0.0024	0.0000	NA
4	σ^2_e	0.5041	0.0102	0.7047	0.0092
5	σ^2_C	0.0547	0.0068	0.0829	0.0067
5	σ^2_{CP}	0.0125	0.0077	0.0280	0.0068
5	$r_{AF, AP}$	-0.2334	0.7446	0.9024	NA
5	σ^2_{AF}	0.1780	0.0195	0.0873	0.0177
5	σ^2_{AP}	0.0009	0.0015	0.0002	0.0006
5	σ^2_e	0.5001	0.0102	0.6974	0.0092
6	σ^2_C	0.0511	0.0066	0.0816	0.0065
6	σ^2_{CP}	0.0018	0.0076	0.0228	0.0065
6	$r_{AF, AP}$	-0.4091	0.2498	0.9163	NA
6	σ^2_{AF}	0.1667	0.0189	0.0807	0.0167
6	σ^2_{AP}	0.0149	0.0070	0.0003	0.0007
6	σ^2_e	0.5013	0.0102	0.6990	0.0091

Table S3. Variance component estimates (±se) extracted from univariate mixed models of average voluntary wheel-running **speed** on six consecutive days, including direct common (maternal) environmental variance (σ^2_{CF}), indirect common (maternal) environmental variance (σ^2_{CP}), direct additive genetic variance (σ^2_{AF}), indirect additive genetic variance (σ^2_{AP}), correlation between σ^2_{AF} and σ^2_{AP} ($r_{AF, AP}$), and residual variance (σ^2_e), all fitted separately for control and selected mice. All traits were standardized to a mean of 0 and a variance of 1 for each generation, such that the estimates represent the proportion of total phenotypic variance.

Day	Component	Control mice		Selected mice	
		Estimate	SE	Estimate	SE
1	σ^2_{CF}	0.0685	0.0094	0.0968	0.0070
1	σ^2_{CP}	0.0072	0.0104	0.0000	NA
1	$r_{AF, AP}$	-0.5146	NA	-0.1695	0.4828
1	σ^2_{AF}	0.3644	0.0316	0.1619	0.0238
1	σ^2_{AP}	0.0000	0.0005	0.0016	0.0013
1	σ^2_e	0.6744	0.0147	0.6864	0.0097
2	σ^2_C	0.0711	0.0093	0.1058	0.0074
2	σ^2_{CP}	0.0000	NA	0.0037	0.0059
2	$r_{AF, AP}$	-0.5108	1.5852	0.1856	0.4690
2	σ^2_{AF}	0.3595	0.0320	0.1667	0.0240
2	σ^2_{AP}	0.0003	0.0011	0.0019	0.0014
2	σ^2_e	0.6920	0.0146	0.6487	0.0096
3	σ^2_C	0.0611	0.0092	0.1037	0.0073
3	σ^2_{CP}	0.0000	NA	0.0054	0.0058
3	$r_{AF, AP}$	0.8463	NA	0.5621	0.4889
3	σ^2_{AF}	0.3551	0.0320	0.1582	0.0231
3	σ^2_{AP}	0.0001	0.0005	0.0011	0.0010
3	σ^2_e	0.7157	0.0150	0.6443	0.0094
4	σ^2_C	0.0667	0.0094	0.0920	0.0069
4	σ^2_{CP}	0.0000	NA	0.0034	0.0057
4	$r_{AF, AP}$	0.8663	NA	0.4556	0.4816
4	σ^2_{AF}	0.3259	0.0309	0.1801	0.0236
4	σ^2_{AP}	0.0003	0.0009	0.0011	0.0010
4	σ^2_e	0.7260	0.0149	0.6392	0.0094
5	σ^2_C	0.0718	0.0094	0.0875	0.0067
5	σ^2_{CP}	0.0000	NA	0.0055	0.0057
5	$r_{AF, AP}$	0.9402	NA	0.3734	0.6144
5	σ^2_{AF}	0.3223	0.0307	0.1779	0.0223
5	σ^2_{AP}	0.0006	0.0011	0.0006	0.0007
5	σ^2_e	0.7228	0.0148	0.6305	0.0091
6	σ^2_C	0.0717	0.0095	0.0865	0.0067
6	σ^2_{CP}	0.0000	NA	0.0033	0.0057
6	$r_{AF, AP}$	0.9300	1.1884	0.2406	0.4851
6	σ^2_{AF}	0.3259	0.0309	0.1726	0.0219
6	σ^2_{AP}	0.0006	0.0013	0.0011	0.0010
6	σ^2_e	0.7215	0.0148	0.6318	0.0091

Fig. S1 Same as Fig 2 without the z-transformation. Voluntary wheel-running **A** distance, **B** duration, and **C** speed in focal male (triangles) and female (circles) mice from control lines (C, blue) and high-runner lines (HR, red) as a function of the sex of the mouse previously occupying the same wheel (clean wheel, female, or male). Wheel running behavior was measured over six consecutive days (each shown in a separate panel). Symbols denote the average and the 95% confidence intervals (CI) of distance (number of wheel revolutions), duration (number of 1-min intervals with at least one revolution), and speed (the average number of revolutions in active 1-min intervals).

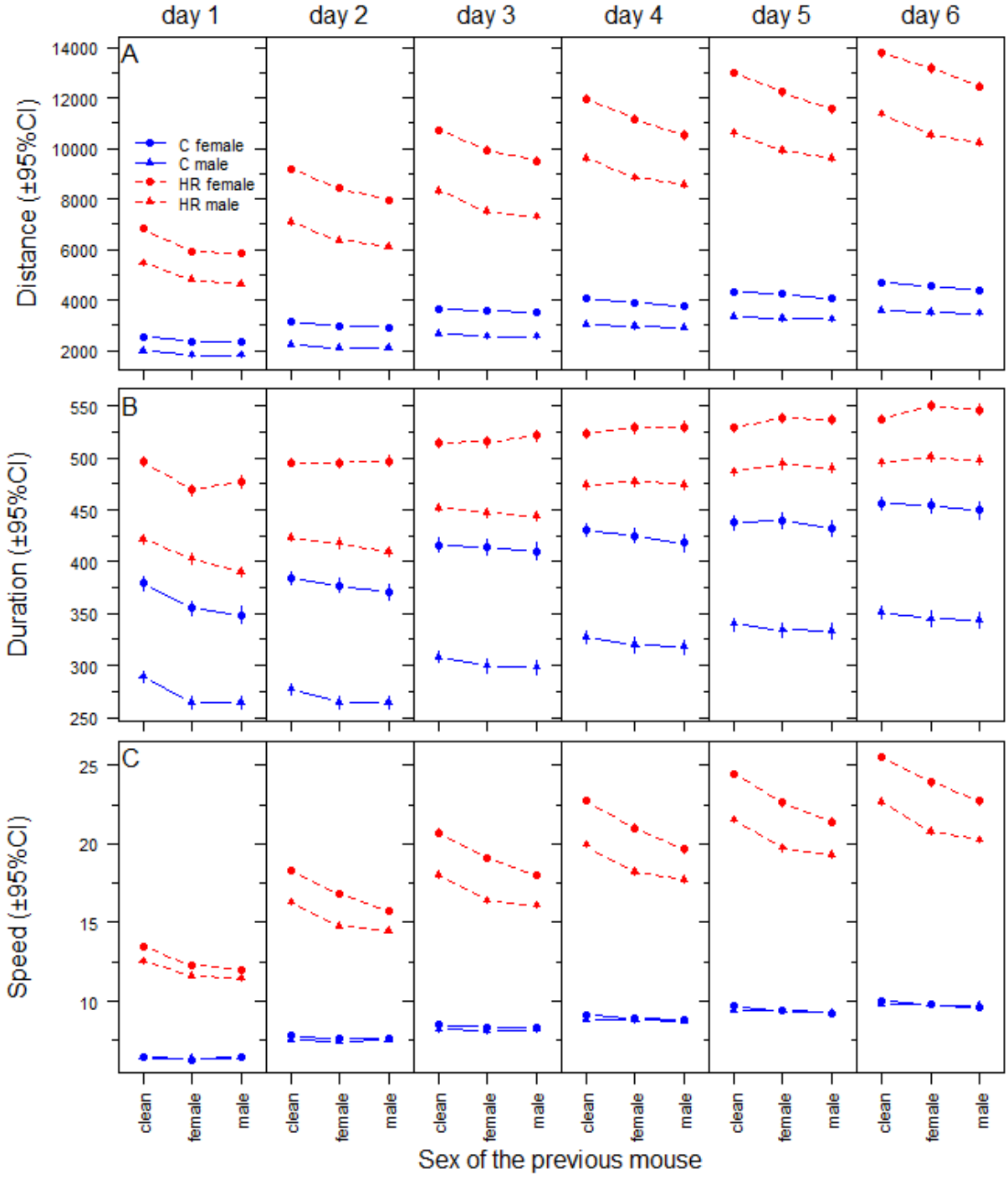

Fig. S2 Same as Fig. S1, showing the effect of the linetype (instead of sex) of the previous mouse. Voluntary wheel-running **A** distance, **B** duration, and **C** speed in focal males (triangles) and females (circles) from control lines (C, blue) and high-runner lines (HR, red) as a function of the *linetype* of the mouse previously occupying the same wheel (clean wheel, control, or selected). Wheel running behavior was measured over six consecutive days (each shown in a separate panel). Symbols denote the average and the 95% confidence intervals (CI) of distance (number of wheel revolutions), duration (number of 1-min intervals with at least one revolution), and speed (the average number of revolutions in active 1-min intervals).