I smell a mouse: indirect genetic effects on voluntary wheel-running distance, duration and speed

Ian Dewan[1,2,*], Theodore Garland, Jr.[3], Layla Hiramatsu[3,4], Vincent Careau[1,5]

[1]Department of Biology, University of Ottawa, Ottawa, ON, Canada

[2]Present address: Department of Biology, Carleton University, Ottawa, ON, Canada

[3]Department of Evolution, Ecology, and Organismal Biology, University of California, Riverside, California, USA

[4]Present address: Friedrich Miescher Laboratory of the Max Planck Society, Tübingen, Germany.

[5]Canada Research Chair in Functional Ecology

* Author for correspondence:

idewa069@uottawa.ca

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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 six 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 ae easy to comprehend in the context of aggressive interactions between conspecifics because the behaviour of a focal individual will likely depend on the behavior of the individual with whom it interacts (Wilson et al. 2009b).

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, 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. 2014; Wilson et al. 20wilson 200909b; Wilson et al. 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. 2009b). 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

84 running (e.g., Drickamer and Evans 1996), heritable indirect effects on wheel-running distance,
85 duration, and speed have not been considered.

86 In this study, we tested whether individual variation in voluntary wheel running can be
87 affected by indirect genetic effects using a large dataset from a long-term artificial selection
88 experiment on voluntary wheel running in house mice (*Mus musculus*) (Swallow et al. 1998;
89 Swallow et al. 2009; Wallace and Garland 2016; Garland et al. 2016). Because mice were wheel-
90 tested in batches (see Methods below), a mouse's wheel running could be affected by the
91 previous mouse measured in the same wheel enclosure in a prior batch within a given generation.
92 Using the data for the first 78 generations of the experiment, we examined how wheel running of
93 focal mice was influenced by attributes of the preceding mouse, specifically sex and selection
94 treatment (i.e., whether the mouse was from one of the four non-selected control lines or from
95 one of the four lines bred for high wheel running). We used a type of mixed model known as an
96 "animal model" (Henderson 1973; Lynch and Walsh 1998; Wilson et al. 2010) to integrate
97 pedigree and phenotypic information and estimate such key parameters as direct additive genetic
98 variance, indirect additive genetic variance, and their covariance (or correlation). Finally, we
99 replicated our analyses for time spent running (duration) and average running speed, the two
100 components of distance run as measured in the current experiment.
101
102 **Methods**
103 *Selection experiment*
104 The experiment, ongoing since 1993 (Swallow et al. 1998), used eight lines of mice bred from
105 224 outbred Hsd:ICR mice, divided into four non-selected control lines and four lines selected
106 for high wheel running (HR, for "high-runners"). Wheel running was measured over six days,

and the selected trait was the average number of 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 a plateau 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; Careau et al. 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 six days for each mouse. Time spent (duration) running 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 six 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 four days added in Careau et al. (2015). Here, we added data on all six days (one to six) for generations 35 to 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 four days). We also deleted all data from generation 0 to 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 six 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. Note that 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% (2,781) 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 to 6) was standardized to z-scores (mean=0, sd=1) separately in control and HR lines within each generation. This standardisation 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 models 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 such key parameters as direct additive genetic variance, indirect additive genetic variance, and their covariance (or correlation). Separate models were run for each day, because the six 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 six 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 that 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:

$$y_{ki} = \mu + \text{fixed effects} + ce_{Fi} + ce_{Pj} + a_{Fi} + a_{Pj} + \text{Cor}(a_F, a_P) + e$$

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 wheel running, fixed effects are described below, and e the residual error (see also Fig 1). The random effects included were ce_{Fi}, the identity of the focal mouse's dam (i.e., common environment); ce_{Pi}, the identity of the previous mouse's dam (i.e., common indirect environment); a_{Fi}, the additive genetic contribution of the focal mouse (i.e., direct 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_{Pi}, 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 the `proLik()` function the nadiv 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 data set (Careau et al. 2013; Careau et al. 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 vs 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% confidence intervals 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; Kelly et al. 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 (Fig. 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 day 1 (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

244 previous and focal mouse was only a significant on days 1 and 3 (Table 1C). All of the
245 significant interactions detected for running distance (see previous section) were also present for
246 average running speed, with even stronger effect sizes (Table 1). Mice ran at a slower speed on
247 previously occupied wheels than clean wheels (Fig 2C, S1, S2), and the effect was more
248 pronounced in females than males (Fig 2C; dots vs triangles), and in HR than control mice (Fig
249 2C, red vs blue symbols).

250 For running duration, estimates for indirect genetic variance (σ^2_{AP}) were considerably
251 higher for days 1-2 than days 3-6, and relatively similar to σ^2_{AP} estimates for running distance
252 (Table 2B; Fig 3C). By contrast, σ^2_{AP} were much smaller for average running speed (Table 2C;
253 Fig 3E). In the selected lines, the correlation between direct and indirect genetic variance ($r_{AF, AP}$)
254 was significant and positive for running duration on days 1-2 (Table 3B; Fig 3D). The $r_{AF, AP}$ was
255 not significant on any day for average running speed (Table 3C; Fig 3F). Estimates of the other
256 components of phenotypic variance (common environment, direct additive genetic, and residual
257 variance) are shown in Table S2 for running duration and Table S3 for average running speed.
258

259 **Discussion**

260 We tested for the presence of IGEs on voluntary wheel-running behavior that might occur when
261 mice are tested in wheel enclosures that were previously occupied by a conspecific. It is worth
262 emphasizing that these IGEs are doubly indirect because (1) they come from other individuals
263 (as in the definition of IGEs) and (2) no direct interaction with those individuals ever occurs –
264 only scents and odors from urine and feces are encountered by the focal animal. This is different
265 from the commonly-considered case of conspecific aggressive interactions (e.g., Wilson et al.

2009b). 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 (Fig 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 mostly running speed; 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. These contrasting results may be explained by methodological differences: 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. Given that Drickamer and Evans (1996) suggest that at least some of the increase in wheel running can be explained

289 due to avoidance of another mouse, it might be expected that the opposite results would have
290 been obtained in the present study. In the selection experiment, in order for a mouse to avoid the
291 scent it must avoid the wheel, whereas in Drickamer and Evans (1996), a mouse avoiding the
292 scent would avoid the cage, and thus probably spend more time in the wheel. Similarly, Vargas-
293 Pérez et al. (2009) reported that male Balb/c mice attempt to avoid potential conflict with other
294 individuals that they detect through urine or fecal matter, and are more prone to avoiding males
295 than females. Subordinate mice ran less in the presence of a dominant mouse than on their own,
296 but only when provided with a single wheel: when provided with two wheels, subordinate mice
297 ran more than their dominant cage-mate. Hence, our results for wheel-running distance concur
298 with those of Drickamer and Evans (1996) and Vargas-Pérez et al. (2009) and are consistent with
299 the idea that access to and amount of wheel running are modulated by social interactions, and it
300 is plausible that mice might avoid a wheel that has been previously used. However, analysis of
301 the underlying components is more complicated, because one would expect that avoidance
302 effects might manifest themselves more through wheel-running duration than speed. Indeed, the
303 sex of the previous mouse only significantly influenced speed, whereas most of the IGEs
304 detected occurred through duration.
305 Given that odors from other mice can affect wheel-running behavior, and more
306 specifically can suppress wheel running in the context of the present selection experiment, it is of
307 considerable interest that a recent genome-wide single nucleotide polymorphism (SNP)
308 association study detected SNPs related to vomeronasal organ genes that are significantly
309 differentiated in frequency between HR and control lines at generation 61 (Xu and Garland
310 2017). One possible explanation is that sensitivity to conspecific scents via the vomeronasal
311 organ is also a heritable trait that evolved as a correlated response to selection for high voluntary

312 wheel-running behavior. Indeed, if mice that are less sensitive to the smell of conspecifics are
313 less suppressed from entering the wheel on the initial day, then training effects could "snow ball"
314 into major differences during the following days, thus increasing the likelihood of being selected
315 based on wheel running on days 5&6. This is inconsistent, however, with the presence of a
316 significant interaction between selection treatment of the focal mouse and sex of the previous
317 mouse (Table 1E), which indicates that, compared to control mice, wheel running in selected
318 mice is more strongly influenced by the sex of the previous mouse. One would expect the
319 opposite (i.e., if alleles reducing sensitivity are favored in selected mice, it should translate to
320 less inhibition of wheel running). More experiments are needed to verify if changes in
321 vomeronasal organ gene frequencies detected in the current selection experiment are related to
322 the inhibitory effects of the scents left by the previous mouse occupying the wheel enclosure.
323 Although we found evidence of IGEs on daily wheel-running distance, the variance
324 components associated with IGEs were perhaps only large enough to be biologically relevant on
325 days 1 and 2 (Fig 3A). That IGEs would only be present on the first two days could be explained
326 by the dissipation of scent signals of the previous mouse after two days; however, this is
327 rendered less plausible by the fact that there is no such diminution in the effect of the sex of the
328 previous mouse (Table 1A). Alternatively, the IGEs detected as variance component in running
329 distance (Table 2) could simply be most important in the initial/exploratory/acclimatory phase of
330 wheel running. A previous study on the first 31 generations of the current experiment found that
331 wheel-running distance on different days are all positively genetically correlated, but the genetic
332 correlations can be substantially smaller than 1, especially between days 1-2 vs 5-6 (Careau et al.
333 2015). Such differences in the underlying quantitative genetic architecture of wheel running
334 across days were hypothesized to be related to anxiety or fear upon initial exposure to a novel

335 environment (i.e., wheel attached to the home cage). The factors related to this initial exposure
336 were proposed to be changes in housing (in same-sex group vs. alone) and/or exposure to the
337 new environment (the wheel enclosure), to which we add chemosignals left by previous mice
338 occupying the wheel for the majority of individuals in the current experiment.

339 The magnitude of the indirect genetic variance was relatively small: it accounted for only
340 ~1-2% of the total phenotypic variation (Table S1-S3). However, one should not conclude that
341 IGEs are unimportant for voluntary wheel running in this system. Indeed, the indirect genetic
342 variance components for running duration were up to ~10% of the direct additive genetic
343 variance (Table 2). Moreover, it was possible to detect IGEs in this data set, despite mice being
344 able to interact only by scent (and possibly the physical presence of dried urine or feces in the
345 wheels), which suggests that IGEs may be an important contributor to variation in physical
346 activity when mice are able to interact more directly, as in wild populations. Future experimental
347 work to elucidate the mechanism and importance of IGEs on activity in familiar vs novel
348 environments could involve experimental manipulations with more possibility of interactions
349 between individuals (e.g., housing mice with access to a common wheel) or applying urine to
350 wheels.

351 Our findings eliminate IGEs as a potential explanation for the selection limits observed in
352 HR lines (Careau et al. 2013; see also Careau et al. 2015). To constrain response to selection, the
353 correlation between direct and indirect genetic variance ($r_{AF, AP}$) would have to be negative (e.g.,
354 see Wilson et al. 2011), so that selecting for high running distance would cause HR mice to
355 increasingly suppress wheel running in the subsequent mice (including other HR mice). In the
356 current study, however, the correlation was never statistically significant for running distance
357 (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

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Table 1. Estimates (in units of standard deviations) and 95% confidence intervals (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	0.061	**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	-0.026	-0.126	0.075	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**	0.006	-0.044	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	0.049	-0.055	0.154	-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**	0.003	-0.046	0.053	**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	0.043	-0.011	0.096	0.021	-0.037	0.078

Table 2. Direct genetic variance (σ^2_{AF}±se) and indirect genetic variance (σ^2_{AP}±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% confidence intervals 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 Table S1-3 for all other variance components included in these models.

	Control mice						Selected mice					
					95%CI						95%CI	
	σ^2_{AF}	se	σ^2_{AP}	se	Lower	Upper	σ^2_{AF}	se	σ^2_{AP}	se	Lower	Upper
A) distance												
Day 1	0.2580	0.0259	0.0032	0.0030	0.0006	0.0113	0.0996	0.0198	0.0022	0.0015	0.0009	0.0049
Day 2	0.2289	0.0248	0.0070	0.0049	0.0011	0.0178	0.1098	0.0207	0.0027	0.0019	0.0012	0.0065
Day 3	0.2041	0.0235	0.0000	NA	NA	NA	0.0982	0.0200	0.0004	0.0006	0.0002	0.0019
Day 4	0.2182	0.0242	0.0011	0.0018	0.0005	0.0061	0.0997	0.0199	0.0005	0.0006	0.0002	0.0021
Day 5	0.2179	0.0238	0.0001	0.0011	0.0001	0.0030	0.0904	0.0185	0.0001	0.0004	0.0001	0.0009
Day 6	0.2233	0.0243	0.0040	0.0031	0.0008	0.0104	0.0881	0.0181	0.0003	0.0006	0.0001	0.0018
B) duration												
Day 1	0.2039	0.0218	0.0045	0.0036	0.0009	0.0147	0.0849	0.0188	0.0017	0.0014	0.0007	0.0048
Day 2	0.1574	0.0191	0.0162	0.0078	0.0054	0.0307	0.0838	0.0185	0.0017	0.0015	0.0007	0.0054
Day 3	0.1167	0.0166	0.0000	NA	NA	NA	0.0894	0.0187	0.0000	NA	NA	NA
Day 4	0.1715	0.0195	0.0020	0.0024	0.0005	0.0086	0.0833	0.0179	0.0000	NA	NA	NA
Day 5	0.1779	0.0195	0.0009	0.0014	0.0004	0.0047	0.0862	0.0176	0.0000	NA	NA	NA
Day 6	0.1666	0.0189	0.0150	0.0070	0.0059	0.0276	0.0792	0.0165	0.0000	NA	NA	NA
C) speed												
Day 1	0.3644	0.0316	0.0000	NA	NA	NA	0.1618	0.0238	0.0016	0.0013	0.0007	0.0045
Day 2	0.3595	0.0320	0.0003	0.0011	0.0001	0.0033	0.1667	0.0240	0.0019	0.0014	0.0008	0.0044
Day 3	0.3553	0.0320	0.0000	NA	NA	NA	0.1584	0.0231	0.0011	0.0010	0.0005	0.0033
Day 4	0.3264	0.0310	0.0000	NA	NA	NA	0.1805	0.0236	0.0011	0.0010	0.0005	0.0029
Day 5	0.3222	0.0307	0.0006	0.0010	0.0002	0.0034	0.1778	0.0223	0.0006	0.0007	0.0003	0.0023
Day 6	0.3259	0.0309	0.0006	0.0013	0.0002	0.0036	0.1726	0.0219	0.0011	0.0010	0.0005	0.0034

Table 3. Correlation between direct and indirect additive genetic variance ($r_{AF,\,AP}$±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% confidence intervals 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			
			95%CI				95%CI	
	$r_{AF,\,AP}$	se	Lower	Upper	$r_{AF,\,AP}$	se	Lower	Upper
A) distance								
Day 1	0.2684	0.5131	-0.5112	0.9995	-0.0008	0.4765	-0.5647	0.5872
Day 2	0.3395	0.3552	-0.1630	0.9995	0.3544	0.4223	-0.1897	0.8318
Day 3	1.0000	NA	NA	NA	0.9496	0.8169	-0.4121	0.9993
Day 4	-0.0253	0.6866	-0.7199	0.9994	1.0000	NA	NA	NA
Day 5	0.2246	1.9986	-0.2359	0.9996	1.0000	NA	NA	NA
Day 6	-0.2241	0.4274	-0.7319	0.3589	0.8060	0.9704	-0.6479	0.9994
B) duration								
Day 1	0.4473	0.4504	-0.2565	0.9993	0.7982	0.4594	0.0566	0.9994
Day 2	0.3168	0.2601	-0.0521	0.6666	0.9099	0.4374	0.5849	0.9994
Day 3	1.0000	NA	NA	NA	1.0000	NA	NA	NA
Day 4	-0.1562	0.5228	-0.9995	0.5186	1.0000	NA	NA	NA
Day 5	-0.2124	0.7516	-0.9994	0.7073	1.0000	NA	NA	NA
Day 6	-0.4034	0.2497	-0.7266	-0.0556	1.0000	NA	NA	NA
C) speed								
Day 1	-1.0000	NA	NA	NA	-0.1704	0.4827	-0.7505	0.4562
Day 2	-0.5106	1.5844	-0.9994	0.3798	0.1856	0.4690	-0.3996	0.7734
Day 3	1.0000	NA	NA	NA	0.5657	0.4882	-0.0573	0.9995
Day 4	1.0000	NA	NA	NA	0.4597	0.4805	-0.1520	0.9994
Day 5	1.0000	NA	NA	NA	0.3728	0.6144	-0.3367	0.9996
Day 6	0.9300	1.1882	-0.5271	0.9994	0.2406	0.4851	-0.3149	0.8966

Figure legends

Fig 1 Diagram of the quantitative genetic model fitted to the data (cf. Fig 1 in Wilson et al. 2009b). 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_{Pi}), 3) direct genetic effects (a_{Fi}, which are assumed to be entirely additive in nature: see Methods), 4) indirect genetic effects of the previous (a_{Pj}), and 5) a correlation between direct and indirect genetic effects (Cor(a_F, a_P)).

Fig 2 Voluntary wheel-running A) distance, B) duration, and C) speed in focal male (triangles) and female (dots) 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 behaviour 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 (revolutions/duration) after standardisation 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).

Fig 3 Indirect genetic variance (σ^2_{AP}±se; left panels) and its correlation with direct genetic variance ($r_{AF,AP}$±se; right panels) in voluntary wheel-running behaviour, 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 dots) and selected (blue squared) mice. All traits were standardised 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.

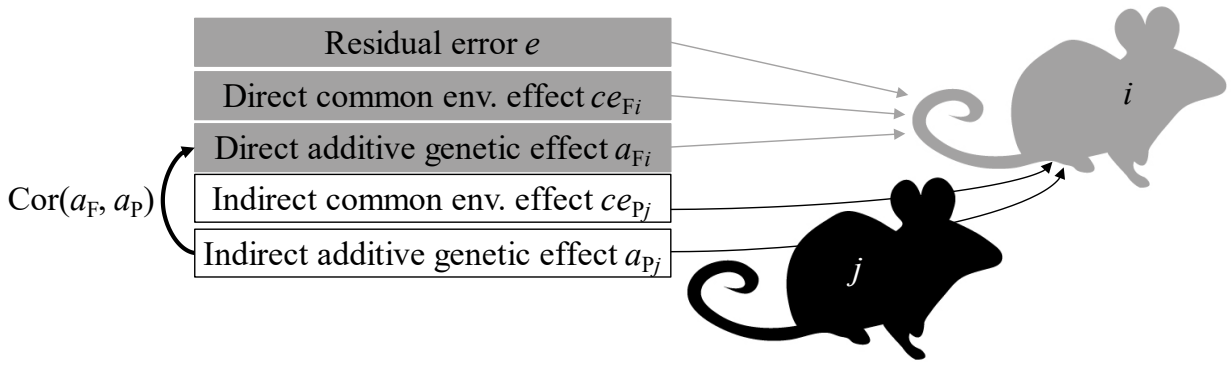
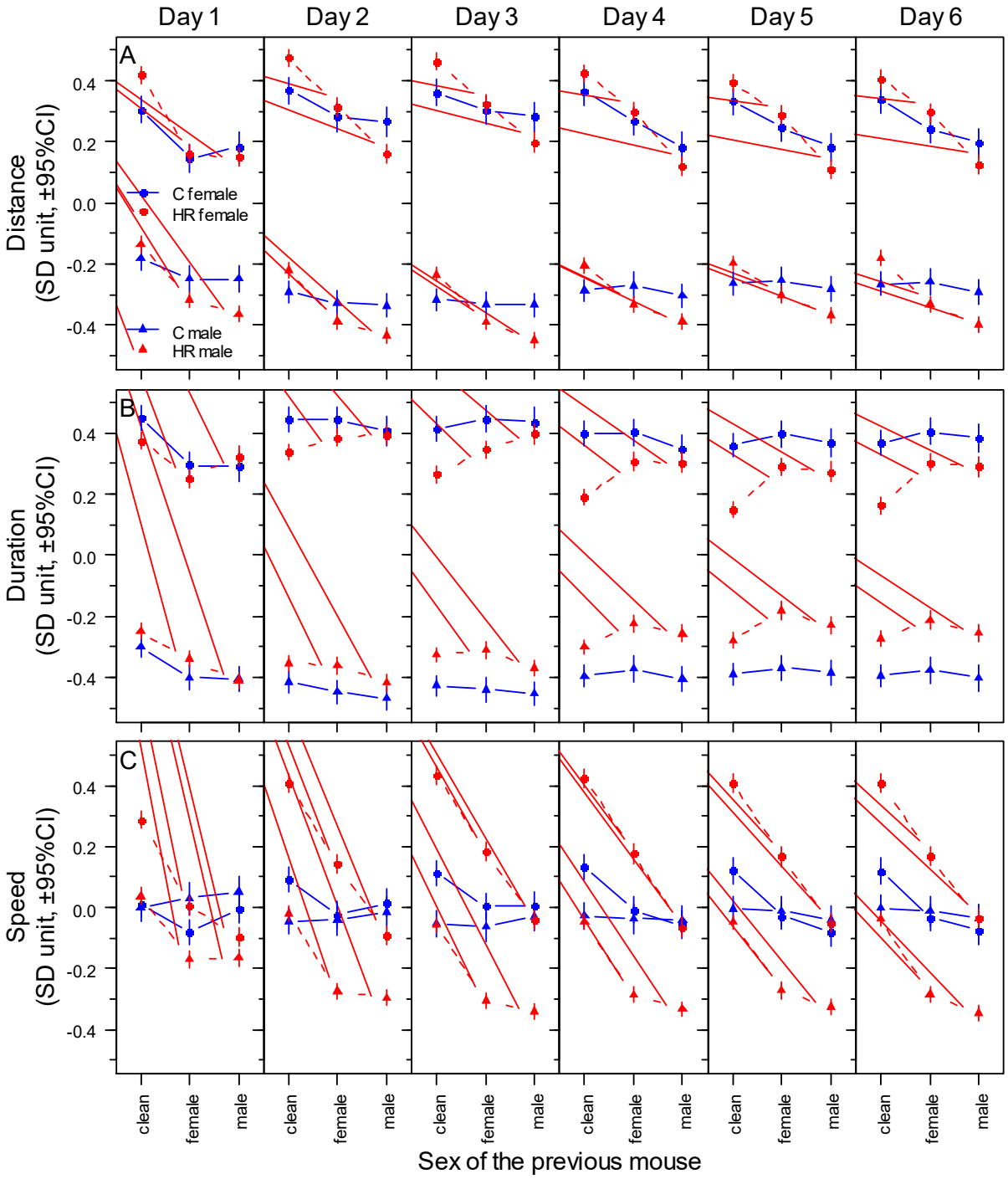

Residual error e
Direct common env. effect ce_{Fi}
Direct additive genetic effect a_{Fi}
Indirect common env. effect ce_{Pj}
Indirect additive genetic effect a_{Pj}
Cor(a_F, a_P)
i
j

Figure 1



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537 Figure 2
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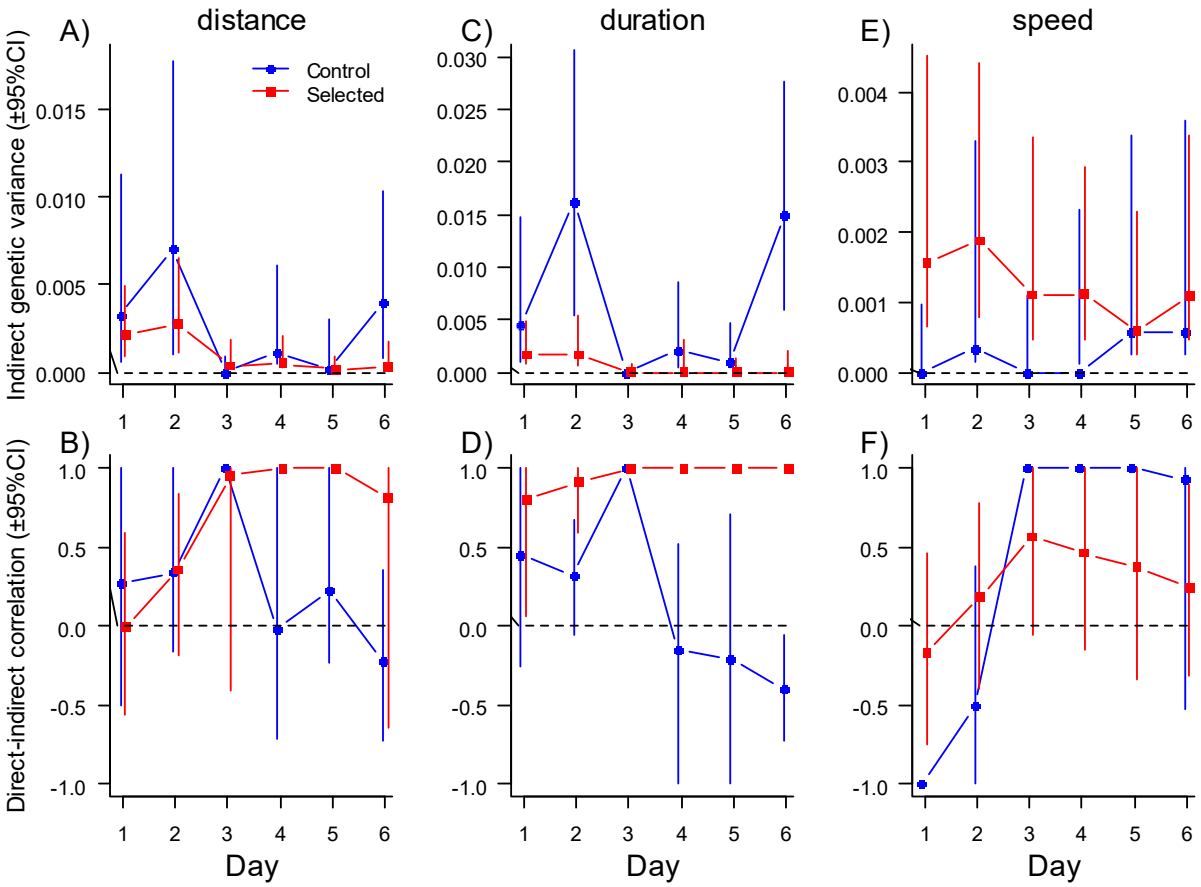

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540 Figure 3.

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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 standardised 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	$r_{AF, AP}$	0.2684	0.5131	-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	$r_{AF, AP}$	0.3395	0.3552	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	$r_{AF, AP}$	1.0000	NA	0.9496	0.8169
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	$r_{AF, AP}$	-0.0253	0.6866	1.0000	NA
4	σ^2_{AF}	0.2182	0.0242	0.0997	0.0199
4	σ^2_{AP}	0.0011	0.0018	0.0005	0.0006
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	0.0232	0.0068
5	$r_{AF, AP}$	0.2246	1.9986	1.0000	NA
5	σ^2_{AF}	0.2179	0.0238	0.0904	0.0185
5	σ^2_{AP}	0.0001	0.0011	0.0001	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 standardised 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	$r_{AF, AP}$	0.4473	0.4504	0.7982	0.4594
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	0.0272	0.0069
2	$r_{AF, AP}$	0.3168	0.2601	0.9099	0.4374
2	σ^2_{AF}	0.1574	0.0191	0.0838	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	0.0801	0.0066
3	σ^2_{CP}	0.0187	0.0080	0.0256	0.0068
3	$r_{AF, AP}$	1.0000	NA	1.0000	NA
3	σ^2_{AF}	0.1167	0.0166	0.0894	0.0187
3	σ^2_{AP}	0.0000	NA	0.0000	NA
3	σ^2_e	0.5341	0.0101	0.6951	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	0.5228	1.0000	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	0.0831	0.0067
5	σ^2_{CP}	0.0125	0.0077	0.0281	0.0068
5	$r_{AF, AP}$	-0.2124	0.7516	1.0000	NA
5	σ^2_{AF}	0.1779	0.0195	0.0862	0.0176
5	σ^2_{AP}	0.0009	0.0014	0.0000	NA
5	σ^2_e	0.5001	0.0102	0.6978	0.0091
6	σ^2_C	0.0511	0.0066	0.0819	0.0065
6	σ^2_{CP}	0.0017	0.0076	0.0231	0.0065
6	$r_{AF, AP}$	-0.4034	0.2497	1.0000	NA
6	σ^2_{AF}	0.1666	0.0189	0.0792	0.0165
6	σ^2_{AP}	0.0150	0.0070	0.0000	NA
6	σ^2_e	0.5013	0.0102	0.6995	0.0090

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 standardised 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}	0.0684	0.0094	0.0968	0.0070
1	σ^2_{CP}	0.0073	0.0104	0.0000	NA
1	$r_{AF, AP}$	-1.0000	NA	-0.1704	0.4827
1	σ^2_{AF}	0.3644	0.0316	0.1618	0.0238
1	σ^2_{AP}	0.0000	NA	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	$r_{AF, AP}$	-0.5106	1.5844	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	$r_{AF, AP}$	1.0000	NA	0.5657	0.4882
3	σ^2_{AF}	0.3553	0.0320	0.1584	0.0231
3	σ^2_{AP}	0.0000	NA	0.0011	0.0010
3	σ^2_e	0.7156	0.0150	0.6442	0.0094
4	σ^2_C	0.0668	0.0094	0.0919	0.0069
4	σ^2_{CP}	0.0000	NA	0.0034	0.0057
4	$r_{AF, AP}$	1.0000	NA	0.4597	0.4805
4	σ^2_{AF}	0.3264	0.0310	0.1805	0.0236
4	σ^2_{AP}	0.0000	NA	0.0011	0.0010
4	σ^2_e	0.7260	0.0149	0.6391	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	$r_{AF, AP}$	1.0000	NA	0.3728	0.6144
5	σ^2_{AF}	0.3222	0.0307	0.1778	0.0223
5	σ^2_{AP}	0.0006	0.0010	0.0006	0.0007
5	σ^2_e	0.7229	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	1.1882	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 behavior in focal males (triangles) and females (dots) 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 behaviour was measured over six consecutive days (each shown in a separate panel). Symbols denote the average and the 95% confidence intervals (CI) of A) the number of wheel revolutions (distance), B) number of 1-min intervals with at least one revolution (duration), and C) the average number of revolutions in active 1-min intervals (speed).

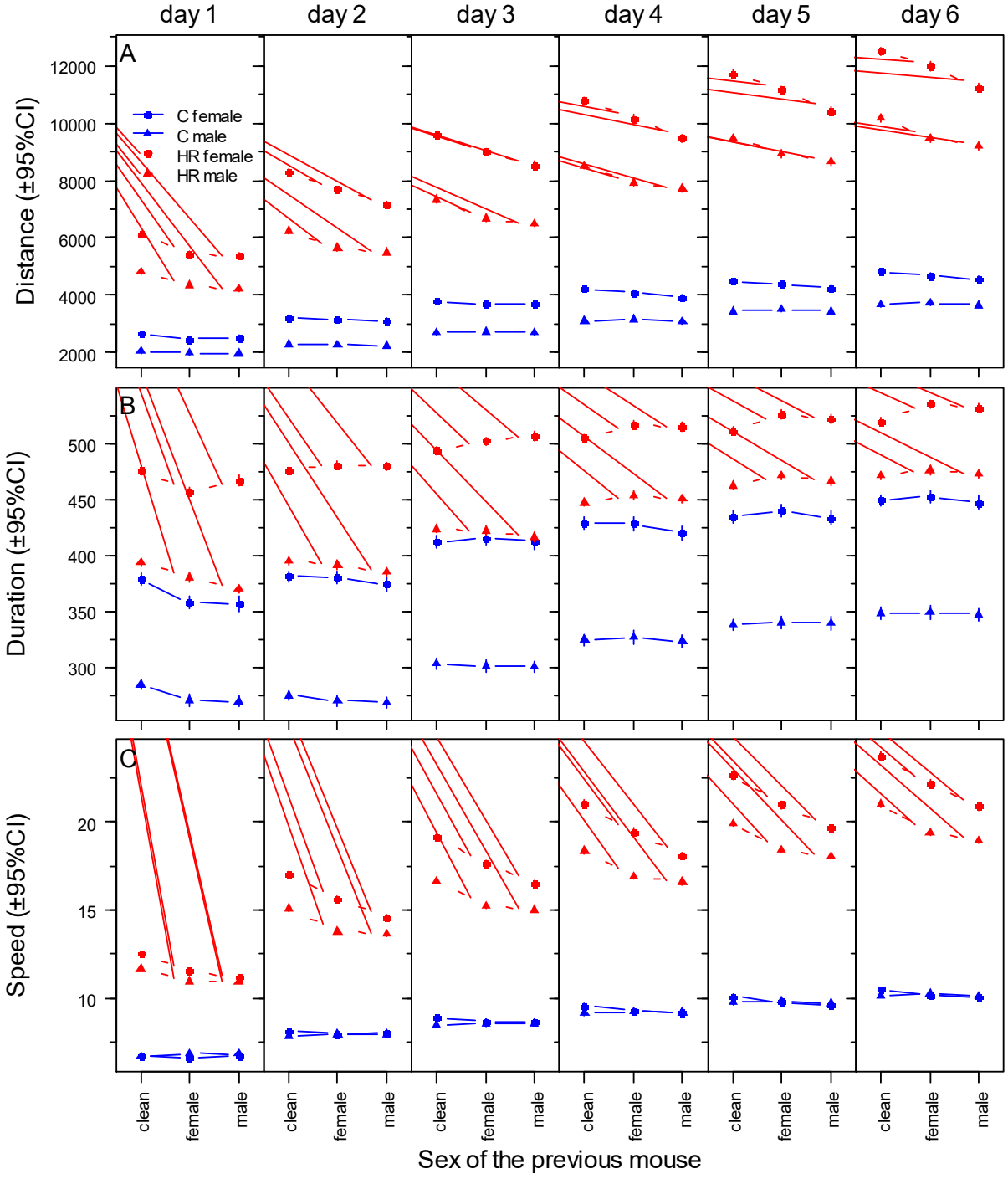


Fig S2 Same as Fig S1, showing the effect of the linetype (instead of sex) of the previous mouse. Voluntary wheel-running behavior in focal males (triangles) and females (dots) from control lines (C; blue) and high-runner lines (HR, red) as a function of the *linetype* of the mouse previously occupying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

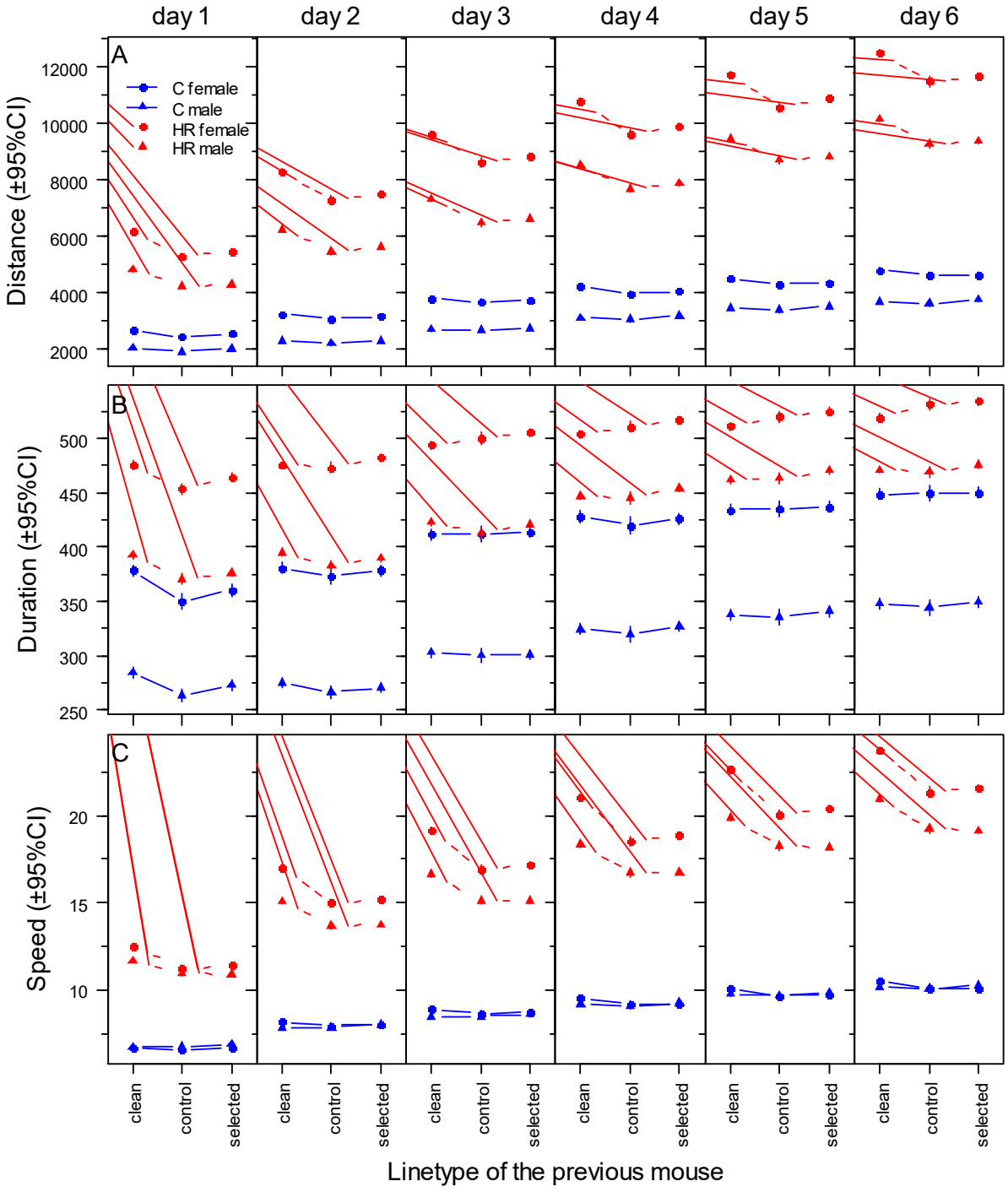


Table S1. Variance component estimates (±se) extracted from univariate mixed models of voluntary wheel-running distance on six consecutive days, including direct common (maternal) environmental variance (σ^2_{CF}), indirect common (maternal) environmental variance (σ^2_{CP}), direct additive genetic 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 standardised 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 standardised 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 standardised 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 behavior in focal males (triangles) and females (dots) 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 behaviour was measured over six consecutive days (each shown in a separate panel). Symbols denote the average and the 95% confidence intervals (CI) of A) the number of wheel revolutions (distance), B) number of 1-min intervals with at least one revolution (duration), and C) the average number of revolutions in active 1-min intervals (speed).

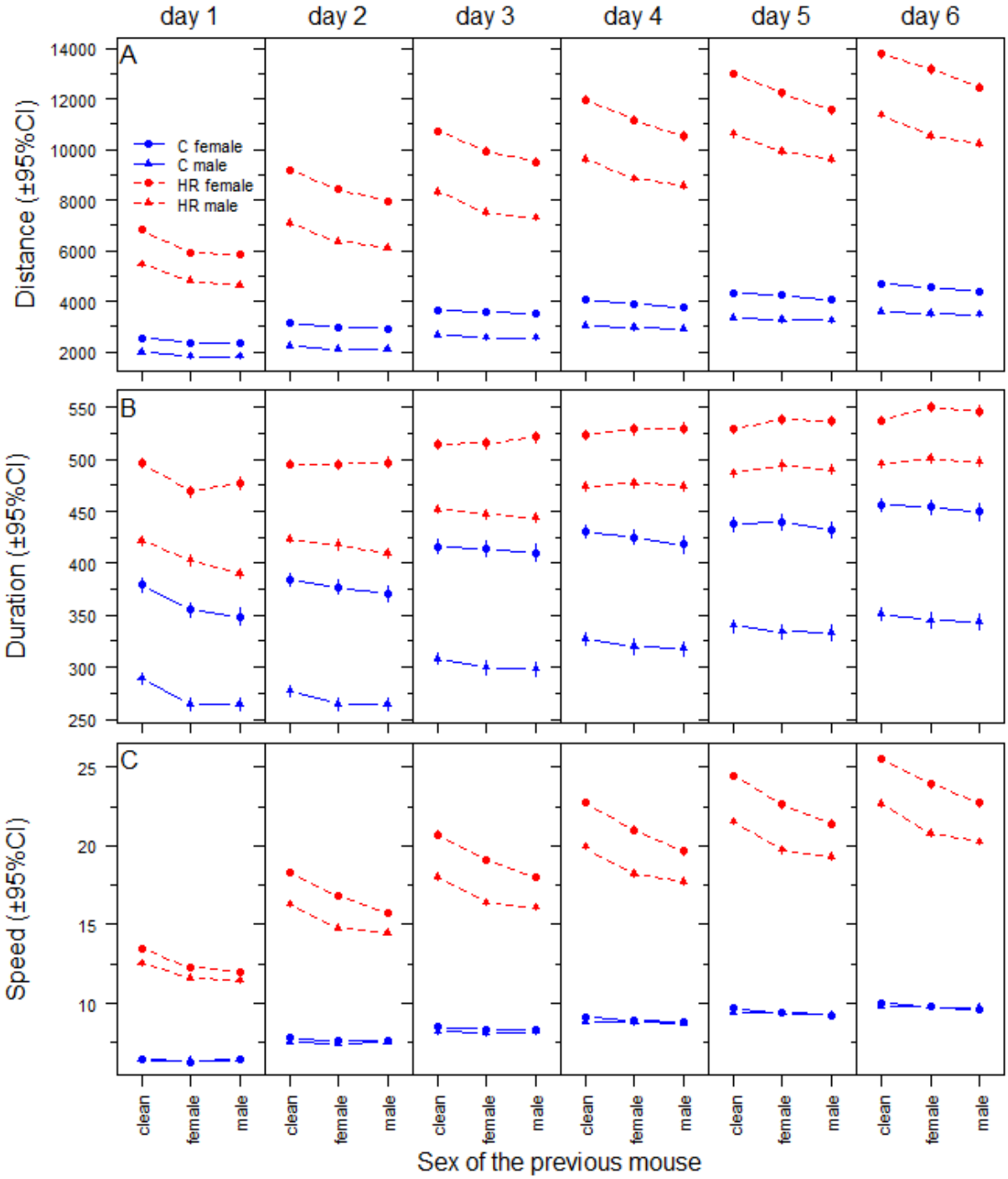
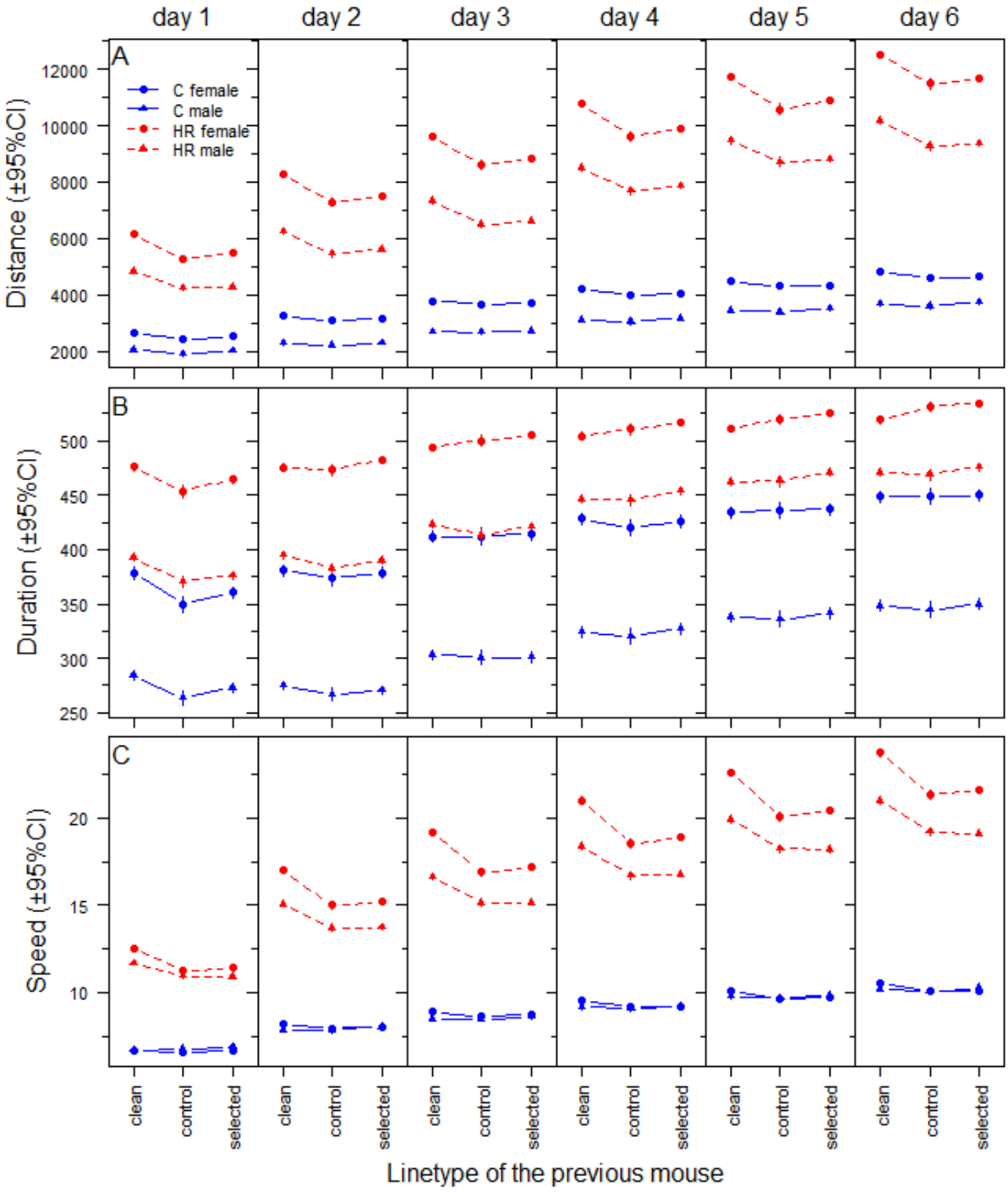


Fig S2 Same as Fig S1, showing the effect of the linetype (instead of sex) of the previous mouse. Voluntary wheel-running behavior in focal males (triangles) and females (dots) from control lines (C; blue) and high-runner lines (HR, red) as a function of the *linetype* of the mouse previously occupying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



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