Selective Breeding and Short-Term Access to a Running Wheel Alter Stride Characteristics in House Mice

Gerald C. Claghorn[1]
Zoe Thompson[1]
Jarren C. Kay[1]
Genesis Ordonez[1]
Thomas G. Hampton[2]
Theodore Garland Jr.[1],*

[1]Department of Biology, University of California, Riverside, California 92521; [2]Mouse Specifics, Framingham, Massachusetts 01701

ABSTRACT

Postural and kinematic aspects of running may have evolved to support high runner (HR) mice to run approximately threefold farther than control mice. Mice from four replicate HR lines selectively bred for high levels of voluntary wheel running show many differences in locomotor behavior and morphology as compared with four nonselected control (C) lines. We hypothesized that HR mice would show stride alterations that have coadapted with locomotor behavior, morphology, and physiology. More specifically, we predicted that HR mice would have stride characteristics that differed from those of C mice in ways that parallel some of the adaptations seen in highly cursorial animals. For example, we predicted that limbs of HR mice would swing closer to the parasagittal plane, resulting in a two-dimensional measurement of narrowed stance width. We also expected that some differences between HR and C mice might be amplified by 6 d of wheel access, as is used to select breeders each generation. We used the DigiGait Imaging System (Mouse Specifics) to capture high-speed videos in ventral view as mice ran on a motorized treadmill across a range of speeds and then to automatically calculate several aspects of strides. Young adults of both sexes were tested both before and after 6 d of wheel access. Stride length, stride frequency, stance width, stance time, brake time, propel time, swing time, duty factor, and paw contact area were analyzed using a nested analysis of covariance, with body mass as a covariate. As expected, body mass and treadmill speed affected nearly every analyzed metric. Six days of wheel access also affected nearly every measure, indicating pervasive training effects, in both HR and C mice. As predicted, stance width was significantly narrower in HR than C mice. Paw contact area and duty factor were significantly greater in minimuscle individuals (subset of HR mice with 50%-reduced hind limb muscle mass) than in normal-muscled HR or C mice. We conclude that stride characteristics of house mice are adaptable in response to both selective breeding and changes in daily locomotor behavior (activity levels) that occur during as few as 6 d. These results have important implications for understanding the evolution and coadaptation of locomotor behavior and performance.

Keywords: artificial selection, body size, experimental evolution, gait, kinematics, locomotion, morphology.

Introduction

Locomotion is critical to the life of most animals (Hansson and Åkesson 2014; Irschick and Higham 2015), and the specific characteristics of locomotor adaptations are incredibly diverse, even among terrestrial vertebrate quadrupeds (e.g., Vanhooydonck et al. 2014; de Albuquerque et al. 2015; Higham et al. 2015). Variation along the gradient of sedentary to highly mobile animals is correlated with many suborganismal traits (Wallace and Garland 2016), and broad, interspecific trends suggest a few key traits for supporting high mobility (Biewener 1989; Garland and Janis 1993; Carrano 1999; Christiansen 2002; Bonine 2005).

Much of the existing literature on kinematic and morphological adaptations for high locomotor performance focus on so-called cursors (Carrano 1999). Cursorial animals are generally defined as those that can run long distances swiftly and/or easily (see references within Gregory 1912; Stein and Casinos 1997). Morphological features that typify a cursor include long, parasagittally oriented limbs; lightening and lengthening of distal limb elements (often reported as increased metatarsal/femur length ratio); and digitigrade or unguligrade locomotion. The functional description of cursorial adaptations is intuitively satisfying and well reasoned using simple models of the physics of running. However, most cursors are relatively large animals and belong to a few clades, namely, Carnivora, Perissodactyla, and Artiodactyla (but see Lovegrove and Mowoe 2014; Young et al. 2014), which potentially confounds the apparent trends.

Theoretically, comparing the kinematics of animals of disparate size and shape (such as comparing a large cursor to a mouse)

is complicated by the scaling and transferability of kinematics and posture between different limb morphologies (Gatesy and Pollard 2011). Empirically, apparent broad-scale trends linking cursoriality (i.e., aspects of enhanced locomotor behavior and performance) with morphology can break down when comparing animals of different size within a clade (Day and Jayne 2007). Additionally, morphological characteristics that appear to be correlated with high locomotor activity are sometimes more closely tied to phylogeny. For example, metatarsal/femur ratio is well correlated with sprint speed among species in the broad sense (after accounting for phylogeny and body size), but when the pool is limited to cursors, the pattern is greatly diminished (Garland and Janis 1993). However, simultaneous analyses of multiple putative skeletal adaptations for speed may increase the strength of associations (Christiansen 2002).

Regardless of empirical results, most of the proposed adaptions for cursoriality are well vetted in biomechanical models. For example, all else being equal, longer limbs increase stride length and also (given the physics of lever systems) increase the velocity of the distal end of the limb compared to a shorter limb (Gregory 1912). Aligning the limbs in a parasagittal plane (parallel to the plane that divides the body into left and right) aligns the long axis of limb bones with the forces of gravity and braking-propulsive forces and thus limits potentially injury-inducing forces to one plane, reducing torsional loading in favor of bending (Biewener 1989; Blob 2001). Functionally, the upright, parasagittal orientation of limbs results in the reduction of lateral forces, limiting the propulsive and braking forces to the fore-aft directions (Chen et al. 2006), where they translate directly to forward movement.

Studying the evolution of locomotor behavior and performance is a difficult task, for several reasons. For example, persuading wild animals to behave normally under controlled and reproducible conditions is fraught with complications. Moreover, broad-scale comparisons, even if conducted within a phylogenetically informed context (e.g., Vanhooydonck et al. 2014; de Albuquerque et al. 2015; Higham et al. 2015), are always based on correlational evidence (Garland et al. 2016). In addition, sometimes the precise nature of selection in the wild is difficult to determine, even in the case of well-known animal athletes (Wilson et al. 2013; Irschick and Higham 2015). Therefore, modeling (e.g., Cespedes and Lailvaux 2015) or experimental evolution (Swallow et al. 2009; Wallace and Garland 2016) can provide useful alternatives.

Accordingly, we have used selective breeding to develop the high runner (HR) mouse model for study of coordinated evolutionary changes in locomotor behavior and performance (Garland 2003; Wallace and Garland 2016). This experimental system is reasonably well suited for study of the evolution of kinematics because HR mice (1) have been bred for, and have evolved, great voluntary daily movement distances; (2) have evolved to be good endurance runners in forced treadmill exercise tests (Meek et al. 2009; Claghorn et al. 2016); (3) have evolved to have some anatomical differences relevant to kinematics, including reductions in muscle mass (Garland et al. 2002) and increased hind limb symmetry and larger femoral heads (Garland and

Freeman 2005; Kelly et al. 2006); and (4) are well studied in terms of physiological adaptations to high activity as well as patterns of wheel-running behavior (Girard et al. 2001; Rezende et al. 2009; Garland et al. 2011a, 2011b). Furthermore, HR mice and their counterpart control (C) lines of mice have a known evolutionary history, differ only slightly in body mass, and do not differ meaningfully in such skeletal dimensions as femur length (Garland and Freeman 2005; Kelly et al. 2006), so the model does not share the same confounding effects of disparate body size, extreme morphological differences, or phylogenetic effects that complicate many other comparisons.

HR mice run approximately three times as many wheel revolutions per day as C mice (fig. 1). The difference in total wheel revolutions is caused primarily by an increase in average speed of running, rather than the amount of time spent running (fig. 1; Koteja et al. 1999), although male HR mice do run for significantly longer per day than C males (Garland et al. 2011b). A selection limit of unknown origin was reached around generations 16–28, depending on line and sex (Garland et al. 2011a; Careau et al. 2013b), and wheel running has not increased in the HR lines for at least the next 40 generations of selective breeding. This result may indicate that HR mice are running as far as they are physically capable, although no physical, physiological, or psychological limits to wheel running have been explicitly identified (Rhodes et al. 2005; Rezende et al. 2009; Kolb et al. 2010; Meek et al. 2010; Claghorn et al. 2016). The possibility of kinematic or biomechanical factors coming into play at the selection limit has not yet been explored.

HR mice have evolved a number of correlated features, including increases in two whole-organism indicators of exercise capacity: endurance during forced treadmill exercise (Meek et al. 2009; Claghorn et al. 2016) and maximal exercise-induced oxygen consumption (Swallow et al. 1998b; Rezende et al. 2006a, 2006b; Kolb et al. 2010; Dlugosz et al. 2013; Claghorn et al. 2017). HR mice are also smaller and leaner, achieve higher maximum speeds during wheel running, and have a reduced incremental cost of transport on a whole-animal basis, although not on a mass-adjusted basis (Swallow et al. 1999, 2001; Rezende et al. 2006c). Relevant suborganismal traits that have evolved in HR mice include increased symmetry of hind limb bone lengths, larger femoral heads, heavier foot bones, and altered semicircular canal shape (Garland and Freeman 2005; Kelly et al. 2006; Schutz et al. 2014).

A subset of HR mice has the minimuscle phenotype, which is characterized by a 50% mass reduction in the triceps surae and total hind limb muscle mass (in homozygotes), caused by a drastic reduction of type IIb muscle fibers (Guderley et al. 2006; Talmadge et al. 2014). The minimuscle phenotype is caused by a Mendelian recessive allele that was present at a low frequency (~7%) in the original base population but has increased in frequency in two of the four HR lines, eventually becoming fixed in one (Garland et al. 2002; Hannon et al. 2008; Kelly et al. 2013). In minimuscle individuals, the medial gastrocnemius has a reduced force per cross sectional area (Syme et al. 2005), but hind limb muscles have double the per-gram oxidative capacity (Houle-Leroy et al. 2003). The gastrocnemius also has elevated capil-
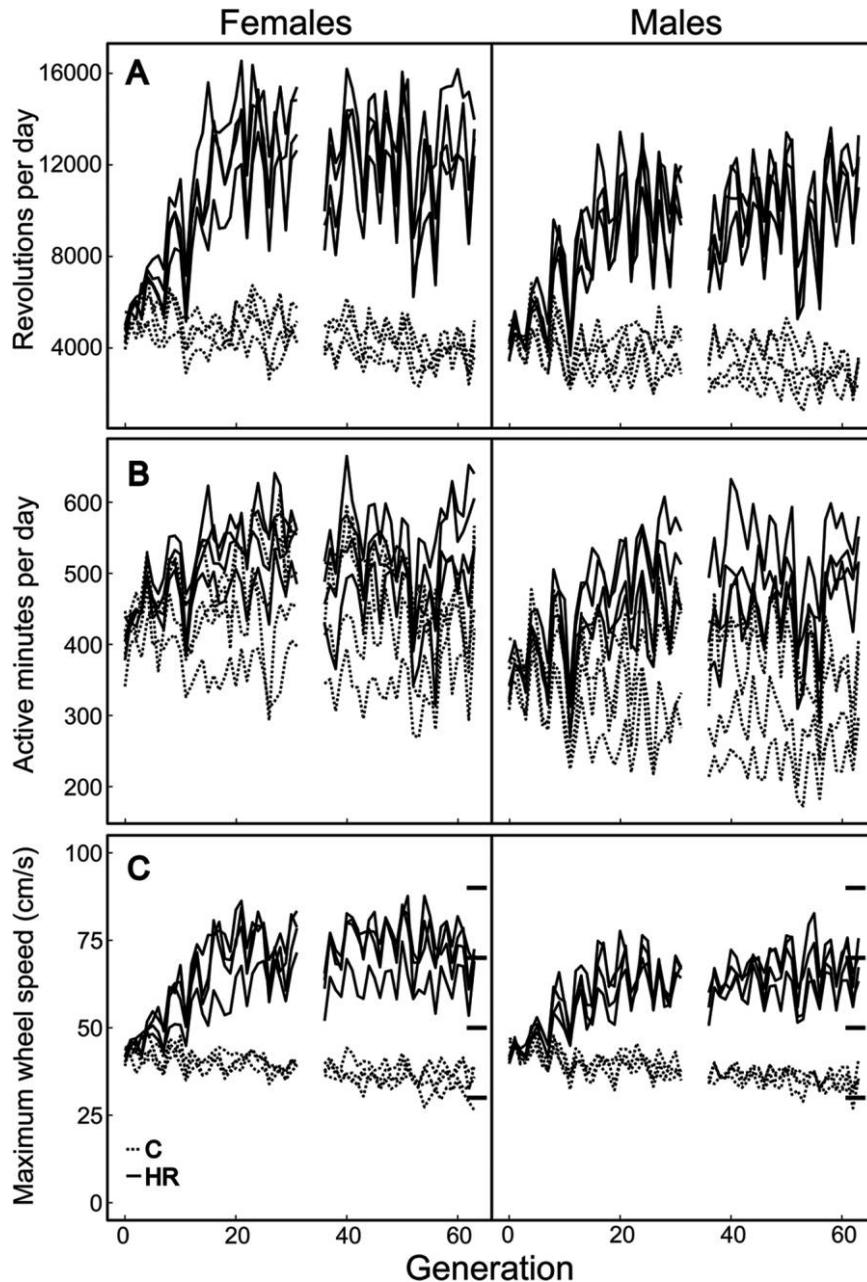
Figure 1. High runner (HR) mice have evolved to run more wheel revolutions than control (C) mice primarily by running faster. *A*, Revolutions per day for female mice from all eight lines averaged between days 5 and 6 of a 6-d period of wheel access. Once reaching their selection limits, HR mice (solid lines) run about three times as many wheel revolutions as C mice (dotted lines), and total wheel revolutions for HR mice have not appreciably increased since generation 31 (Kolb et al. 2013). The differential between HR and C mice is similar for males, but all males run less on average. *B*, Time spent running has not diverged significantly between female HR and C mice. Male HR mice do spend more time running, but the differential in total wheel revolutions is primarily due to increased speed, and not time spent running, for both males and females. *C*, Maximum wheel running speed, extrapolated from the highest number of wheel revolutions in a single minute, has also significantly diverged. Black horizontal bars represent the four testing speeds at which we characterized gait. *A–C*, Data for generations 0–31 compiled by Careau (2013*a*); subsequent generations represent unpublished data. The gap in data from generations 32–35 was from when the lab moved from Madison, Wisconsin, to Riverside, California. Seasonal variation is apparent, with mice tending to run more during winter generations and less during the summer (see Careau et al. 2013*a*).

larization (Wong et al. 2009) and increased glycogen storage (Gomes et al. 2009). Minimuscle mice have elevated whole-animal $\dot{V}_{O_2}$ max when tested in hypoxia (Rezende et al. 2006*a*)

but have reduced maximal sprint speeds (as measured on a photocell-timed racetrack) and elevated cost of locomotion on wheels (Dlugosz et al. 2009). Several of these differences might

Table 1: Stride characteristics measured

Stride characteristic	Description
Stride length (cm)	Distance between contacts of the same paw in one limb cycle
Stride frequency (Hz)	Number of complete limb cycles within a second of running
Duty factor (%)	Percent of stride time spent in stance
Brake time (s)	Time from initial paw contact to maximal paw contact
Propel time (s)	Time from maximal paw contact to paw liftoff
Paw area (cm^2)	Maximal paw contact area with the treadmill during stance
Stance width (cm)	Lateral distance between left and right paws during stance
Stance time (s)	Time within a limb cycle in which the paw is in contact with the treadmill
Swing time (s)	Time within a limb cycle in which the paw is not in contact with the treadmill

lead one to expect differences in stride characteristics between minimuscle and wild-type individuals, although the direction is difficult to predict.

We hypothesized that HR mice would show stride alterations that have coadapted with locomotor behavior, morphology, and physiology. Specifically, we obtained estimates of stride length, stride frequency, stance time, swing time, duty factor, brake time, and propel time (table 1). We measured stance width as a proxy for running posture along the sprawled/erect spectrum and predicted that HR mice should have narrower stances, which would be in line with cursorial animals and would suggest a reduction in lateral forces during straight-line running. We also measured paw contact area as an index of foot posture along the plantigrade/digitigrade or possibly crouched/erect continuum, predicting that HR mice would run more on their toes, thus having a smaller paw contact area. We also predicted that HR mice would take longer strides than C mice, similar to how cursorial animals have evolved long strides.

Methods

Animals

The starting population for the HR selection experiment was 224 individuals from a widely used outbred strain (Hsd:ICR), purchased from Harlan-Sprague-Dawley (Indianapolis, IN). Mice were bred randomly for two generations, then separated into eight closed lines, four of which were subsequently bred for high voluntary wheel-running behavior on days 5 and 6 of a 6-d period of wheel access as young adults (Swallow et al. 1998a). The four replicate HR mouse lines have been bred for high voluntary wheel-running activity for nearly 70 generations and have

a variety of behavioral, morphological, and physiological adaptations as compared with four nonselected C lines (Rhodes et al. 2005; Swallow et al. 2009; Garland et al. 2011a, 2011b; Wallace and Garland 2016). However, kinematic analyses of HR mice have not yet been attempted, primarily due to experimental complications of capturing quality kinematic data from large numbers of animals as small as house mice. For example, digitization of markers affixed to skin can involve significant error in the characterization relative to bones (Filipe et al. 2006; Bauman and Chang 2010), and the use of biplanar X-ray motion analysis (Brainerd et al. 2010) requires equipment and expertise that is available at only a few sites.

We studied 50 male and 50 female mice from generation 68 of the HR selective breeding experiment (Swallow et al. 1998a), with six males and six females from each of seven of the eight lines and eight males and eight females from line 6, an HR line that remains polymorphic for the minimuscle phenotype (e.g., Syme et al. 2005; T. Garland Jr., unpublished results). Mice were raised under the typical conditions of the selection experiment (Swallow et al. 1998b; Careau et al. 2013b), with the exception that they were not toe-clipped for identification and were housed singly from the time of weaning. Mice did not have access to wheels at any point before the first measurements. Food and water were available ad lib. throughout the course of the project, and the photoperiod was 12L:12D, with the light phase beginning at 0700 hours and the dark phase beginning at 1900 hours. The University of California–Riverside Institutional Animal Care and Use Committee approved all experimental conditions and protocols.

DigiGait Imaging System

We used the DigiGait Imaging System (Mouse Specifics; Nagy et al. 2017) to accomplish high-throughput phenotyping and allow comparison of the two-dimensional stride characteristics of both sexes and all eight lines of HR and C mice running on a treadmill at speeds that are typically seen during voluntary wheel running. At approximately 6 wk of age, all mice were tested on the DigiGait Imaging System. This system uses high-speed (148 frames/s) ventral-plane videography to image the subjects as their limbs advance to and retreat from a motorized transparent treadmill belt. Software determines the area of the paws for each of the four limbs to determine spatial and kinematic indexes throughout sequential strides. More than 30 metrics of posture and locomotion are reported to characterize the gait. Based on our initial hypotheses, we chose to analyze stride length, stride frequency, duty factor, swing time, stance time, propel time, brake time, stance width, and paw contact area (contact surface area with treadmill). To separate functional from morphological paw size, we measured hind foot sizes by digital photography for each individual that was treadmill tested ($N = 100$).

We recognize that treadmill locomotion necessarily differs to some degree from locomotor behavior for which the HR lines of mice have been selectively bred. In the future, it would be of great interest to obtain comparable data from mice as they run voluntarily on wheels over many hours. However, we do not currently have the technology to do this for large numbers of ani-

mals. Thus, the present data should be viewed as a first step toward understanding the evolution of gait characteristics in these animals.

Tests were conducted during the light phase, between 0800 and 1800 hours. Each mouse was removed from its cage, weighed, and immediately placed onto the treadmill inside of a transparent acrylic enclosure, which was adjusted in length such that the mouse had enough room to run but was kept in frame of the camera. The mouse had about 30 s to acclimate while the software controlling the camera was being prepared. The treadmill was started suddenly and stopped immediately if the mouse failed to orient or keep pace with the treadmill. This was repeated until the mouse was able to maintain pace in frame for 1–3 s, with the minimum requirement for a successful trial being 10 or more in-frame strides. Nearly every mouse in the sample was able to successfully run at all test speeds. Mice were tested at successive speeds of 30, 50, 70, and 90 cm/s with no incline and rested only for the time that it took to start the next trial (<2 min). Speeds were chosen to bracket the speeds that mice run on wheels (fig. 1). Total handling time for each animal was 6–15 min. Each mouse was tested once (per speed) before and once immediately after 6 d of wheel access. In total, we analyzed eight videos each of almost all of the 100 mice, for 797 total videos analyzed. All mice used a trot gait at all measured speeds, with the exception of a small number of mice that used one to three strides of a galloping gait when adjusting to the highest two test speeds. Because this behavior was so rare, these strides were excluded from our analysis.

Wheel Access

After the initial DigiGait testing, mice were individually housed in cages with access to the same wheels used in the routine selective breeding protocol (Swallow et al. 1998a; wheel circumference, 1.12 m) for 6 d. Mice had no wheel exposure before this. As during routine wheel testing for the selection experiment, revolutions were recorded in 1-min intervals by a photocell counter attached to the wheel and compiled via customized software (San Diego Instruments, San Diego, CA) for 23 h per day over 6 d.

Photographs were taken to measure paw size in all mice. Mouse feet were gently pressed against a glass slide postmortem and photographed ventrally with a consumer-grade digital single-lens reflex camera (Nikon D60 with a Nikon 50-mm lens). The paw photographs were outlined in ImageJ once each for size analysis. In keeping with the rest of this report, left and right paws were averaged for each mouse, and only hind paw data are shown.

Dissection

Males were dissected about 6 wk after the second DigiGait trial, and, for logistical reasons, females were dissected about 3 wk after the males. Of chief interest was the mass of the triceps surae, to verify the minimuscle phenotype of mice from the fixed minimuscle line and to determine the phenotype of mice from the polymorphic line.

Statistics

Group comparisons were performed using SAS PROC MIXED (SAS 9.4, SAS Institute, Cary, NC). The four primary factors of analysis were line type (HR vs. C), sex, training (before vs. after wheel access), and treadmill speed. All possible interaction terms were also included, except where noted, and we mostly reported least squares means $\pm$ standard errors from the full models. For the one HR line that contained both minimuscle and normal-muscled mice phenotypes (lab designation HR#6), minimuscle status was determined by dissection (Garland et al. 2002), and then minimuscle status for those mice as well as mice within the fixed minimuscle line (HR#3) was used as an additional explanatory variable. As in previous analyses of these lines of mice, replicate line was used as a random effect nested within line type. Individual was an additional factor for repeated measures. Body mass was used as a covariate in all analyses. Wheel freeness (a measure of how many revolutions the wheel spins after being accelerated to a standard speed) was measured before and after the wheel trials but was not predictive of wheel running and thus was not included as a covariate (models that included freeness did not yield results different from what is reported here).

Results

Additional tables and figures, as well as some results not described here (e.g., for repeatability of stride characteristics before vs. after 6 d of wheel access), can be found in appendixes A–C, available online.

Body Mass

Female HR mice were nearly the same body mass as female C mice, but HR males were significantly smaller than C males, as indicated by a significant sex–by–line type interaction ($P = 0.0250$; table A1; fig. 4). On average, mice from all groups gained mass during the period of wheel access (training, $P = 0.0045$; fig. 4). Minimuscle mice were significantly smaller than normal-muscled mice (minimuscle, $P = 0.0173$; fig. 4). Body mass was used as a covariate in the analysis of all stride characteristics, and it had a statistically significant effect on stance width, paw contact area, swing time, stance time, duty factor, and propel time ($P < 0.05$ for all; fig. 4; tables 2, A5).

Wheel Running

On days 5 and 6 of the 6 d of wheel access (the values used in the routine selective breeding protocol), HR mice ran significantly more wheel revolutions per day than C mice ($P < 0.0001$; fig. 5), females ran significantly more than males ($P = 0.0088$; fig. 5), and the line type–by–sex interaction was also significant ($P = 0.0412$; fig. 5). The HR versus C fold difference in daily running was 3.1 for females and 2.7 for males (tables A3, A4; fig. 5). HR mice ran for significantly more time than C mice ($P = 0.0346$) and significantly faster ($P < 0.0001$). The differences between HR and C speeds were larger for females than for males (sex–by–line type interaction for average speed, $P = 0.0151$) and for

Table 2: Effects of line type, sex, training, treadmill speed, minimuscle, and body mass on key stride characteristics: results of nested ANCOVAs

	Stride frequency			Stance width			Duty factor			Stride length			Paw contact area			Swing time		
Factor	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Line type	.01	1, 6	.9139	11.64	1, 6	**.0143**	.5	1, 6	.5045	.04	1, 6	.8481	.01	1, 6	.9140	.04	1, 6	.8413
Sex	4.59	1, 6	.0758	11.12	1, 6	**.0157**	.02	1, 6	.8975	4.07	1, 6	.0902	6.07	1, 6	**.0488**	2.83	1, 6	.1434
Training	87.54	1, 6	**<.0001**	20.41	1, 6	**.0040**	.97	1, 6	.3632	78.79	1, 6	**.0001**	26.17	1, 6	**.0022**	14.65	1, 6	**.0087**
Speed	6,177.67	3, 18	**<.0001**	114.12	3, 18	**<.0001**	894.83	3, 18	**<.0001**	3,805.22	3, 18	**<.0001**	1.28	3, 18	.3128	57.96	3, 18	**<.0001**
Minimuscle	.01	1, 655	.9283	1.39	1, 655	.2394	4.94	1, 655	**.0266**	.01	1, 655	.9043	7.67	1, 550	**.0058**	1.93	1, 728	.1647
Line type × sex	3.66	1, 6	.1042	.57	1, 6	.4775	.95	1, 6	.3679	2.07	1, 6	.2004	.28	1, 6	.6179	2.85	1, 6	.1422
Line type × training	.18	1, 6	.6898	.63	1, 6	.4568	2.61	1, 6	.1571	.09	1, 6	.7779	0	1, 6	.9958	1.31	1, 6	.2968
Line type × speed	.39	3, 18	.7614	2.49	3, 18	.0934	.97	3, 18	.4275	.19	3, 18	.9048	.18	3, 18	.9095	.29	3, 18	.8311
Sex × training	.94	1, 6	.3704	1.46	1, 6	.2718	.03	1, 6	.8608	1.63	1, 6	.2487	0	1, 6	.9571	.73	1, 728	.3929
Sex × speed	2.46	3, 18	.0955	.6	3, 18	.6206	3.54	3, 18	**.0358**	1.82	3, 18	.1805	.73	3, 18	.5461	4.02	3, 728	**.0074**
Training × speed	1.35	3, 18	.2889	3.42	3, 18	**.0397**	5.58	3, 18	**.0069**	1.55	3, 18	.2360	5.1	3, 18	**.0099**	1.26	3, 728	.2878
Line type × sex × training	2.27	1, 6	.1824	.01	1, 6	.9272	.04	1, 6	.8571	1.75	1, 6	.2338	.01	1, 6	.9261			
Line type × sex × speed	.39	3, 18	.7633	.91	3, 18	.4545	1.23	3, 18	.3263	.16	3, 18	.9190	.17	3, 18	.9168			
Linetype × training × speed	.5	3, 18	.6859	1.99	3, 18	.1515	1.51	3, 18	.2448	.62	3, 18	.6132	.7	3, 18	.5648			
Sex × training × speed	.48	3, 18	.7011	.19	3, 18	.9030	.02	3, 18	.9971	.37	3, 18	.7729	.61	3, 18	.6198			
Four-way interaction	1.77	3, 18	.1892	.33	3, 18	.8067	.92	3, 18	.4488	1.7	3, 18	.2035	1.02	3, 18	.4090			
Body mass	.14	1, 655	.7108	39.41	1, 655	**<.0001**	28.3	1, 655	**<.0001**	.3	1, 655	.5858	26.7	1, 550	**<.0001**	12.89	1, 728	**.0004**

Note. Graphs of these stride characteristics can be found in figure 2. Statistically significant P values are shown in bold. Empty data cells indicate that the data did not converge.
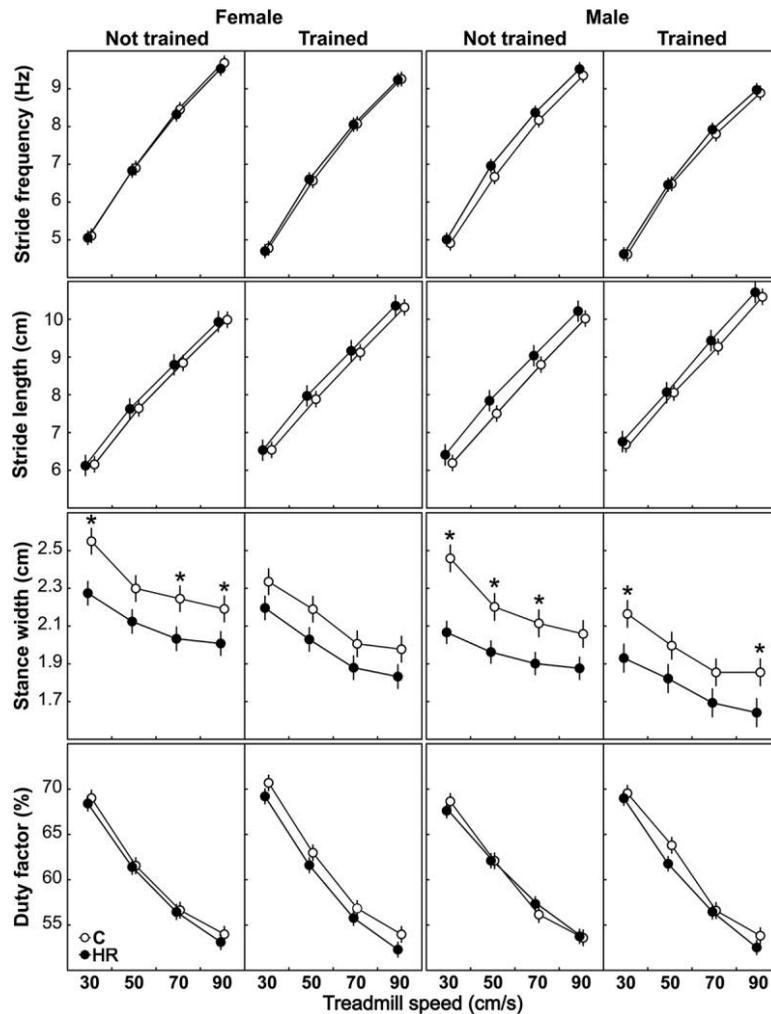
Figure 2. High runner (HR) mice use narrower stances while running on a treadmill but do not differ from control (C) mice in stride frequency or duty factor. Speed was tightly controlled, but graphs are offset along the *X*-axis for clarity of the error bars. The results of a nested ANCOVA are shown in table 2, and least squares means are available in appendix A, available online. None of the characteristics shown was affected by training (6 d of wheel access). *Top*, stride frequency is not affected by line type, but strides become more frequent with increasing treadmill speed and longer (*second row*) and less frequent after wheel access. *Third row*, stance width is significantly smaller in HR mice under all test conditions and for both sexes, as indicated by asterisks. Stance width also becomes smaller with increasing speed and decreases after wheel access. *Bottom*, duty factor is not significantly different between HR and C mice but decreases with increasing speed.

maximum speed ($P = 0.0132$). Female HR mice ran about 150% faster on average and reached top speeds that were 104% faster than those of female C mice. Male HR mice ran about 89% faster on average and reached top speeds 61% faster than those of male C mice. The ranges of speeds observed were comparable to previous generations and to the treadmill speeds used in the kinematic analysis (see "Methods" and fig. 1). On average, minimuscle individuals ran significantly faster on wheels (table A3; fig. 5).

Effects of Speed on Stride

As expected, treadmill speed greatly affected nearly every metric of stride that we analyzed. As treadmill speed increased, both stride length and frequency increased; stance, swing, brake, and propel times decreased; duty factor decreased; and stance width

decreased (all $P < 0.0001$; table 2; fig. 2). Paw contact area was the only measurement not significantly influenced by treadmill speed. In a few cases, there was a significant interaction between speed and line type, sex, or training. In most cases, this indicated that the measurement was different between line types, sexes, or training status at low speeds but then converged at high speeds. For example, brake time was significantly affected by training, but at the highest speed, brake time was nearly identical before and after wheel access (table 2; fig. 2).

Effect of Selective Breeding on Stride

Adjusted for body mass and as predicted, stance width was significantly narrower in HR than in C mice (effect of line type, $P = 0.0143$; fig. 2). However, stride length, stride frequency, paw
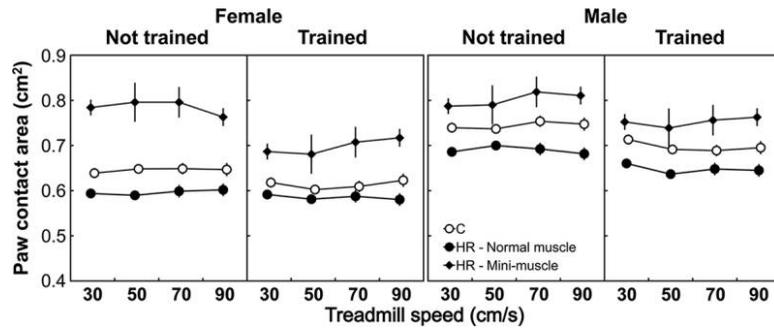
Figure 3. Minimuscled mice have larger paw contact area while running on a treadmill, but the paw contact area of normal-muscled high runner (HR) mice does not differ significantly from controls (C). Simple means and standard errors (not accounting for repeated measures) for paw contact area are shown. Speed was tightly controlled, but graphs are offset along the X-axis for clarity. The results of a nested ANCOVA are shown in table 2, and least square means are available in appendix A, available online.

contact area, swing time, stance time, brake time, and propel time were not significantly affected by selective breeding (effect of line type, $P > 0.05$ for all; tables 2, A5).

Effects of Sex on Stride

Females showed a trend for shorter, more frequent strides ($P = 0.0902, 0.0758$ for sex effect on stride length and stride frequency, respectively; table 2; fig. 2). The increase in frequency was related to a trend for decreased stance time ($P = 0.0701$), which was in turn attributable to a significantly smaller amount of time spent in the propel phase ($P = 0.0201$). Females had significantly wider stances ($P = 0.0157$) and smaller paw contact areas ($P = 0.0488$) than males, regardless of line type. No sex–by–line type interactions were found for any measurement, suggesting that selection for wheel running has not affected the sexes differently in terms of gait during these treadmill tests.

Effects of 6 d of Wheel Access on Stride

After 6 d of wheel access, stride length significantly increased ($P = 0.0001$; table 2; fig. 2); stride frequency decreased ($P < 0.0001$); stance width decreased ($P = 0.0040$); paw contact area decreased ($P = 0.0022$); and stance, swing, brake, and propel time increased ($P = 0.0001$, $P = 0.0087$, $P = 0.0050$, $P = 0.0004$, respectively). One interaction of interest was a line type–by–training effect on brake time ($P = 0.0197$), indicating that while HR mice did not significantly differ from C mice overall, they were relatively more different from one another after wheel access (table A6).

Effects of Minimuscle Phenotype on Stride

Paw contact area during running on the treadmill was significantly greater in the subset of HR mice with the minimuscle phenotype (8.46% larger paw area; $P = 0.0058$; fig. 3). However, digitized photographs of hind paws of these same individual mice did not reveal any statistical difference in paw

area for minimuscle individuals, nor between HR and C mice (see app. B). Duty factor was also significantly greater in minimuscle mice ($P = 0.0266$; table 2), owing, at least partly, to a significantly longer brake time ($P = 0.0064$).

Discussion

Overview of Results

Although they run voluntarily at much higher speeds on wheels (fig. 1), mice from the selectively bred HR lines were quite similar to C mice in most stride characteristics that we quantified during treadmill locomotion. The one notable difference between line types was that, as predicted, HR mice had narrower stance widths (ran with feet closer to one another and to the sagittal plane) than nonselected controls. We also found that minimuscle individuals (a subset of the HR mice with a genetically determined 50% reduction in hind limb muscle mass) have larger
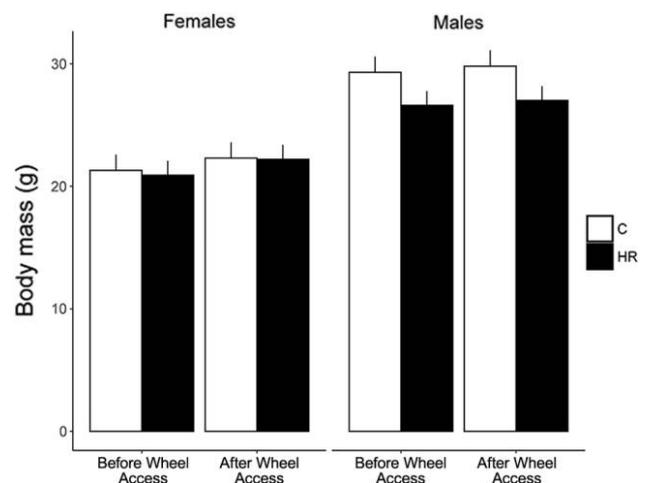

Figure 4. Body mass for high runner (HR) and control (C) mice, both before and after 6 d of wheel access. Least squares means ± standard errors from a nested ANCOVA are shown in table A2, available online.
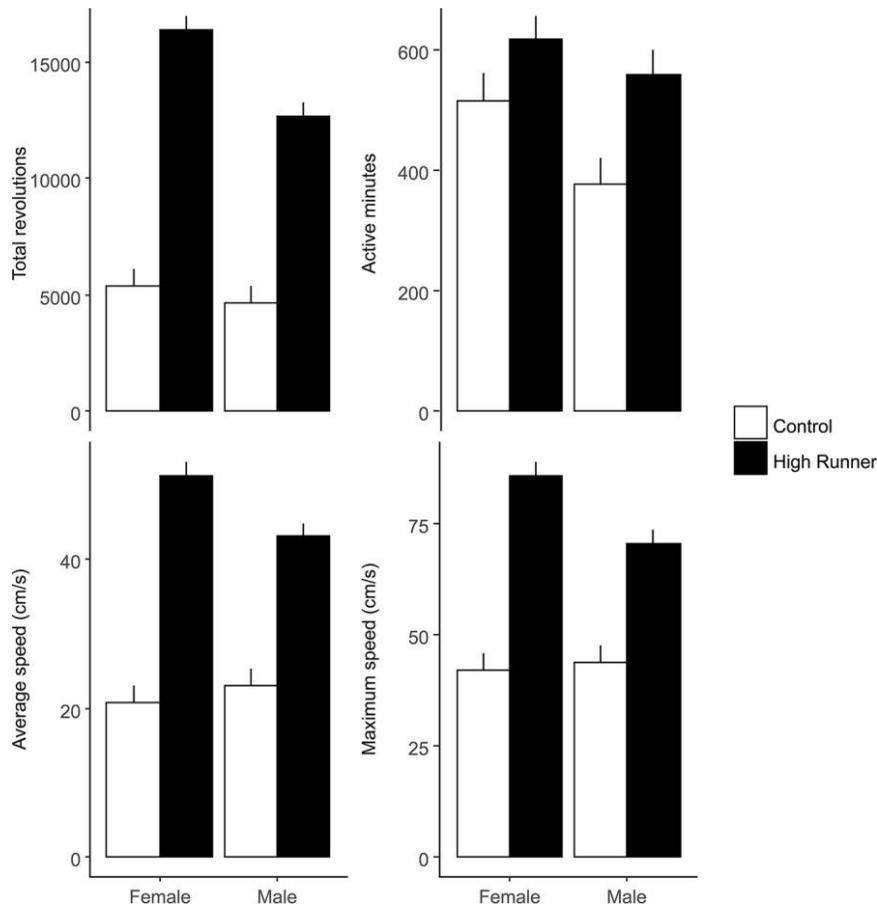
Figure 5. Wheel running for high runner (HR) and control (C) mice. Least squares means ± standard errors from a nested ANCOVA are shown in table A3, available online.

paw contact areas—but not larger paws—and higher duty factor across running speeds. We found no statistically significant effect of selective breeding on any of the temporal measurements of stride.

Although relatively few of the traits tested herein have evolved in HR mice, almost every measured stride characteristic was significantly affected by 6 d of wheel access, thus demonstrating pervasive training, conditioning, learning, or plasticity (Garland and Kelly 2006; Kelly et al. 2012), as well as the power of the methods employed to detect even relatively small variations. In addition, sex affected a number of stride characteristics, even after accounting statistically for the effects of body size (males are larger than females). Interestingly, even though Garland and Freeman (2005) showed that, at generation 11, males had shorter leg bones than females (adjusted for body mass), we found an opposite trend of females taking shorter, more frequent strides ($P = 0.0902, P = 0.0758$ for sex effect on stride length and stride frequency, respectively). Thus, known morphological differences between HR and C mice did not translate into detectable differences in stride in this study. This may be viewed as one of many examples of etho-eco-morphological mismatches (Diogo 2017). Minimuscle mice have longer hind limbs (longer tibiafib-

ula and sum of hind limb bones [femur, tibiafibula, and metatarsals]; Kelly et al. 2006) but did not have significantly longer strides, again showing an apparent mismatch. HR mice have heavier foot bones, with no statistically significant effect of minimuscle beyond the HR effect (Kelly et al. 2006), but here the minimuscle mice had significantly greater paw contact areas, whereas paw contact area did not differ between HR and C mice. These results serve as a cautionary tale for attempts to infer function solely from form.

Stance Width

All mice narrowed their stance in response to increased speeds, but HR stances were narrower at all speeds (fig. 2). All else being equal, a narrower stance should result in a more vertical alignment of limbs and therefore a more erect posture (Biewener 1989; Carrano 1999). Such alignment is purported to be an adaptation to align the long bones with the direction of the ground reaction force, thus reducing strain (Carrano and Biewener 1999). Therefore, the narrowed stance in HR mice could be related to the increased wheel-running speeds (fig. 1) that have evolved as the primary mechanism by which HR mice have increased wheel

running, because this change should increase ground reaction forces. Force plate and three-dimensional kinematic data would be required to test this idea.

Paw Contact Areas

Interestingly, paw contact area was the only characteristic unaffected by treadmill speed. The minimuscle phenotype may cause larger paw contact areas during loading because individuals with this phenotype rely more on two-legged support, as in rat aging models (Horner et al. 2011). Alternatively, because minimuscles have reduced total force-generating capacity (see Syme et al. 2005), affected individuals may use a relatively crouched posture and longer stance time to generate the work necessary for push-off (Usherwood 2013). Either possibility is consistent with the finding that minimuscle mice also have significantly greater duty factor (fig. 2; table 2). However, the present data indicate that the significant difference is derived from an increase in the time spent in the braking phase of stance rather than the propulsive stage (see table A5). Again, force plate data would clarify these findings, but photographic evidence indicates that although the effective paw area during stance is larger, the actual paws of minimuscle mice are not larger than those of non-minimuscle mice, nor do HR mice differ from C mice (see app. B).

Training Effects

Training effects were pervasive, with mice using longer, less frequent (fig. 2) strides after 6 d of wheel access and spending more time in both the stance and swing phases. Furthermore, stance width (fig. 2) and paw contact area decreased following wheel access. Training effects from wheel access have been observed in numerous rodent studies, including several involving the HR and C mice, but we have typically used much longer training periods, usually 6–10 wk (e.g., Rhodes et al. 2003; Kelly et al. 2006, 2017; Middleton et al. 2008; Meek et al. 2014). However, training effects at the molecular level have been observed in as few as 4 d of wheel access (Gomes et al. 2009). In a study of ground reaction forces and gait during wheel running, training was found to lower duty factors while raising hind limb peak forces (Roach et al. 2012). Contrary to that finding, we found a training-by-speed interaction effect only on duty factor, where duty factor was increased after wheel running and only at low speeds. This may indicate that wheel training changes kinematics in ways that differ by running platform (wheel vs. treadmill).

Effects of Running Speed

As expected, treadmill speed had widespread effects (Herbin et al. 2007), with increasing speed eliciting longer, more frequent strides, owing to both decreased stance and swing times (see table A5). Stance time decreased more dramatically than swing time with increasing speed, and, thus, duty factor decreased. Increasing speed also resulted in decreased stance width. The stance width for C mice at the highest speed was smaller than, or comparable to, that of the HR mice at low speeds (fig. 2),

which shows that C mice are physically capable of a narrower stance but choose to use a wider stance than HR mice across all tested speeds. Treadmill speeds were chosen to capture the range of speeds that are normally seen during wheel running (fig. 1; also see table A4), so we are confident that the chosen treadmill speeds were sufficient to compare stride characteristics at speeds relevant to voluntary wheel running. However, as discussed below, we do recognize that treadmill running likely differs somewhat from wheel running.

Potential Effects of Methodology on Stride Characteristics

Running HR and C mice on a treadmill most likely yielded somewhat different results than would be obtained if kinematics were measured during running either overground or on a wheel. For instance, Herbin et al. (2007) found that, at the same speeds, mice took shorter, more frequent strides when running on a treadmill as compared with running overground, though changes with speed were consistent under either condition. Within the selection experiment, the effect of running platform is apparent from the evolution of the minimuscle phenotype, which seems to be adaptive for wheel running (Garland et al. 2002) but detrimental to some other measures of locomotor performance. Specifically, minimuscle mice run as fast as or faster (sometimes for fewer minutes per day) than other HR mice on wheels, but the HR line in which minimuscle is fixed is no better at graded treadmill endurance trials than C mice and is worse than the other HR lines (Meek et al. 2009). Mice from that same minimuscle line are also slower overground sprinters and have a higher cost of transport than the two HR lines that do not have minimuscles (Dlugosz et al. 2009). Other than this specific example, voluntary wheel running and treadmill endurance measurements appear to be positively genetically correlated, as HR mice have evolved greater endurance (Meek et al. 2009) and rats that were selectively bred for high treadmill endurance also run more on wheels voluntarily (Waters et al. 2008). Running in an upright wheel is likely different from both flat ground and treadmill running, but that specific comparison has not yet been performed. Gait and ground reaction force studies of mice running on upright (Roach et al. 2012) and saucer-style (Smith et al. 2015) running wheels revealed expected force and kinematic changes with speed but did not explicitly compare those gaits to overground or treadmill running.

Evolution of Cursoriality Outside of Carnivores and Ungulates

Cursoriality is not a binary condition but rather one end of a spectrum of locomotor behaviors, performance, and associated morphology and kinematics (Carrano 1999). Therefore, if the characteristics that typify a cursor improve locomotor economy, performance, or safety factors, then selection for enhanced locomotor behaviors (high speed of locomotion, long daily movement distance, etc.) should result in some degree of cursor-like morphology and kinematics in any group. For instance, within primates, lineages leading to humans evolved long legs and a very upright and bipedal running posture, along with a suite of skeletal adaptations (reviewed in Bramble and Lieberman

2004), but humans remain plantigrade and have a relatively small metatarsal/femur ratio compared to quadrupedal cursors. In another example, elephant shrews have evolved a high metatarsal/femur length ratio, upright posture, and scapular morphology coincident with greater speed than similar-sized mammals, including closely related species (Seckel and Janis 2008; Lovegrove and Mowoe 2014). Kelly et al. (2006) argued that HR mice could be considered cursors based on behavioral and performance characteristics. Considering all of the existing evidence, we conclude that HR mice are similar to many other so-called cursors in that their anatomy and kinematics constitute an incomplete set of possible cursorial adaptations.

Acknowledgements

Literature Cited

Bauman J.M. and Y.-H. Chang. 2010. High-speed X-ray video demonstrates significant skin movement errors with standard optical kinematics during rat locomotion. J Neurosci Methods 186:18–24.

Biewener A.A. 1989. Scaling body support in mammals: limb posture and muscle mechanics. Science 245:45–48.

Blob R.W. 2001. Evolution of hindlimb posture in nonmammalian therapsids: biomechanical tests of paleontological hypotheses. Paleobiology 27:14–38.

Bonine K.E. 2005. Muscle fiber-type variation in lizards (Squamata) and phylogenetic reconstruction of hypothesized ancestral states. J Exp Biol 208:4529–4547.

Brainerd E.L., D.B. Baier, S.M. Gatesy, T.L. Hedrick, K.A. Metzger, S.L. Gilbert, and J.J. Crisco. 2010. X-ray reconstruction of moving morphology (XROMM): precision, accuracy and applications in comparative biomechanics research. J Exp Zool A 313:262–279.

Bramble D.M. and D.E. Lieberman. 2004. Endurance running and the evolution of *Homo*. Nature 432:345–352.

Careau V., M.E. Wolak, P.A. Carter, and T. Garland Jr. 2013*a*. Data from: Limits to behavioral evolution: the quantitative genetics of a complex trait under directional selection. Dryad Data Repository. http://dx.doi.org/10.5061/dryad.37b28.

———. 2013*b*. Limits to behavioral evolution: the quantitative genetics of a complex trait under directional selection. Evolution 67:3102–3119.

Carrano M.T. 1999. What, if anything, is a cursor? categories versus continua for determining locomotor habit in mammals and dinosaurs. J Zool 247:29–42.

Carrano M.T. and A.A. Biewener. 1999. Experimental alteration of limb posture in the chicken (*Gallus gallus*) and its bearing on the use of birds as analogs for dinosaur locomotion. J Morphol 240:237–249.

Cespedes A.M. and S.P. Lailvaux. 2015. An individual-based simulation approach to the evolution of locomotor performance. Integr Comp Biol 55:1176–1187.

Chen, J., A. Peattie, K. Autumn, and R. Full. 2006. Differential leg function in a sprawled-posture quadrupedal trotter. J Exp Biol 209:249–259.

Christiansen P.E.R. 2002. Locomotion in terrestrial mammals: the influence of body mass, limb length and bone proportions on speed. Zool J Linn Soc 136:685–714.

Claghorn G.C., I.A. Fonseca, Z. Thompson, C. Barber, and T. Garland Jr. 2016. Serotonin-mediated central fatigue underlies increased endurance capacity in mice from lines selectively bred for high voluntary wheel running. Physiol Behav 161:145–154.

Claghorn G.C., Z. Thompson, K. Wi, L. Van, and T. Garland Jr. 2017. Caffeine stimulates voluntary wheel running in mice without increasing aerobic capacity. Physiol Behav 170:133–140.

Day L.M. and B.C. Jayne. 2007. Interspecific scaling of the morphology and posture of the limbs during the locomotion of cats (Felidae). J Exp Biol 210:642–654.

de Albuquerque R.L., K.E. Bonine, and T. Garland Jr. 2015. Speed and endurance do not trade off in phrynosomatid lizards. Physiol Biochem Zool 88:634–647.

Diogo R. 2017. Etho-eco-morphological mismatches, an overlooked phenomenon in ecology, evolution and evo-devo that supports ONCE (organic nonoptimal constrained evolution) and the key evolutionary role of organismal behavior. Front Ecol Evol 5:3. doi:10.3389/fevo.2017.00003.

Dlugosz E.M., M.A. Chappell, D.G. McGillivray, D.A. Syme, and T. Garland Jr. 2009. Locomotor trade-offs in mice selectively bred for high voluntary wheel running. J Exp Biol 212:2612–2618.

Dlugosz E.M., H. Schutz, T.H. Meek, W. Acosta, C.J. Downs, E.G. Platzer, M.A. Chappell, and T. Garland Jr. 2013. Immune response to a *Trichinella spiralis* infection in house mice from lines selectively bred for high voluntary wheel running. J Exp Biol 216:4212–4221.

Filipe V.M., J.E. Pereira, L.M. Costa, A.C. Maurício, P.A. Couto, P. Melo-Pinto, and A.S.P. Varejão. 2006. Effect of skin movement on the analysis of hindlimb kinematics during treadmill locomotion in rats. J Neurosci Methods 153:55–61.

Garland T., Jr. 2003. Selection experiments: an under-utilized tool in biomechanics and organismal biology. Pp. 23–56 in V.L. Bels, J.-P. Gasc, and A. Casinos, eds. Vertebrate biomechanics and evolution. BIOS Scientific Publishers, Oxford.

Garland T., Jr., and P.W. Freeman. 2005. Selective breeding for high endurance running increases hindlimb symmetry. Evolution 59:1851–1854.

Garland T., Jr., and C.M. Janis. 1993. Does metatarsal/femur ratio predict maximal running speed in cursorial mammals? J Zool 229:133–151.

Garland T., Jr., and S.A. Kelly. 2006. Phenotypic plasticity and experimental evolution. J Exp Biol 209:2344–2361.

Garland T., Jr., S.A. Kelly, J.L. Malisch, E.M. Kolb, R.M. Hannon, B.K. Keeney, S.L. Van Cleave, and K.M. Middleton. 2011*a*. How to run far: multiple solutions and sex-specific responses to selective breeding for high voluntary activity levels. Proc R Soc B 278:574–581.

Garland T., Jr., M.T. Morgan, J.G. Swallow, J.S. Rhodes, I. Girard, J.G. Belter, and P.A. Carter. 2002. Evolution of a small-muscle polymorphism in lines of house mice selected for high activity levels. Evolution 56:1267–1275.

Garland T., Jr., H. Schutz, M.A. Chappell, B.K. Keeney, T.H. Meek, L.E. Copes, W. Acosta, et al. 2011*b*. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. J Exp Biol 214:206–229.

Garland T., Jr., M. Zhao, and W. Saltzman. 2016. Hormones and the evolution of complex traits: insights from artificial selection on behavior. Integr Comp Biol 56:207–224.

Gatesy S.M. and N.S. Pollard. 2011. Apples, oranges, and angles: comparative kinematic analysis of disparate limbs. J Theor Biol 282:7–13.

Girard I., M.W. McAleer, J.S. Rhodes, and T. Garland Jr. 2001. Selection for high voluntary wheel-running increases speed and intermittency in house mice (*Mus domesticus*). J Exp Biol 204:4311–4320.

Gomes F.R., E.L. Rezende, J.L. Malisch, S.K. Lee, D.A. Rivas, S.A. Kelly, C. Lytle, B.B. Yaspelkis, and T. Garland Jr. 2009. Glycogen storage and muscle glucose transporters (GLUT-4) of mice selectively bred for high voluntary wheel running. J Exp Biol 212:238–248.

Gregory W.K. 1912. Notes on the principles of quadrupedal locomotion and on the mechanism of the limbs in hoofed animals. Ann N Y Acad Sci 22:267–294.

Guderley H., P. Houle-Leroy, G.M. Diffee, D.M. Camp, and T. Garland Jr. 2006. Morphometry, ultrastructure, myosin isoforms, and metabolic capacities of the "mini muscles" favoured by selection for high activity in house mice. Comp Biochem Physiol B 144:271–282.

Hannon R.M., S.A. Kelly, K.M. Middleton, E.M. Kolb, D. Pomp, and T. Garland Jr. 2008. Phenotypic effects of the "mini-muscle" allele in a large HR × C57BL/6J mouse backcross. J Hered 99:349–354.

Hansson L.-A. and S. Åkesson. 2014. Animal movement across scales. Oxford University Press, Oxford.

Herbin M., R. Hackert, J.-P. Gasc, and S. Renous. 2007. Gait parameters of treadmill versus overground locomotion in mouse. Behav Brain Res 181:173–179.

Higham T.E., A.V. Birn-Jeffery, C.E. Collins, C.D. Hulsey, and A.P. Russell. 2015. Adaptive simplification and the evolution of gecko locomotion: morphological and biomechanical consequences of losing adhesion. Proc Natl Acad Sci 112:809–814.

Horner A.M., D.W. Russ, and A.R. Biknevicius. 2011. Effects of early-stage aging on locomotor dynamics and hindlimb muscle force production in the rat. J Exp Biol 214:3588–3595.

Houle-Leroy P., H. Guderley, J.G. Swallow, and T. Garland Jr. 2003. Artificial selection for high activity favors mighty mini-muscles in house mice. Am J Physiol 284:R433–R443.

Irschick D.J. and T.E. Higham. 2015. Animal athletes: an ecological and evolutionary approach. Oxford University Press, New York.

Kelly S.A., T.A. Bell, S.R. Selitsky, R.J. Buus, K. Hua, G.M. Weinstock, T. Garland Jr., F. Pardo-Manuel de Villena, and D. Pomp. 2013. A novel intronic single nucleotide polymorphism in the *myosin heavy polypeptide 4* gene is responsible for the mini-muscle phenotype characterized by major reduction in hind-limb muscle mass in mice. Genetics 195:1385–1395.

Kelly S.A., P.P. Czech, J.T. Wight, K.M. Blank, and T. Garland Jr. 2006. Experimental evolution and phenotypic plasticity of hindlimb bones in high-activity house mice. J Morphol 267:360–374.

Kelly S.A., F.R. Gomes, E.M. Kolb, J.L. Malisch, and T. Garland Jr. 2017. Effects of activity, genetic selection, and their interaction on muscle metabolic capacities and organ masses in mice. J Exp Biol 2017. doi:10.1242/jeb.148759.

Kelly S.A., T.M. Panhuis, and A.M. Stoehr. 2012. Phenotypic plasticity: molecular mechanisms and adaptive significance. Compr Physiol 2:1417–1439.

Kolb E.M., S.A. Kelly, K.M. Middleton, L.S. Sermsakdi, M.A. Chappell, and T. Garland Jr. 2010. Erythropoietin elevates VO_2max but not voluntary wheel running in mice. J Exp Biol 213:510–519.

Kolb E.M., E.L. Rezende, L. Holness, A. Radtke, S.K. Lee, A. Obenaus, and T. Garland Jr. 2013. Mice selectively bred for high voluntary wheel running have larger midbrains: support for the mosaic model of brain evolution. J Exp Biol 216:515–523.

Koteja P., T. Garland Jr., J.K. Sax, J.G. Swallow, and P.A. Carter. 1999. Behaviour of house mice artificially selected for high levels of voluntary wheel running. Anim Behav 58:1307–1318.

Lovegrove B.G. and M. Mowoe. 2014. The evolution of micro-cursoriality in mammals. J Exp Biol 217:1316–1325.

Meek T.H., J.C. Eisenmann, and T. Garland Jr. 2010. Western diet increases wheel running in mice selectively bred for high voluntary wheel running. Int J Obes 34:960–969.

Meek T.H., J.C. Eisenmann, B.K. Keeney, R.M. Hannon, E.M. Dlugosz, and T. Garland Jr. 2014. Effects of early-life exposure to Western diet and wheel access on metabolic syndrome profiles in mice bred for high voluntary exercise. Genes Brain Behav 13:322–332.

Meek T.H., B.P. Lonquich, R.M. Hannon, and T. Garland Jr. 2009. Endurance capacity of mice selectively bred for high voluntary wheel running. J Exp Biol 212:2908–2917.

Middleton K.M., S.A. Kelly, and T. Garland Jr. 2008. Selective breeding as a tool to probe skeletal response to high voluntary locomotor activity in mice. Integr Comp Biol 48:394–410.

Nagy N., R.J. Nonneman, T. Llanga, C.F. Dial, N.V. Riddick, T. Hampton, S.S. Moy, et al. 2017. Hip region muscular dystrophy and emergence of motor deficits in dysferlin-deficient Bla/J mice. Physiol Rep 5:e13173. doi:10.14814/phy2.13173.

Rezende E.L., T. Garland Jr., M.A. Chappell, J.L. Malisch, and F.R. Gomes. 2006*a*. Maximum aerobic performance in lines

of *Mus* selected for high wheel-running activity: effects of selection, oxygen availability and the mini-muscle phenotype. J Exp Biol 209:115–127.

Rezende E.L., F.R. Gomes, M.A. Chappell, and T. Garland Jr. 2009. Running behavior and its energy cost in mice selectively bred for high voluntary locomotor activity. Physiol Biochem Zool 82:662–679.

Rezende E.L., F.R. Gomes, J.L. Malisch, M.A. Chappell, and T. Garland Jr. 2006b. Maximal oxygen consumption in relation to subordinate traits in lines of house mice selectively bred for high voluntary wheel running. J Appl Physiol 101:477–485.

Rezende E.L., S.A. Kelly, F.R. Gomes, M.A. Chappell, and T. Garland Jr. 2006c. Effects of size, sex, and voluntary running speeds on costs of locomotion in lines of laboratory mice selectively bred for high wheel-running activity. Physiol Biochem Zool 79:83–99.

Rhodes J.S., S.C. Gammie, and T. Garland Jr. 2005. Neurobiology of mice selected for high voluntary wheel-running activity. Integr Comp Biol 45:438–455.

Rhodes J.S., H. van Praag, S. Jeffrey, I. Girard, G.S. Mitchell, T. Garland Jr., and F.H. Gage. 2003. Exercise increases hippocampal neurogenesis to high levels but does not improve spatial learning in mice bred for increased voluntary wheel running. Behav Neurosci 117:1006–1016.

Roach G.C., M. Edke, and T.M. Griffin. 2012. A novel mouse running wheel that senses individual limb forces: biomechanical validation and in vivo testing. J Appl Physiol 113:627–635.

Schutz H., H.A. Jamniczky, B. Hallgrimsson, and T. Garland Jr. 2014. Shape-shift: semicircular canal morphology responds to selective breeding for increased locomotor activity. Evolution 68:3184–3198.

Seckel L. and C. Janis. 2008. Convergences in scapula morphology among small cursorial mammals: an osteological correlate for locomotory specialization. J Mamm Evol 15:261–279.

Smith B.J.H., L. Cullingford, and J.R. Usherwood. 2015. Identification of mouse gaits using a novel force-sensing exercise wheel. J Appl Physiol 119:704–718.

Stein B.R. and A. Casinos. 1997. What is a cursorial mammal? J Zool 242:185–192.

Swallow J.G., P.A. Carter, and T. Garland Jr. 1998a. Artificial selection for increased wheel-running behavior in house mice. Behav Genet 28:227–237.

Swallow J.G., T. Garland Jr., P.A. Carter, W.Z. Zhan, and G.C. Sieck. 1998b. Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). J Appl Physiol 84:69–76.

Swallow J.G., T. Garland Jr., P. Koteja, and P.A. Carter. 1999. Locomotor activity, food consumption, and body composition in house mice selected for high wheel-running activity. FASEB J 13:A419–A419.

Swallow J.G., J.P. Hayes, P. Koteja, and T. Garland Jr. 2009. Selection experiments and experimental evolution of performance and physiology. Pp. 301–351 in T. Garland Jr. and M.R. Rose, eds. Experimental evolution: concepts, methods, and applications of selection experiments. University of California Press, Berkeley.

Swallow J.G., P. Koteja, P.A. Carter, and T. Garland Jr. 2001. Food consumption and body composition in mice selected for high wheel-running activity. J Comp Physiol B 171:651–659.

Syme D.A., K. Evashuk, B. Grintuch, E.L. Rezende, and T. Garland Jr. 2005. Contractile abilities of normal and "mini" triceps surae muscles from mice (*Mus domesticus*) selectively bred for high voluntary wheel running. J Appl Physiol 99:1308–1316.

Talmadge R.J., W. Acosta, and T. Garland Jr. 2014. Myosin heavy chain isoform expression in adult and juvenile mini-muscle mice bred for high-voluntary wheel running. Mech Dev 134:16–30.

Usherwood J.R. 2013. Constraints on muscle performance provide a novel explanation for the scaling of posture in terrestrial animals. Biol Lett 9:20130414.

Vanhooydonck B., R.S. James, J. Tallis, P. Aerts, Z. Tadic, K.A. Tolley, G. Measey, and A. Herrel. 2014. Is the whole more than the sum of its parts? evolutionary trade-offs between burst and sustained locomotion in lacertid lizards. Proc R Soc B 281:20132677.

Wallace I.J., and T. Garland Jr. 2016. Mobility as an emergent property of biological organization: insights from experimental evolution. Evol Anthropol 25:98–104.

Waters R.P., K.J. Renner, R.B. Pringle, C.H. Summers, S.L. Britton, L.G. Koch, and J.G. Swallow. 2008. Selection for aerobic capacity affects corticosterone, monoamines and wheel-running activity. Physiol Behav 93:1044–1054.

Wilson A.M., J. Lowe, K. Roskilly, P.E. Hudson, K. Golabek, and J. McNutt. 2013. Locomotion dynamics of hunting in wild cheetahs. Nature 498:185–189.

Wong L.E., T. Garland Jr., S.L. Rowan, and R.T. Hepple. 2009. Anatomic capillarization is elevated in the medial gastrocnemius muscle of mighty mini mice. J Appl Physiol 106:1660–1667.

Young J.W., R. Danczak, G.A. Russo, and C.D. Fellmann. 2014. Limb bone morphology, bone strength, and cursoriality in lagomorphs. J Anat 225:403–418.