

**Run far, gain weight, repeat:
seasonal cycles in locomotor activity and body mass of laboratory mice**

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Abstract: Seasonal rhythms are widely documented in wild organisms across diverse species. Seasonality in laboratory rodents, however, remains an intriguing phenomenon, considering that laboratory conditions are supposed to be constant. Here, I study seasonality in locomotor activity and body mass measurements taken as part of a long-term artificial selection experiment involving ~36,002 house mice (Hsd:ICR) spanning 78 generations across 23 years. The selection experiment consists of four control (C) lines, and four replicates of selected (S) lines bred specifically for high voluntary wheel running. Continuous wavelet transforms (CWT) revealed strong circannual rhythmicity with inverted 12-month cycles in both wheel-running distance and body mass, although amplitude declined in the last 5 years. I applied generalized additive mixed models to test group differences between sex, line type (S vs C), and laboratory location (University of Wisconsin–Madison, where the experiment started and ran through 31 generations, versus University of California, Riverside) in running distance, duration, speed, and body mass (measured before and after 6 days of wheel running). Running distance showed greater amplitude in S than C lines and in males than females. Running duration and body mass cycles were stronger in Wisconsin than Riverside, again with males showing higher amplitudes than females. For running speed, S lines displayed more seasonality than C lines. These results unambiguously demonstrate seasonal cycles in behaviour and morphology under controlled laboratory conditions, without, however, being able to provide a definitive explanation (i.e., endogenous circannual mechanisms maintained across generations versus subtle environmental cues). This thesis contributes to the understanding of seasonal cycles and stresses the importance of considering them to improve reproducibility and reliability of research using laboratory mice.

Résumé: Les rythmes saisonniers sont bien documentés chez de nombreuses espèces, mais restent mystérieux chez les rongeurs de laboratoire, pour lesquels les conditions sont supposées constantes. J'étudie la saisonnalité de l'activité locomotrice et de la masse corporelle dans le cadre d'une expérience de sélection artificielle à long terme impliquant ~36 002 souris domestiques (Hsd:ICR) sur une période de 23 ans (78 générations). L'expérience est constituée de quatre lignées contrôles (C) et quatre lignées sélectionnées (S) pour une haute activité volontaire sur roue. Une analyse par transformées en ondelettes continues (CWT) révèle des cycles annuels inversés de 12 mois pour la distance parcourue et la masse corporelle. Des modèles additifs mixtes généralisés ont été employés pour tester si la saisonnalité était différente entre mâles vs femelles, lignées C vs S, et à l'Université du Wisconsin–Madison, où l'expérience a débuté et s'est déroulée pendant 31 générations, versus l'Université de Californie à Riverside. La distance présente une amplitude plus élevée chez les lignées S que C et chez les mâles que les femelles. Pour la vitesse de course, les lignées S sont plus saisonnières que les lignées C. La durée de course et les cycles de masse corporelle sont plus marqués au Wisconsin qu'à Riverside, avec également les mâles démontrant une amplitude saisonnière plus prononcée que les femelles. Ces résultats démontrent sans ambiguïté la persistance de cycles saisonniers en comportement et morphologie en conditions contrôlées, sans toutefois pouvoir fournir d'explication définitive, suggérant soit des mécanismes circannuels endogènes maintenus à travers les générations, soit l'influence d'indices environnementaux subtils. Cette thèse contribue à la compréhension des cycles saisonniers et souligne l'importance de les prendre en compte pour améliorer la reproductibilité et la fiabilité des études sur les souris de laboratoire.

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Introduction

Circannual rhythms in biology

All living beings, from unicellular protists (Anderson & Keafer, 1987) to humans (Beltran-Frutos et al., 2022; Bronson, 1995; Nelson & Demas, 1996; Shephard & Aoyagi, 2009), possess a sense of time which they use to sequence and regulate biological activities to specific times of the day or year (Kumar, 2017). Biological rhythms have been categorized into ultradian (shorter than daily cycles), circadian (daily cycles), tidal/lunar (aligned with ocean tides and lunar day), and circannual (annual cycles). Annual biological cycles are typically measured as recurrent changes in physiology, metabolism, and behaviour over the course of a year. Such cycles are often interpreted as adaptations that allow organisms to anticipate and prepare for seasonal variations (Helm & Lincoln, 2017; Leise, 2013; Schwartz & Andrews, 2013). Typical examples include migration in birds, hibernation or breeding seasons in mammals, and molting as winter or summer approaches. By timing their life history to favorable seasons and resource availability, organisms can maximize their Darwinian fitness (Boyce, 1979; Nylin & Gotthard, 2025; Stearns, 1998).

Although many wild animals can respond to cyclic environmental changes, observed seasonal changes alone are insufficient to imply the presence of “true” endogenous circannual rhythms. The literature suggests that seasonal cycles are shaped by both environmental cues and internal time-keeping mechanism that maintain a ~12-month period over the lifetime of individuals (Helm & Lincoln, 2017; Kumar, 2017). The terms “seasonal”, “annual”, and “circannual” are sometimes used as mutual definitions for 12-month rhythms. Dark & Zucker (1983) categorized seasonal rhythms into three

types. Type I rhythms are endogenous but do not typically persist beyond a single cycle in the absence of environmental cues. Type II rhythms are considered "true" circannual rhythms only if it persists with a near-yearly period for at least two consecutive cycles under constant environmental conditions (Gwinner, 1981). This definition distinguishes true circannual endogenous rhythms (Type II circannual oscillators) from cycles dependant and triggered by the detection of external cues. Type III rhythms, by contrast, are driven entirely by environmental cues and lack a major endogenous component (e.g., pollen production and seasonal allergic responses in humans).

The environment provides cues for seasonal changes, acting as Zeitgebers ("time givers") to synchronize physiology and behaviour with the most favorable time of the year (Sar   et al., 2021). Seasonal organisms typically rely on changes in day length (i.e., photoperiod) and other key Zeitgebers, such as temperature, food availability, social factors, and rainfall (Bradshaw & Holzapfel, 2007; Foster & Kreitzman, 2009; Kumar, 2017; Sar   et al., 2021). Photoperiod has long been recognized as a primary zeitgeber driving seasonal rhythms in mammals, including rodents. Controlled experiments have demonstrated that changes in day length can significantly influence circadian behaviours and circannual patterns, including reproduction, metabolism, and physical activity (Goldman, 2001; Gorman & Zucker, 1995). Changing day lengths can induce changes in body fat composition and activity levels, as observed in the wild vole *Microtus montanus* (Hoffmann, 1982; Petterborg, 1978). Additionally, locomotor activity may be influenced by fluctuations in food availability and the low temperatures of winter, as observed in South African hedgehogs (*Atelerix frontalis*) (Gillies et al., 1991) and North American

heteromyid desert rodents (*Dipodomys microps*, *D. merriami*, and *Perognathus longimembris*) (Kenagy, 1973).

Evidence of internally generated, circannual rhythms has been found in long-lived or strongly seasonal species, indicating the presence of a self-sustained annual timer independent of environmental input (Type II). For instance, studies under constant conditions have shown the persistence of circannual cycles in laboratory stonechats (*Saxicola torquata*) (Gwinner & Dittami, 1990) and golden-mantled ground squirrels (*Citellus lateralis*) (Pengelley et al., 1978). However, the annual cycles began to drift away from the 12-month period, with periods shorter (~10.5 months) than the true calendar year (termed “free running”). Free-running cycles demonstrates the true endogenous nature of the cycles and suggests that Zeitgebers are required to accurately time circannual cycles. In some cases, when animals are deprived of such major Zeitgebers as photoperiod or temperature, annual rhythms lose coherence or cease entirely (Type I) (Gwinner, 1981; Joy & Mrosovsky, 1985). Circannual timing mechanisms remain less well understood than the circadian clock. Nevertheless, it is generally accepted that internal rhythms interact with environmental cues (particularly photoperiod) to trigger phenotypic changes that synchronize with annual environmental changes (Beltran-Frutos et al., 2022; Edery, 2000; Helm & Lincoln, 2017; Suckow & Tirado-Muñiz, 2023).

Seasonality in laboratory animals

Laboratory animals, particularly rodents, are typically maintained under constant, controlled conditions to minimize extrinsic variability and improve experimental

reproducibility (Suckow & Tirado-Muñiz, 2023). Typically, laboratory rodents are held on a 12:12 hour light-dark cycle, at room temperature (e.g., 21°C), and are fed with the same food and housed similarly throughout the year. If laboratory animals experience no change in day length or temperature throughout the year, then they should not show annual oscillations; hence, many researchers assume that such controlled conditions effectively eliminate seasonal influences on laboratory animals. Indeed, although experiments routinely account for such factors such as strain, age, sex, and circadian variation (Perissin et al., 2003), the time of the year, or season, is rarely considered a variable of concern in the design of experiments and is often overlooked in laboratory research.

However, some studies have uncovered 12-month cycles in laboratory animals, hinting at the presence of seasonal effects even in controlled settings (Gwinner & Dittami, 1990; Kondo et al., 2006). For example, one 2-year study of laboratory rats documented that mating success and litter production peaked each year between May and August, and declined between December and February (Lee & McClintock, 1986). Similarly, long-term studies on breeding colonies of house mice (*Mus musculus* and *domesticus*) kept under constant laboratory conditions showed that females produced larger litters in spring and summer than in autumn and winter (Drickamer, 1977, 1990). Drickamer (1977) proposed the existence of an inherited annual rhythm in reproduction, supported by its persistence across generations despite constant environmental conditions. Comparable results were found in *Peromyscus*, where four of eight taxa showed reproductive patterns in the laboratory that corresponded closely with those of their wild populations (Drickamer & Vestal, 1973).

Although the research cited above suggests the presence of circannual cycles in reproduction of laboratory rodents, the empirical evidence for other phenotypes, such as behaviour, is mixed. A review by Ferguson and Maier (2013) concluded that, overall, few robust circannual changes could be detected in laboratory rodents housed in standard laboratory settings. Behavioural tests (response to pain, depression- and anxiety-like behaviour) showed inconsistent or only minor seasonal trends across studies. None of the studies reviewed by Ferguson and Maier (2013) reported a statistically significant effect of season on activity, learning or sexual behaviour. They pointed out that if seasonal rhythms truly exist in these rodents under constant conditions, then the effect sizes are likely small, which might explain why some experiments detect them while others do not. In a study on *Microtus* voles published after Ferguson and Maier (2013), two behaviours demonstrated seasonal patterns: boldness (indicated by shorter latencies in barrier activity level) and open-field activity peaked in spring and summer relative to other times of the year, and the seasonal cycles were consistently greater in males than in females (Eccard & Herde, 2013). Another study found recurring patterns of 2-4 months in cage floor activity and feeding behaviour, but not in body mass (Pernold et al., 2021).

Some research on laboratory rodents reported persistent seasonal rhythms in body mass under constant environmental conditions. Young female mice (21 days old) have higher body mass during spring and summer compared to other seasons (Drickamer, 1977). Similarly, in the golden-mantled ground squirrel (hibernating mammal), body mass follows a robust 10.5-month cycle peaking in winter (Pengelley et al., 1978; Pengelley & Asmundson, 1974). The transition between winter and summer phenotypes persisted for consecutive years (2-3 years) regardless of environmental lighting

conditions, whether short or long days, constant light, or constant darkness, suggesting a true endogenous type II rhythm (Zucker, 2001). A study on the golden-mantled ground squirrel, conducted over two years at the University of California, Riverside, showed annual variation in body mass and voluntary wheel-running distance (Pengelley & Fisher, 1966). They showed a peak in activity in spring followed by a steady decline in the summer to reach a minimum in winter. Wheel activity was in inverse ratio to the body mass, which increased during summer and fall and decreased in summer. Pengelley & Fisher (1966) proposed an endogenous annual rhythm: “The problem, therefore is to determine which exogenous factors, or combination of them, acting through sensory apparatus, keep the endogenous rhythms in phase with the seasons.”

Collectively, studies on laboratory rodents hints that seasonal variations may occur in physiology, morphology, and behaviour despite stable photoperiod, temperature, and food. This has been interpreted by some authors as that laboratory rodents retain a vestige of an endogenous annual timing mechanism (a circannual clock), even after generations of breeding under stable conditions. Alternatively, others speculated that subtle seasonal cues are infiltrating the housing facilities and influencing the animals. Possible seasonal cues can vary depending on the housing facilities, but may include shifts in humidity, heating and ventilation equipment, ambient noise, pollen entering through the clothes of the staff, or even the behaviour of human personnel working with the mice (Suckow & Tirado-Muñiz, 2023). If true, then one could expect that the seasonality of laboratory rodents should vary from one facility to another depending on the efficiency of the filtering systems, local procedures, and the surrounding environment (e.g., laboratories in seasonally variable versus more annually stable climates).

Challenges of studying circannual rhythms

Despite growing interest in seasonal biology in laboratory rodents, robust evidence for circannual rhythms remains limited. The limited evidence for seasonal or circannual rhythms in laboratory rodents come mainly from the challenges of studying biological cycles on a 12-month scale. A complete circannual cycle spans 365 days from peak to peak, and to meet the formal definition of an endogenous circannual rhythm, at least two full cycles must be observed under constant conditions (Gwinner, 1981). Hence, studying seasonality requires long-term efforts and data spanning multiple generations and years, making them resource-intensive and logistically demanding compared to circadian experiments. These challenges often lead to studies with small sample sizes within uncontrolled and unreplicated experimental setups, which severely reduce the statistical power needed to detect subtle annual fluctuations (Pernold et al., 2021). Signals of a circannual rhythm might be low in amplitude making them hard to distinguish from noise without very large sample sizes and statistical power.

In addition to logistical and statistical constraints, studies on seasonality in laboratory rodents suffer from sampling limitations. For example, Ferguson and Maier (2013) noted that several studies assessed behavioural endpoints at only two time points within a single year. With only two measurements, any apparent trend (or lack thereof) could simply reflect random variation, cohort differences, or subtle environmental changes, making it insufficient to test for annual rhythms. To properly study biological cycles, researchers must not only collect data over multiple years but also sample frequently and evenly throughout the year. Furthermore, most studies have relied

on simple comparisons of group means or linear models, which are not well suited to detect subtle, nonlinear, or time-varying rhythms.

Importance of studying seasonality in laboratory rodents

Studying seasonality in laboratory animals has significant implications not only for chronobiology, but for any field that relies heavily on laboratory models (e.g., medicine, pharmacology, animal behaviour). Given that annual rhythms are widespread across biological systems, they provide a common structure for research across disciplines (Stevenson et al., 2015). Even if subtle, circannual cycles may cause poor reproducibility by introducing undesirable and uncontrolled variation, and potentially acting as a confound across studies. For example, circannual cycles could cause poor reproducibility by increasing the difficulty in detecting treatment effects in some seasons, but not others. Considering the importance of understanding circannual cycles in laboratory rodents (and other model animals), there remains surprisingly large knowledge and empirical gaps. Upon finding weak support for seasonal or circannual effects on laboratory rodent behaviour in the published literature, Ferguson and Maier (2013) ended their review by stating: “We encourage our fellow researchers to explicitly describe such effects (or the lack thereof) as additional results will further resolve this markedly divisive issue. Specifically, those studies in which the identical behaviours are measured during several seasons and ideally, across several years would substantially contribute to the literature.”

Experimental design of this study

To evaluate potential circannual cycles, I used one of the longest time series ever collected on house mice held under standard laboratory conditions. The dataset consists of behavioural and morphological measurements made over 78 generations spanning 23 years (1993-2016), collected as part of an artificial selection experiment on voluntary wheel running (Swallow et al., 1998). The experiment was conducted at the University of Wisconsin–Madison (hereafter ‘Wisconsin’, or WIS) for 31 generations, after which it was moved to the University of California, Riverside (UCR), where the experiment continued under standard laboratory conditions until generation 112 (T. Garland Jr. personal communications). The selection experiment consists of four control (C) lines, and four replicates of selected (S) lines bred specifically for high voluntary wheel running, measured as the number of wheel revolutions on days 5 and 6 of a 6-day period of wheel access when young adults. As a result of selection, voluntary running distance has substantially increased in all four S lines over the first ~20 generations (~2.5-fold increase compared to C lines; [Figure 1](#)), demonstrating a genetic basis for the selected trait (Kolb et al., 2010). A few remarkable correlated responses have occurred (i.e., as a result of selection on running distance): S mice evolved to be smaller in body length and mass, and leaner, even when wheel access is restricted (Hiramatsu & Garland, 2018; Meek et al., 2010; Swallow et al., 1999, 2001). Also, in females, running distance (measured in wheel revolutions per day) increased mainly through changes in running speed (average wheel revolutions per minute) rather than running duration (minutes per day), whereas in males, the increase in running distance occurred through both changes in duration and speed (Garland et al., 2011). Although the absolute difference in mean running distance between the S and C lines is slightly larger for females, the fold

difference is similar between sexes (Careau et al., 2013; Garland, 2003; Swallow et al., 1998). In a study conducted at generations 21–22, fertility, litter size, mean offspring mass, and most aspects of maternal-care behaviour did not differ statistically between S lines and C lines (Girard et al., 2002; Khan et al., 2024).

One intriguing observation from this experiment is the noticeable oscillations in wheel-running distance and body mass occurring across generations (see Figure 1 in Careau et al. 2013). Upon examination of the time series as a function of date (instead of generations as usual), it becomes evident that both S and C lines have higher activity levels during the winter months compared to the summer months ([Figure 1](#)). Although these seasonal variations were noted before (Careau et al., 2013), they were mostly considered a nuisance variable and have never been the focus of a study.

Objective

This research aims to expand the understanding of seasonal rhythms in laboratory mice. Using the long-term dataset from the selection experiment, I will describe the extent of the seasonal variation occurring in voluntary wheel running and body mass. My analysis consists of two main parts: a first part using the Continuous Wavelet Transform (CWT) method and a second part using generalized additive mixed models (GAMMs). In the first part, I use CWT to provide an objective and qualitative description of the period and amplitude of the generation-to-generation variation in voluntary wheel-running distance and body mass. The second part of my analysis aims to apply GAMMs and use cyclic splines to test for differences in seasonality between sexes (males vs females), line type (control vs selected lines), or universities (Wisconsin vs Riverside). Another objective is

to extend the analysis on the underlying traits of running distance, which is determined by the product of average running speed and running duration. By testing for seasonality in these components, I will determine if a cycle in running distance is primarily driven by a cycle in running duration or speed, or both. This information, in addition to potential differences in the seasonality of males vs females, C vs S lines, and Wisconsin vs Riverside generations, may shed light on the nature of the seasonality observed in laboratory mice.

Methods

Animals

Hsd:ICR mice trace their origins to 100 female and 30 male Swiss-Webster albino mice obtained in 1947 by the Institute for Cancer Research (ICR) in Philadelphia, Pennsylvania (Hauschka & Mirand, 1973). The ICR lines were established through a breeding program aimed at improving litter size and weaning success. Charles River Laboratories later acquired these lines and in 1983, Harlan Sprague Dawley (Hsd) purchased the ICR mice from Charles River Laboratories. Further details on the strain's history are provided by Dohm (1994), see also Girard et al. (2002).

The population for the selection experiment was founded with 224 (112 males and 112 females) outbred Hsd:ICR mice purchased from Harlan Sprague Dawley, Indianapolis, IN. These founder animals were designated generation -2. They were paired randomly, and their offspring, designated generation -1, were randomly assigned to eight replicate lines (Swallow et al., 1998). Their offspring were designated generation 0, the generation at which artificial selection was first applied in four of the eight lines. The

four S lines were selectively bred for high wheel-running activity, measured by total daily wheel revolutions, while the remaining four C lines served as controls with no selection for activity level. In the S lines, the highest-running male and female from each family were designated as breeders, whereas in the C lines, breeders were randomly chosen from each family (sib-matings were prohibited in all lines). In S lines, all offspring from all families were kept and tested on wheels (see below), whereas in C lines only two males and two females were randomly kept from each family and tested on wheels.

A typical generation includes wheel testing of over 400 mice from the S lines, based on approximately 10 families per line and an average of 10.5 offspring per family across four S lines. In the C lines, only two males and two females are randomly chosen at weaning from each family, in four lines, resulting in around 160 control mice typically tested per generation. Selection procedures are further detailed in previous publications (Careau et al., 2013; Garland et al., 2011; Swallow et al., 1998). I studied mice from generations 0 to 78, spanning across October 1993 to June 2016 (~23 years). It is important to note that after generation 31, the mice were moved from the Wisconsin to UCR, and no measures were taken and no selection was applied for four generations (32 to 35) across 15 months during the relocation (see gap in [Figure 1](#)).

Housing and husbandry conditions

Mice were housed in standard clear plastic cages ($27 \times 17 \times 12.5$ cm) with metal or wire tops and wood shavings. Animals were housed in same-sex groups of up to four individuals, except during breeding or measurements of behavioural traits. Mice were maintained on a 12-h light/12-h dark cycle (lights on 0700 h), which is also maintained

during the wheel-running trials. Room temperature was regulated to approximately 22-23°C and humidity remained relatively constant. Throughout the experiment, water, and fixed formula food (Harlan Teklad Laboratory Rodent Diet [W]8604) were provided *ad libitum*. Breeding females were given Teklad S-2335 Mouse Breeder diet (Krugner-Higby et al., 2006).

Wheel-running protocol

Voluntary wheel running was assessed using Wahman-type activity wheels (1.12 m circumference, 0.36 m diameter; Lafayette Instruments, Indiana). The standard housing cages were modified to incorporate a 5.5-cm-long stainless-steel tube, inserted through a 7.7-cm-diameter hole in the side of the cage. This modification allowed free access to a wheel. Each wheel was equipped with a photocell counter, which was connected to an MS-DOS personal computer. The software, sourced from San Diego Instruments (San Diego, CA), recorded activity as the number of revolutions every minute for 6 consecutive days across the 200 wheels. Activity wheels were evenly distributed between two rooms in both Wisconsin and UCR. Each generation, mice were measured in three to four batches of around 200 individuals per batch, over three (or four if needed) consecutive weeks. For each batch, mice of six to nine weeks of age were randomly and individually placed into the testing cages on the morning of the first day, with data collection starting at 13:00 (CST or PST). Body mass was measured immediately before placement and again after removal on the sixth day (Swallow et al., 1998). Every 24 hours, the wheels were inspected to clear food pellets and wood shavings. Each mouse was measured for six days only once. After all the measurements were completed for a

generation, pairs were created and allowed to breed to produce offspring for the subsequent generation. At Wisconsin, generations (0-31) were measured at 3 months intervals (4 generations per year), whereas at UCR (36-78), generations were measured every 3 to 4 months (3 generations per year).

Data processing

Wheel measurements on days 5 and 6 were used for statistical analysis, because the selected trait was the total running distance (revolutions/day) on these two days. Other measurements were compiled from the start of the experiment (generation 0) through generation 78 (total of 79 generations), including running duration (min/day, based on the number of minutes that had at least one full revolution), average running speed (revolutions/min) and body mass (g; measured on the day 1 and again on day 6). Data from generation 52 were entirely excluded because mice were substantially older than a typical generation when wheel running was measured. Generations 63, 64, and 67 were also excluded because technical problems yielded unusable measurements for many individuals. No measures were taken, and no selection was applied for four generations (32, 33, 34, and 35) across 15 months during the relocation of mice to UCR ([Figure 1](#)). The resultant data set for wheel-running distance, duration, and speed is of 36,002 observations from 71 generations, comprised of 10,878 C and 25,124 S mice. For body mass, the dataset consists of 35,028 observations across 69 generations, including 10,567 C mice and 24,461 S mice.

Statistical analyses

a) CWT

The periodicity of wheel-running distance and body mass was assessed with Continuous Wavelet Transform (CWT) method in the `analyze.wavelet()` function from the `WaveletComp` package (Roesch & Schmidbauer, 2014) in R (Version 2024.09.1+394; R Core Team, 2025). Wavelet analysis is a powerful tool used to detect rhythmicity and measure periodicity in various types of molecular and behavioural data (Bajaj, 2021; Halali et al., 2021). For example, CWT was used to analyze bioluminescence rhythms of mRNA and proteins in cell cultures (Price et al., 2008).

A CWT analysis yields both amplitude and phase information over time using a Morlet wavelet (Grinsted et al., 2004; Torrence & Compo, 1998). Unlike traditional methods, such as Fourier analysis, CWT analysis allows for the examination of localized intermittent periodicities within time series data allowing a more precise understanding of signals. Furthermore, the time series data does not require prior detrending or smoothing to eliminate baseline effects and noise. This feature of the wavelet analysis is particularly useful, considering the initial response to selection and the subsequent limit reached in generations ~20-30 in all S lines (Careau et al., 2013).

The wavelet power spectrum provides a visual characterization of the energy distribution (spectral density) of a time series across a two-dimensional time-frequency figure. The horizontal axis represents time since the start of the experiment, while the vertical axis represents the frequency domain (period), expressed in months. The color intensity on the spectrum corresponds to the amplitude of the signal, where higher color intensity indicates greater power. The detected periods are indicated as solid black lines and can move on the y-axis as the period(s) detected changes. I used CWT to identify and

characterize periodicities present within the data in running distance and body mass for each line separately. To allow a combined (cross-wavelet, see below) analysis of activity and body mass, I excluded data from mice for which wheel-running measurements and body mass on a given day was missing. Body mass measurements were unavailable for two generations (60 and 73) during which running distance was recorded. Those generations were excluded from the dataset for the CWT analysis only. The mean trait value was computed for each line in each generation, resulting in 69 observations (generation means) per line.

After conducting separate CWT analyses for running distance and body mass, I conducted a cross-wavelet analysis using the `analyze.coherency()` function from the `WaveletComp` package, a technique that provides insights into the synchronization of two variables within a time series. This analysis determines whether running distance and body mass are synchronized or oscillating out of phase. Additionally, the coherency analysis quantifies the timing and nature of any phase difference, identifying which variable leads and which lags. For detailed theoretical background, refer to Cazelles et al. (2008).

Although the CWT provides an objective description of the periodicity in running distance and body mass, the technique has several limitations when applied to my dataset. For computational efficiency, CWT necessitates regularly spaced time points in the data series. However, measurements were taken approximately every 3 to 4 months, resulting in uneven temporal spacing across generations. To address this, linear interpolation was applied using the `approx()` function in R to obtain even monthly-spaced measurements of running distance and body mass (see [Figure 2](#)). Although imputation is a common

practice in wavelet-based time series analysis (Lee & Meng, 2007), it can reduce statistical power and represent a major limitation when applied to my dataset. For example, the lack of data in generations 31 to 35 (during the university change, see above) forced linear imputation between 2002 and 2003; hence, one would expect no cycle during that period (note, this also applies to excluded generations). Additionally, the CWT approach operates on generation-level averages, must be applied for each line separately, does not support random effects (hence the separate analysis for each line, using generation means), and does not allow for formal hypothesis testing or experimental group comparisons.

b) GAMM

Generalized Additive Mixed Models (GAMMs; Wood, 2017) were used to model nonlinear and cyclic patterns in behaviour (voluntary wheel-running distance, speed, and duration) and body mass across the experiment in R (Version 2024.09.1+394). GAMMs are useful for analyzing biological time series with complex temporal structures while accounting for both fixed and random effects. Compared to such methods as Locally Estimated Scatterplot Smoothing (LOESS) or Generalized Linear Models (GLMs), GAMMs provide greater flexibility by allowing unspecified nonlinear functions (smooths) for predictors. This enables the modeling of long-term trends and cyclic seasonal components. GAMMs can also handle irregular sampling intervals (Pedersen et al., 2019), such as the current time series. GAMMs also facilitate hypothesis testing and model selection through such criteria as the Akaike Information Criterion (AIC) (Akaike, 2011; S. N. Wood et al., 2016). Models were fitted using R package `gamm4` (Wood &

Scheipl, 2009), which implements the `mgcv` smoothing framework and estimates random effects via `lme4` package. This approach was chosen over the `gamm` function (which estimates random effects via `nlme` package) to reduce computing time with the large number of random effects in the models.

To examine annual cycles in behaviour (voluntary wheel-running distance, speed, and duration) and body mass, I fitted GAMMs assuming a Gaussian error distribution. All models were fitted on the raw data and included a series of fixed effects chosen based on prior knowledge of the system, including age at measurement, sex, line type (C vs S), and the interaction between sex and line type. These variables were included to control for known sources of variation in running distance due to developmental, genetic, and sex-specific differences. University location was included as a fixed effect to allow distinct intercepts between the two environments, which was necessary because it was also used as a grouping factor for smooth terms (see below).

A series of random effects were also used to account for the nested and hierarchical structure of the experiment. These included dam ID (to control for nonindependence in offspring that come from the same family), replicate line identity (8 levels, C1, C2, C4, C5, S3, S6, S7, and S8), and generation (to capture unknown effects that could apply to all lines in a specific generation). To avoid pseudoreplication associated with the spatiotemporal structure of the behavioural measurements (i.e., ~400 to 600 mice tested per generation using 200 wheels across two rooms), I included a random effect of test batch nested within generation (to capture unknown effects that could apply to a specific batch within a generation), test room nested within batch and generation (to capture unknown effects that could apply to a specific room within a

batch), and wheel number nested within generation (to capture unknown effects that could apply to a specific wheel within a generation, e.g., defective bearing). The spatiotemporal random effects that apply to wheel measurements do not apply to body mass measurements; therefore, random effects of batch, room, and wheel were not fitted to models of body mass.

Two smoothing terms were included in the models. The first is a smoothing term for the *Date* variable “days since the start of the experiment”, which varied continuously from 0 to 8,245 days, fitted separately for control and selected lines using the `by=` argument in the `gam4 ( )` function. This structure allows the model to estimate long-term temporal trends separately for S and C lines, capturing the different non-linear temporal changes occurring in S and C lines. The basis dimension was set to $k = 4$ to model long-term trends while avoiding overfitting. Model checks with `gam.check ( )` confirmed that this choice did not under-smooth the model.

To model seasonal effects, I fitted cyclic cubic regression splines (`bs = "cc"`) to the variable “day of the year” (DOY), which varied from 1 (January 1st) to 365 (December 31st). This approach allows smooth, continuous modeling of annual cycles by maintaining continuity between December 31 and January 1. Leap years (6) were handled using the `yday` function from the `lubridate` package (Grolemund & Wickham, 2011). In a seasonal cycle, a change in curvature occurs twice within a year, resulting in two inflection points. To capture this pattern while avoiding overfitting, I set the basis dimension to $k = 4$. For cyclic cubic splines, the maximum effective degrees of freedom (edf) is $k - 1$, because one basis spline is lost due to cyclic constraints of matching the end and start of the year smoothly. Setting $k = 4$ allows for up to three edf, providing

enough flexibility to model typical annual patterns (e.g., a sinusoidal curve or a more complex shape), without excessive “wiggleness” that captures non-seasonal variation. The edf reflects the smooth’s flexibility rather than the magnitude of seasonal change, where a higher edf indicates a more flexible fit, not necessarily a stronger seasonality.

To test for potential differences in cyclicity in males vs females, C vs S, and Wisconsin vs Riverside, I compared different models and selected the most parsimonious based on AIC (Burnham & Anderson, 2004; Wood, 2017). While keeping the same fixed and random effects, I fitted GAMMs with different numbers of cyclic splines, ranging from the simplest model with a single cyclic spline fitted for the entire dataset, to the most complex model with eight different cyclic splines fitted to each combination of sex, line type, and laboratory. If the simplest model has the lowest AIC, then the conclusion would be that a common cyclic spline appropriately describes the annual variation in the trait. By contrast, a lower AIC in the model that fits the cyclic splines separately by sexes would suggest a difference in the seasonal cycles between males and females. Although this represents a test of different hypotheses in a statistical sense, I re-iterate that my study is descriptive: my aim is to search for potential differences in the cyclic amplitude in different groups.

Model assumptions were evaluated by examining the distribution and independence of residuals by visual inspection of Q-Q plots, which assess whether residuals follow a normal distribution, and residual vs. fitted plots, which reveal heteroscedasticity. They all showed slight but acceptable deviations from a normal fit. For GAMMs, it is assumed that residuals are independently distributed, with constant

variance (homoscedasticity) and normality. Given the nested nature of the experimental design and the inclusion of random effects, I assumed that autocorrelation was negligible.

Although the effective degrees of freedom (edf) associated with each smooth term reflects the estimated effect size, their biological interpretation is not intuitive. To ease the interpretation of the differences in cyclical variation between groups and traits, I used three metrics. First, I extracted the amplitude of the fitted cyclic spline smooth for each trait and group: this amplitude provides a raw effect size regarding the absolute extent of cyclical variation in a trait/group. Second, I calculated the percent difference between two groups as:

$$\text{Percent difference} = \frac{|V_1 - V_2|}{\left[\frac{(V_1 + V_2)}{2} \right]} \times 100$$

where V_1 and V_2 are the amplitudes being compared. Third, I calculated the "coefficient of cyclic variation" (ccv), which captures the magnitude of cyclical variation by adapting the standard coefficient of variation for periodic data (Fulford, 2014). For each trait and group, the ccv was computed as the standard deviation of the estimated cyclic cubic spline divided by the mean of the corresponding raw data. Like the conventional coefficient of variation, the ccv is dimensionless, providing a standardized measure of the amplitude of periodicity that can be compared across traits and groups independently of units and mean differences.

Results

CWT analysis

Wavelet power spectra and average power plots for running distance showed strong periodicity, indicating the presence of a single dominant frequency by a color band recurring every 12 months in both S and C lines ([Figure 3](#); [Figure S1](#) and [S2](#)).

Additionally, the modal frequency at 12 months is denoted by a solid black line showing its synchronicity to a yearly cycle. Body mass (measured before mice were given access to wheel) also showed a strong periodicity over a 12-month period, which seems to synchronically occur in both S and C mice ([Figure 4](#); [Figure S3](#) and [S4](#)). However, the cyclic fluctuation in body mass seems weaker than for voluntary wheel running (note: [Figure 3](#) and [4](#) are plotted on different scales). In addition, for running distance and body mass, some S and C lines showed power in a secondary band near 24 months denoted by black lines. These 24-month features possibly represent harmonics or subharmonic structures of the 12-month cycle rather than evidence for a true biannual rhythm, as interpolation, uneven sampling, slightly asymmetric cycles, and edge effects could generate 24-month artifacts.

Closer inspection suggests temporal changes in power in both running distance and body mass throughout the experiment. Besides the two-year gap (i.e., missing data due to university change), the wavelet analysis plots demonstrate decreasing amplitude over time, denoted by the fading intensity of color. For running distance, 5 of the 8 lines (line C1, S3, S6, S7, S8) showed a decline in amplitude, but only line C1 completely lost its 12-month periodicity (distinguishable by the absence of a black line on the graph after 2013). For body mass, weak to no power was estimated after 2010 in all 8 lines, but only S6 had showed a loss of periodicity entirely in the last 5 years. However, some of those

patterns may reflect sampling artifacts (i.e., interpolation across months without measurements, and generations excluded).

Cross-wavelet coherency

Wavelet power spectra for cross-wavelet analysis indicated that running distance and body mass before wheel measurements were in synchronicity and showed higher average correlation at the 12-month period ([Figure 5](#)). However, no correlation was detected between wheel running and body mass in the last five years in all four S lines and two of the C lines. The arrows pointing to the left indicate running distance and body mass exhibiting a contrary co-movement (i.e., negatively linked/with anti-cyclical effects). This supports preliminary analyses indicating that mice are smaller in winter than in summer and the opposite for running distance. Hence, body mass follows an annual cycle that is in anti-phase to that of wheel-running behaviour. Across all eight lines, running distance tends to “lead” (arrows pointing downwards) in Wisconsin generations, while body mass tends to lead (arrows pointing upwards) in Riverside generations. However, it remains unclear how causality can be established from correlation, and there is no formal way to test whether this change is statistically significant.

GAMM

a) Temporal trends throughout the experiment

The GAMM’s smoothing function for the long-term trend in running distance successfully modeled the distinct temporal patterns in the C and S lines ([Figure S5](#)). In S lines, the model captured the relatively fast response to selection in the first 10 years,

followed by a plateau and/or a slower increase, especially in running duration. The C lines remained relatively constant throughout the experiment in running distance and speed, but showed a minor increase in running duration. By contrast, the long-term trend smooth function for body mass in S lines showed a decrease body mass over the first 10 years, followed by a stable plateau, whereas C lines displayed a relatively constant body mass ([Figure S5](#)).

b) Seasonal cycle in running distance

After accounting for the fixed effects and long-term temporal trends, a cyclic smooth function captured a significant recurring seasonal variation across years. Of all GAMMs fitted for running distance, the most parsimonious one (i.e., lowest AIC) included a cyclic spline estimated separately for each combination of sex and line type ([Table 1](#)). This implies that differences in seasonal variation in running distance are best explained by modelling an interaction between sex and line type, while modelling differences in cyclicity between university location or other interactions does not significantly contribute to model fit.

A closer look at the fitted cyclic spline functions in the lowest-AIC model confirmed that the peak activity occurred in late January, reached a minimum in early August, and that the amplitude of the seasonal cycle differed across all four combinations of sex and line type ([Figure 6](#), [Table 2](#)). The difference between line types were clear, in which S lines showed greater seasonal amplitude than C lines, for both males and females ([Figure 6](#)). Also, males displayed stronger seasonal pattern than females in both line types, but the relative difference in edf between sexes is more pronounced in C lines than

in S lines ([Table 2](#)). Overall, the amplitude of the seasonal cycle in running distance was 114 and 335 revs/day in control females and males, respectively (a 98% difference), and 865 and 1229 revs/day for selected females and males (a 35% absolute difference; [Table 2](#)). Consistent with these amplitudes, the ccv indicated stronger seasonality in males than females, with ccv values of 1.77% and 6.59% in C females and males, and 5.33% and 9.36% in S females and males ([Table 3](#)).

c) Seasonal cycle in running duration

As a component of running distance, running duration followed a similar seasonal pattern, peaking in late January and reaching a minimum in early August. AIC-based model comparison indicated that the model with separate cyclic splines for each sex and university combination provided the lowest AIC ([Table 1](#)), suggesting some heterogeneity in seasonal patterns across groups. However, the second-best model (with separate splines for sex only), had a Δ AIC of just 0.4. Models within 2 Δ AIC units are often considered equally parsimonious. As the addition of university contributed little to model performance, and because the sex-only model explained slightly more deviance (0.286), I selected the sex-specific cyclic spline model for inference. Based on this model, the resulting smooths revealed significant differences in seasonal patterns and amplitudes between sexes ([Figure 7](#), [Table 2](#)). Both sexes displayed a significant smooth term, but males had a much more pronounced cycle ([Figure 7](#)). The amplitude of seasonal variation in running duration was 8.68 min/day for females and 27.62 min/day for males, respectively (a 104% difference; [Table 2](#)). Similarly, the ccv reflected stronger seasonal

cycling in males, with females showing a ccv of 1.24% compared to 4.54% in males ([Table 3](#)).

d) Seasonal cycle in running speed

Similarly to running distance and running duration, cyclicity was also present in running speed, with a peak in late January and a minimum in August. The lowest-AIC model included a separate cyclic spline for C and S mice ([Table 1](#)). Therefore, seasonal variation in running speed is best explained by differences between C and S lines, and does not vary significantly between sexes, experimental locations, or their interactions. The cyclic smooth terms showed clear differences in both amplitude and significance between line types ([Figure 8](#), [Table 2](#)). C lines displayed weak but significant seasonality, whereas S lines displayed a significant and highly seasonal pattern. The amplitude of seasonal variation was 0.45 and 1.85 revolutions/min for C and S line types, respectively (a 122% difference; [Table 2](#)). Consistent with this pattern, the ccv was still relatively greater in S (ccv= 6.27%) than C mice (ccv= 3.17%), indicating greater cyclicity in running speed among S lines ([Table 3](#)).

e) Seasonal cycle in body mass before wheel running

Body mass followed a 12-month pattern with a peak attained in late August and a minimum reached in February. The most parsimonious model for body mass had separate cyclic splines for the interaction of all four combinations of sex and universities ([Table 1](#)). Significant differences were revealed in seasonal patterns and amplitude between males and females in Wisconsin, but not at UCR ([Figure 9](#), [Table 2](#)). In Wisconsin, a

significant seasonal pattern was observed in both sexes, with males having a greater amplitude (0.99 g) than females (0.66 g; a 40% difference). In UCR, no significant cycle was detected in both sexes, and amplitudes were low in both sexes (0.04 g in females, 0.00 g in males). Consistent with these amplitudes, the ccv indicated stronger seasonality in males than in females and in Wisconsin compared to UCR, with ccv values of 1.96% and 2.34% for Wisconsin females and males, and 0.13% and 0.00% for UCR females and males([Table 3](#)).

f) Seasonal cycle in body mass after wheel running

A separate comparison of GAMM for body mass measured after the six days of wheel running showed the same patterns as body mass before (see above), but amplified. The best competing model fitted a separate spline for each combination of sex and universities ([Table 1](#)). A significant seasonal pattern was observed in both sexes and universities ([Figure 9](#), [Table 2](#)). Seasonality in body mass after wheel access was stronger in Wisconsin than UCR. In Wisconsin, males had a greater amplitude (1.18 g) than females (0.74 g; a 46% difference). In UCR, males had a similar amplitude (0.33 g) to females (0.29 g; a 13% difference). Consistent with these amplitudes, the ccv also showed stronger seasonality in Wisconsin, with ccv values of 2.25% for females and 2.83% for males, compared to much lower values at UCR of 0.86% for females and 0.78% for males ([Table 3](#)).

Discussion

Main findings

In this study, I provide unequivocal evidence for seasonal rhythms in both behavioural and morphological traits of laboratory house mice maintained under putatively constant conditions. Seasonal rhythms were synchronized to the solar year across eight replicate time series, with wheel-running activity peaking in winter, and reaching a minimum in summer, in an anti-cyclic pattern relative to body mass. Although many studies have reported seasonal effects in laboratory rodents (see introduction), these typically reflect differences between seasons rather than long-term, repeatable annual patterns. Here, the detection of an annual cycle across 23 years and 78 generations of laboratory mice demonstrates that seasonal variation can persist over decades, even under stable photoperiod, temperature, and food access. This persistent annual pattern has important implications for chronobiology and for studies using rodents as model organisms, and stresses the importance of considering season as a confounding variable and its potential impact on reproducibility across facilities.

The data from the long-term selection experiment (Careau et al., 2013; Swallow et al., 1998) allowed me to explore some factors that could affect the presence and amplitude of seasonal cycles, including laboratory facility, sex, and adaptive genetic differences produced by artificial selection. I found significant differences in the amplitude of the seasonal cycle between male vs female and C vs S mice. Seasonality in running distance was stronger in males than females, a difference that was primarily driven by seasonal variation in running speed. Seasonality in running distance was also stronger in S than C lines, a difference that was driven by seasonal variation in running duration. Seasonal variation in body mass was more pronounced in the Wisconsin than the Riverside era, and males displayed stronger seasonality than females. To my

knowledge, this is the first study to detect sex, environment, and genetic background differences in the seasonality of a laboratory animal, and these results represent an important first step towards better understanding the nature of the seasonal variation observed in laboratory rodents.

Line type differences in seasonality

Considering that the four replicate S lines and four C lines were all sampled from a common genetic pool at the start of the experiment (i.e., derived from the same founding population), with a sample size of 10 breeding pairs in each line, it is unlikely that the observed greater seasonality in S than C lines are due to founder effects. Therefore, the difference observed between line type seasonality must be a result of selection. However, one could have expected a correlated response in the opposite direction because any genetic variant that would cause a mouse to remain as active in summer than in winter would be highly favoured. Over multiple generations, this would eventually lead to vanishing seasonal variation in S (but not C) mice. It is therefore remarkable that the S mice do display seasonal cycles at all, perhaps due to constraints associated with the annual rhythm and timing of reproduction (note: the diurnal circadian rhythm is also conserved in S mice; see below).

The greater seasonality in running distance observed in S lines compared with C lines was primarily associated with stronger seasonality in running speed rather than running duration. Previous studies have shown that mean running speed increased in both sexes as a result of selection on running distance, with females running relatively faster than males (Garland et al., 2011). Consistent with this, the present results indicate that

seasonal variation in running speed increased in S lines. However, seasonality in running speed did not differ between sexes within both line types, suggesting that males and females have similar seasonality in running speed regardless of selection history.

A plethora of correlated traits have evolved in response to selection (Belke & Garland, 2007; Hillis & Garland, 2023; Kay et al., 2019; Keeney et al., 2012; Kolb et al., 2013; Rhodes, 2005; Thompson et al., 2017, 2024). Hillis & Garland (2023) identified genomic regions that responded to selection, including regions associated with locomotor activity, dopamine signaling, muscle physiology, and neural function. The same genomic changes that enabled increased voluntary running likely also amplified seasonal rhythms in the running speed of the S mice. Circadian timing also showed a correlated response to selection, where S mice have a shorter free-running circadian period than C mice under both constant light and dark lighting conditions (Koteja et al., 2003). Thus, selecting for voluntary wheel running caused correlated changes in the endogenous timekeeping of the circadian rhythm, indicating the presence of a genetic correlation between activity levels and temporal regulation. It would be interesting to test if the results found at the circadian level translate to the circannual scale (i.e., if S and C mice remained synchronized to the solar year throughout the 23 years of the experiment, and if they display their winter/summer behaviour and mass under short/long-day length), but testing this idea would be technically challenging as it requires maintaining the mice under constant darkness or light for multiple years.

An alternative possibility is that the greater seasonality in S than C mice represent a statistical artifact. Indeed, variance generally increases with the mean, and the 2-3-fold higher average activity of S lines could make them appear more seasonal. To test this, I

compared ccv between S and C mice for running distance and running speed ([Table 3](#)). In both traits, the ccv was still higher in S than C mice, meaning that even after standardising for the mean, the difference in cyclical variation remains. Although these comparisons represent no formal statistical testing, the persisting differences in seasonal amplitude support the idea that they represent a biological phenomenon instead of a statistical artefact. However, it remains unclear whether the greater seasonal amplitude in S than C mice reflect an increased plasticity in S mice to adjust activity levels in response to seasonal cues (i.e., a gene-by-environment interaction), or an altered endogenous timing mechanism.

Sex differences in seasonality

Previous work on this selection experiment revealed heterogeneity between the sexes in the correlated responses of wheel-running components (Garland et al., 2011). While running speed increased in both S males and females, running duration significantly increased only in males (Garland et al., 2011). In C mice, males spend less time running than females, but this sex difference vanished in the S lines, indicating that selection for increased voluntary running caused males to increase running duration more than females (Garland et al., 2011). These sex-specific differences in mean running duration did not translate into sex-specific differences in seasonality between line types. Instead, males were consistently more seasonal than females in running duration, regardless of selection history, indicating that even if S males run longer than C males, they both have similar seasonality patterns.

Similarly to running duration, selection did not amplify seasonal variation in body mass. Seasonal variation in body mass did not differ between line types but varied primarily with sex and university. Yet, within each line types, males weighed on average more than females, and the sex difference in mean body mass was smaller in the S lines (Swallow et al., 1999). This parallels the pattern observed in running duration and supports that selection on running distance generated correlated changes of running speed seasonality, but not of time spent running and body mass seasonality.

Consistent with this interpretation, male mice showed stronger seasonal fluctuations in body mass, while females maintained a more constant body mass throughout the year. Previous reports on the selection experiment by Koteja et al. (1999) and Swallow et al. (1999) showed a negative relationship between mass after wheel measurements and running distance in males, but not in females. It does appear that female body mass is maintained despite variation in running activity throughout the year.

Link between wheel running and body mass

In general, behaviour is thought to be more sensitive to environmental variation than morphology (Blomberg et al., 2003), and morphological traits such as body mass and lean mass are generally thought to be less directly linked to Darwinian fitness than higher-level life-history and behavioural traits (Careau et al., 2013; Khan et al., 2024). If behaviour is more sensitive to environmental variation than morphology, then we should observe relatively stronger cycles in running distance than body mass. Comparing the ccv of running distance vs. body mass reveals that seasonality in running distance is relatively greater than in body mass. Although it would be tempting to conclude that the seasonality

in locomotor activity causes seasonality in body mass, I remain aware that my study is correlative, and causal effects cannot be determined without a manipulative experiment. Previously mentioned study on the annual cycle of golden-mantled ground squirrel in wheel activity and body mass (Pengelley & Fisher, 1966) proposed that the seasonal changes in body mass, locomotor activity, hibernation states, and food consumption are interrelated. Interestingly, this study was also conducted in UCR, and found annual cycles in body mass and locomotor activity, peaking in spring and reaching a winter minimum.

The possibility of a mechanistic relationship between the annual cycle in body mass and wheel activity is supported by experimental work on energy balance in S and C mice. Hiramatsu & Garland (2018) found that S and C male mice show stronger relationships between running behaviour and fat change, whereas in females, running behaviour is only weakly related to fat loss. The stronger link between activity and energy balance seen in males could help explain why their body mass also varies more across seasons. If males have a tighter physiological link between activity and fat, then even modest seasonal shifts in running behaviour could generate detectable seasonal changes in body mass. In contrast, the weaker activity-fat relationship in females might help attenuate seasonal oscillations in body mass despite similar variation in activity.

Another study showed that males had both higher cost of transport (COT) and daily locomotor cost (DCOT) than females (Rezende et al., 2009). Consequently, males may have to pay greater daily energetic costs for their locomotion during the most active (winter) phase, resulting in stronger cyclic fluctuations in body mass. Because the COT values refer to wheel running, which they only have access for 6 days with *ad libitum*

food, one could argue that seasonality in body mass is stronger in males because of a higher COT. In support of this idea, the seasonal cycles were greater in body mass measured after the 6 days of wheel running than before ([Figure 9](#)), suggesting that the cycle in wheel running contributes to annual variation in body mass. As to why greater seasonality in male than female is also observed in body mass measured before mice were put on wheels, then one may assume that seasonality observed in wheel-running activity is also present in home-cage locomotor activity (when wheels are not provided). In this case, the higher overall locomotor activity in winter than summer may disproportionately affect male energy budget, which causes stronger seasonality in male than female body mass, even in absence of wheels.

Considering the lack of a seasonal cycle in body mass in the Riverside era, a link between the seasonal cycles of wheel running and body mass would also result in weaker annual cycles of wheel running in UCR. Interestingly, the lowest-AIC GAMM for running duration detected a difference between universities, where Wisconsin mice showed stronger seasonal cycles than Riverside mice ([Table S1](#), [Figure S6](#)). Notably, males in Riverside still showed a (relatively weak) cycle in running duration whereas females showed no seasonality. As the ΔAIC between the selected model and the lowest-AIC model is negligible ($\Delta AIC = 0.4$; Table 1), and the model including university explains slightly less deviance (0.285) than the selected best-fitting model (0.286), the effect of university on the seasonality of running duration is probably small, and needs careful interpretation. Yet, the consistency of differences between universities across both body mass and running duration traits further supports the possible causal relationship in body mass and running duration.

Taken together, these findings challenge the possibility that the more pronounced seasonal cycles in running behaviour could contribute to the greater seasonal variation in body mass. I reiterate that further studies are necessary to identify the possible causal mechanism, and would require manipulative experiments (e.g., controlling food intake or wheel access across seasons). Still, comparing gold curves (UCR) in Figure 9AB versus 9CD highlights a weak cycle in body mass measured after the six days of voluntary wheel running that is inexistent before, suggesting a small, but direct influence of locomotor activity on body mass.

University differences in seasonality

Body mass showed clear seasonality differences between the Wisconsin and Riverside eras. Wavelet analysis of body mass show that Wisconsin generations had a relatively constant 12-month cycle, whereas Riverside generations body mass cycles either deviated from a 12-month period or disappeared entirely. However, it may be a statistical artifact of the intrapolation used, because Wisconsin generations were measured every three months (4 generations per year), whereas the UCR generations were measured approximately every 4 months (3 generations per year). Also, six UCR generations were removed of the statistical analysis (see *Methods*), while none were removed in Wisconsin. Yet, GAMMs show that both sexes showed seasonal variation in body mass during the Wisconsin era, whereas there was no statistically significant seasonality in body mass for both sexes during the Riverside era.

This pattern is consistent with slight differences in running duration seasonality between universities in the second-best GAMM, where Wisconsin mice showed stronger seasonal cycles than Riverside mice ([Table S1](#), [Figure S6](#)). I reiterate that the ΔAIC is negligible (0.4; [Table 1](#)), and the effect of university on the seasonality of running duration is probably small, and needs careful interpretation. Nevertheless, the consistency of differences between universities across both body mass and running duration traits suggests that modest differences in seasonality exist between the two laboratory environments.

Potential explanations of the difference in seasonality between universities include shifts in environmental conditions between Wisconsin vs Riverside eras, and potential alterations in the genetic composition within the mouse populations over time. Interpretation of the causes in the observed differences between universities must be done with caution, because such a comparison between two environments is fraught by many confounding variables, including differences in latitude, HVAC systems, personnel, or bedding used. Handling practices also differed between sites. In Wisconsin, personnel typically wore regular clothing without protective gowns and often handled mice without gloves. At Riverside, protective gowns and gloves were introduced at some point and became part of standard animal-handling procedures. Both universities used the same fixed-formula diet across the 30-year study, and water was provided from the tap in each vivarium throughout the study. Although the water used within each university was always sourced from the same tap, its composition has not been tested for seasonal variation and could potentially cause the observed rhythms. Drickamer (1977) reported seasonal variation in body mass of female mice at weaning, and proposed: “This result

may be explained by variation in water quality or an inherited endogenous rhythm [...]. Alternatively, differences could have resulted from seasonal changes in either the quality or quantity (or both) of nutrition supplied to pre-weaning mice by their dams. Further study is needed regarding monthly variations in dams' milk". However, this explanation remains speculative, and other environmental cues likely play a larger role. It is also possible that continued evolutionary (genetic) changes in Riverside (e.g., higher levels of inbreeding or genetic drift) have disrupted or attenuated the endogenous annual clock, leading to weaker or more irregular seasonal cycles.

Although endogenous circannual clocks exist in some rodents, infiltration of environmental cues in laboratory settings remains a possible factor influencing seasonal patterns. While animal facilities aim to standardize conditions (controlling light, temperature, and feeding regimes), subtle external cues can still reach the animals and entrain physiological rhythms. Suckow (2023) emphasized that unfiltered air, changes in water supply, or relative humidity fluctuations can act as hidden seasonal Zeitgebers. Animal facilities may vary seasonally in air quality, ventilation patterns, and background noise. For example, heating systems, common in winter, may create mechanical vibrations or shift airflow. Additionally, olfactory signals such as changes in ambient odor, pollen, or airborne particulates (especially in facilities not fully sealed) may transmit seasonal information (Bohlen et al., 2014).

A factor to consider is the latitude at which the universities are located. Wisconsin is at a substantially higher latitude (43.07) than UCR (33.95), and experiences much stronger temperate seasonal variation than Riverside, which lies closer to the equator in a semi-desertic environment. If environmental cues (e.g., pollen, odors, humidity) infiltrate

housing facilities, mice in Wisconsin laboratories would be exposed to more seasonal variation in environmental cues than mice in California laboratories, where seasons are more moderate. One compelling example comes from Reichard et al. (2025), who showed that female C57BL/6J mice, housed under constant 14:10 “simulated summer” light cycles, nonetheless exhibited seasonal changes in their estrous cycle that mirrored the external seasons. The authors identified relative humidity as one likely environmental cue, as the facility’s ambient humidity varied seasonally and correlated with changes in estrous phase durations. They concluded that “unidentified external cues” were synchronizing the mice’s physiology with the calendar year. Humidity has also been linked to seasonal responses in rodents. Other studies suggested that pain sensitivity (Winters, 1986) and that thermal nociception behaviour (Chesler et al., 2002) changes with season, speculating that higher humidity in hot months could be a contributing factor. In my own study, we cannot determine whether humidity was a contributing factor to observed seasonal cycles, as humidity logs were not maintained. However, weather station records (Weather Spark, n.d.) clearly show that Madison, Wisconsin, has higher average humidity than Riverside, California, and anecdotal observations suggest that humidity was often higher in the vivarium in Wisconsin during summer months (T. Garland Jr. personal communications). Humidity may explain why mice in Wisconsin have higher amplitude and are more strongly entrained to a 12-month rhythm in body mass and running duration, while the cycles in Riverside are weaker.

An often-overlooked contributor is the human factor. Animal care staff themselves experience seasonal cycles (e.g., shifts in schedules, attire, behaviours), which could indirectly influence the animals via altered handling, noise, or pheromone exposure

(Bohlen et al., 2014; Sorge et al., 2014; Wahlsten et al., 2003). Mice seem to detect human stress through olfaction; indeed, a preliminary study showed that mice exposed to sweat samples from stressed humans showed different physiologic responses when compared to mice exposed to samples from non-stressed humans (Destrez et al., 2021). Chesler et al. (2002) demonstrated that the experimenter's identity had a greater impact on pain response than mouse genotype, and identified season, humidity, cage density, time of day, sex, and social environment as major contributors to behavioural variation. In sum, there is growing recognition that laboratory mice remain sensitive to subtle seasonal changes, despite controlled housing. This means that observed annual rhythms may not always reflect an endogenous clock but may instead originate from unrecognized environmental Zeitgebers. The challenge going forward is to monitor these potential cues more closely, or eliminate them, if possible, to distinguish intrinsic biological timing from external interference.

Strong inference about the mechanisms of seasonality differences between universities remains difficult. However, differences in seasonality between universities highlight the challenge in laboratory biology that even under highly controlled conditions, subtle differences across facilities can shape biological rhythms that may affect reproducibility. Careful consideration of laboratory context is therefore essential when interpreting multi-site studies.

An endogenous timing mechanism?

Given that the Hsd:ICR outbred stock originates from Swiss mice that were originally brought into laboratory culture and domestication in 1936 from sources in eastern Europe

with substantial seasonality (Swallow et al., 1998), is it possible that the observed seasonal rhythms reflect innate seasonal adaptations inherited from wild ancestors?

In natural settings, wild house mice (*Mus musculus*) are more active in winter than in summer. A field study in Germany found that free-ranging *Mus musculus* were active nearly twice as long per day in winter (~11.3 hours) as in summer (~6.7 hours), based on automated detection of visits to enclosures (Robbers et al., 2015). This increased activity is likely driven by the need to forage longer under conditions of low food availability and extended darkness in the winter. Even though nights are colder, the urgency to meet energy needs can drive mice to be more active in winter, whereas in summer the short nights and abundant resources allow them to remain less active overall. Meijer & Robbers (2014) demonstrated seasonal variation in wheel running under semi-natural conditions. When food was present, activity increased steadily through spring and peaked in summer, but when food was absent, activity peaked in April, with minimum reached in midsummer. The observed increase in winter activity in wild and semi-wild rodents likely reflects an adaptive response to seasonal energetic challenges to survive when food is scarce and conditions are harsh. The fact that similar patterns persist in laboratory mice suggests the circannual timing mechanism inherited from wild ancestors is conserved, even when mice are housed under constant photoperiod and temperature, with *ad libitum* access to food.

Conversely, mice tend to be heaviest in summer and lightest in winter, a cycle reflecting seasonal energy balance. Some studies on wild and semi-wild mice show that individuals grow larger during the spring and summer months when food is plentiful, then lose weight over the winter (Bartolommei et al., 2023; Rimbach et al., 2019). This

suggests that during summer, mice can build up fat reserves and body mass (by abundant nutrition and frequent breeding in that season), whereas winter causes a drop in body mass due to limited food and increased energy expenditure. In many temperate-zone rodents, reducing body mass in winter appears adaptive: carrying less weight lowers the animal's caloric needs for maintenance and helps it cope with cold stress. A classic example is the meadow vole: under short winter photoperiods it maintains about 20% lower body weight and consumes much less food than under long-day conditions (previously mentioned; Dark & Zucker, 1983). This photoperiod-driven weight loss is thought to diminish the need for winter foraging and reduce the energy required for thermoregulation. Mice likely share a similar, though perhaps less extreme, physiological response. Even in laboratory experiments, altering day length has a measurable effect on mouse metabolism. Mice exposed to a simulated winter light cycle showed reduced fat mass and shifts in metabolic rhythms compared to those in summer-like light cycles (Small et al., 2023). All of this evidence supports the idea that mice naturally put on weight during long-day (summer) conditions and shed weight during short-day (winter) conditions as part of an adaptive seasonal cycle.

A final point is that litter size could affect body mass or wheel running seasonality. As litter size can strongly determine adult physiology (Parra-Vargas et al., 2023), further analysis on litter size seasonality could explain some of the variation in body mass and/or wheel running. In the meantime, the possibility remains that the observed cycle of high winter activity with low body mass, and low summer activity with high body mass represents an expression of an intrinsic (and adaptive in the wild) seasonal rhythm. These adaptations would have maximized fitness in the wild and are

still evident as cyclic changes in the laboratory. In summary, the seasonal rhythm in activity and body mass of this study on laboratory mice may be a manifestation of an evolved endogenous annual clock, but the possibility and role of environmental factors infiltrating and influencing the colony remains to be explored.

An interaction of endogenous timing and environmental cues?

Although the presence of seasonal cycles in body mass and activity may reflect an endogenous circannual rhythm, results from this study likely suggest an interaction between internal timing mechanisms and subtle environmental cues because of 1) stronger amplitude in S lines, 2) synchronization of rhythm between distinct lines, and 3) their synchronization to the solar year.

The most likely interpretation to the stronger seasonality in S than C lines is that it reflects genotype-by-environment (G×E) interaction, where selection for running distance cause genetic changes that interact with environmental cues to amplify seasonal responses. What possible mechanism may be responsible for the increased sensitivity to environmental cues in S lines? Olfaction plays a key role in how mice perceive their environment (Latham & Mason, 2004) and in regulating circadian behaviour (Abraham et al., 2013). Previous studies indicate differences between the S and C lines in olfactory sensitivity. Mice run less on previously occupied wheels than on clean wheels, and they run less when the wheel was previously occupied by a male than a female (Dewan et al., 2019). Importantly, these reductions in running distance are larger in the S than C lines. This behavioural pattern is consistent with genomic comparisons between S and C lines revealed divergence in olfactory-related genes (Nguyen et al., 2020), suggesting that S

mice may detect external cues differently. If environmental cues reach the mice, S lines may show stronger seasonal variation because they have a heightened olfactory sensitivity.

All eight lines (of both S and C lines) displayed remarkably similar seasonal periods of 12 months in running behaviour and body mass ([Figure 1](#)). Given that these lines have diverged through many generations of selection (including via random genetic drift), such tight rhythm synchronization across genetically distinct lines would be unlikely under a purely endogenous model. If the cycles were strictly genetically determined, we would expect variation in phase or period between lines, yet the observed synchrony suggests that some shared external cue (even if subtle or unidentified) is acting as a Zeitgeber to entrain their internal rhythms. Note, however, that one could disagree and argue that the lines are still very closely related; an endogenous annual rhythm inherited from their common ancestor could be deeply embedded in their genomes, causing little changes in synchrony over a few tens of generations (Ted Garland Jr, personal communications). Synchronicity was also reflected in wavelet analyses conducted per line, although not perfectly consistent, the 12-month period were largely aligned between lines, supporting environmental entrainment. In addition to line-level synchrony, the seasonal rhythms observed in this study align closely with the calendar year: wheel-running activity peaks in winter, and body mass peaks in summer. Under natural conditions, the period of circannual rhythms is approximately 12 months. According to Gwinner (1986), entrainment to an external zeitgeber is demonstrated when one or more of the following criteria are met: 1) the free-running period matches that of the zeitgeber; 2) the zeitgeber can phase-shift the rhythm within several cycles; or 3) the

zeitgeber produces phase shifts at different phases of the annual cycle. Given these criteria, the precise alignment of the observed rhythms in this study with the solar year would not be expected under a purely endogenous model. More specifically, if circadian and circannual timing mechanisms are associated, as is often proposed (Gwinner, 1986), then the shorter circadian period of S mice when deprived of photoperiodic cues (Koteja et al., 2003) would predict that S lines should also express a slightly shorter circannual period when deprived of environmental zeitgebers. If this were the case, their seasonal rhythms should gradually drift out of phase with the calendar year in the absence of entraining cues. However, no such drift was detected. Wavelet analyses showed that both S and C lines remained synchronized with the 12-month solar cycle throughout the study, but less so in UCR, where either zeitgebers are less pronounced or the less strict 3-month interval of generation measurements caused the cycle's attenuation.

Taken together, the synchronized rhythms across genetically diverse lines and their alignment with the solar year suggests that external environmental cues are influencing, if not fully entraining, the observed annual cycles. However, it remains impossible to distinguish between true endogenous rhythms and environmentally driven cycles. As each mouse was only measured once, it is not possible to determine whether individual rhythms would persist over two years and deviate from 12-months without external cues, a criterion necessary to separate between Type I and Type II rhythms (Gwinner, 1981). Longitudinal tracking of individuals over time, and tighter environmental monitoring (e.g., of humidity, noise, or air quality), will be required to disentangle these mechanisms more precisely.

Why this study detected seasonal cycles where others did not?

Previous studies on seasonality in rodent behaviour and physiology have often reported ambiguous or non-significant results, likely due to limitations in sample size, study duration, and statistical resolution. The review from Ferguson & Maier (2013) found that studies detected seasonal effects with as few as 4–40 individuals, and many others failed to do so, possibly lacking the statistical power needed to capture subtle rhythms. In contrast, my study leveraged a large time series spanning 36,002 observations over 23 years, providing exceptional power to detect seasonal patterns in both amplitude and period. Although each generation was tested only every 3–4 months (i.e., ~3–4 sampling points per year), the testing dates were not identical from one year to the next. As a result, when all 23 years are combined, the sampling days collectively span almost the entire 365-day calendar range. In other words, while individual years contain only a few sampling dates, the long-term nature of the experiment yields an almost complete annual coverage of day-of-year values across the dataset. This produces a much finer effective temporal resolution than traditional seasonal average comparisons based on four broad seasons or a few fixed annual sampling days.

Another major strength is the length of the time series. Annual cycles require extended time to detect reliably. Unlike circadian rhythms that recur every 24 hours, circannual rhythms demand long-term data to assess both period consistency and amplitude variation. As Gwinner (1986) emphasized, at least two full years are required to confirm the presence of an annual rhythm, yet most behavioural studies on rodents span only 1–2 years. The 23-year span of this study provides rare insight into long-term biological rhythms, their potential variability over time, and the differences between

sexes, environments, and genetic background. The absence of seasonal cycles in earlier rodent studies may reflect issues in experimental design and data analysis, rather than a true absence of biological rhythms. This work contributes to the chronobiology literature by showing that, with adequate sampling and appropriate analytical tools, seasonal cycles in activity and body mass can be detected even in laboratory rodents.

One thing worthy of note is that despite the high statistical significance of the seasonal effects detected in this study, their effect size is relatively modest, with coefficients of cyclic variation (ccv) generally below 10%. Nevertheless, from a biological perspective, these differences may still be meaningful. Given the circumference of the activity wheel (1.12 m) and the estimated amplitudes of the seasonal cycles, the average difference in running distance between winter and summer corresponds to several additional kilometers of running per mouse. In a natural context, such differences in locomotor activity could translate into substantial variation in energy expenditure, foraging effort, and exposure to environmental risks. Importantly, these seasonal contrasts differ across groups (e.g., selected males versus control females), suggesting that even relatively small proportional changes in activity may have biologically relevant consequences depending on sex and genetic background. Thus, while the absolute effect sizes are modest, they should not be dismissed as trivial, particularly when interpreted in an ecological or evolutionary framework.

Conclusion

To conclude, I found clear evidence of seasonality in both voluntary wheel-running behaviour and body mass in laboratory mice housed under standard laboratory

conditions. Running distance seasonality mainly reflected the combined effects of running speed and duration, whereby seasonality of running duration was different in males vs females, whereas seasonality in running speed was greater in S than C mice. Importantly, body mass seasonality follows an anti-cyclic rhythm with running duration.

Distinguishing between endogenous timing vs environmental entrainment will require more targeted studies that include long-term measures of food intake, energy expenditure, body composition, and measuring mice for more than two years. The present study demonstrates that seasonal variation can introduce unwanted noise into behavioural studies, especially when groups are sampled across different months. Such variation within and among treatments may reduce statistical power, increase the risk of Type II errors, and impact reproducibility if not properly accounted for. Researchers should consider study designs that control for time-of-year effects, or at least incorporate season as a covariate in their statistical models.

Replicated artificial selection experiments on complex traits offer powerful tools to investigate how seasonal physiology interacts with genetics, epigenetics, and environmental sensitivity (Garland, 2003; Hillis & Garland, 2023). A useful way to replicate and complement the findings in this thesis would be to conduct similar analyses of other selection experiments in constant environments. In mice, for example, there are long-term datasets available on body mass (Renne et al., 2003), nesting behaviour (Bult & Lynch, 1996), and metabolic rate (Brzek et al., 2025; Wone et al., 2015). Selection experiments on other species, such as the ones on basal metabolic rate in bank voles (Sadowska et al., 2015) and endurance running capacity in rats (Koch & Britton, 2001),

would provide unique rodent systems to study whether and how selection alters seasonal traits.

References

- Abraham, U., Saleh, M., & Kramer, A. (2013). Odor is a time cue for circadian behavior. *Journal of Biological Rhythms*, 28(1), 26–37. <https://doi.org/10.1177/0748730412469353>
- Akaike, H. (2011). Akaike's Information Criterion. In *International Encyclopedia of Statistical Science* (pp. 25–25). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-04898-2_110
- Anderson DM, & Keafer BA. (1987). *An endogenous annual clock in the toxic marine dinoflagellate Gonyaulax tamarensis*. [https://doi.org/Nature325:616–617](https://doi.org/Nature325:616-617)
- Bajaj, N. (2021). Wavelets for EEG Analysis. In *Wavelet Theory*. IntechOpen. <https://doi.org/10.5772/intechopen.94398>
- Bartolommei, P., Savazza, S., Bonacchi, A., Gasperini, S., Manzo, E., & Cozzolino, R. (2023). *Should I Stay or Should I Go? Seasonal Fluctuations of Wood Mouse Populations in Fields Surrounded by Woodlands*. <https://doi.org/10.21203/rs.3.rs-2642730/v1>
- Belke, T. W., & Garland, T. (2007). A brief opportunity to run does not function as a reinforcer for mice selected for high daily wheel-running rates. *Journal of the Experimental Analysis of Behavior*, 88(2), 199–213. <https://doi.org/10.1901/jeab.2007.62-06>
- Beltran-Frutos, E., Casarini, L., Santi, D., & Brigante, G. (2022). Seasonal reproduction and gonadal function: a focus on humans starting from animal studies. In *Biology of Reproduction* (Vol. 106, Number 1, pp. 47–57). Oxford University Press. <https://doi.org/10.1093/biolre/ioab199>
- Blomberg, S. P., Garland, T., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, 57(4), 717–745. <https://doi.org/10.1111/j.0014-3820.2003.tb00285.x>
- Bohlen, M., Hayes, E. R., Bohlen, B., Bailoo, J. D., Crabbe, J. C., & Wahlsten, D. (2014). Experimenter effects on behavioral test scores of eight inbred mouse strains under the influence of ethanol. *Behavioural Brain Research*, 272, 46–54. <https://doi.org/10.1016/j.bbr.2014.06.017>
- Boyce, M. S. (1979). Seasonality and patterns of natural selection for life histories. In *Am. Nat* (Vol. 114, Number 4).
- Bradshaw, W. E., & Holzapfel, C. M. (2007). Evolution of Animal Photoperiodism. *Annual Review of Ecology, Evolution, and Systematics*, 38(1), 1–25. <https://doi.org/10.1146/annurev.ecolsys.37.091305.110115>
- Bronson, F. H. (1995). THE QUARTERLY REVIEW of BIOLOGY SEASONAL VARIATION IN HUMAN REPRODUCTION: ENVIRONMENTAL FACTORS. In *The Quarterly Review of Biology* (Vol. 70, Number 2).
- Brzek, P., Selewestruk, P., Sadowska, J., G_ł Ebczy'nskiebczy'nski, A. K., Ksi, A., A' Zek, _ł, Kalinovich, A., Nedergaard, J., Konarzewski, M., Physiol, J., & Brz_ł, P. (2025). Divergent selection for basal metabolic rate in mice affects the abundance of UCP1 protein: implications for translational studies 320 Brz_ł ek and others. *The Journal of Physiology*, 603, 319–336. <https://doi.org/10.1113/JP286669#support-information-section>

- Bult, A., & Lynch, C. B. (1996). Multiple selection responses in house mice bidirectionally selected for thermoregulatory nest-building behavior: Crosses of replicate lines. *Behavior Genetics*, 26(4), 439–446. <https://doi.org/10.1007/BF02359488>
- Burnham, K. P., & Anderson, D. R. (Eds.). (2004). *Model Selection and Multimodel Inference*. Springer New York. <https://doi.org/10.1007/b97636>
- Careau, V., Wolak, M. E., Carter, P. A., & Garland, T. (2013). Limits to behavioral evolution: the quantitative genetics of a complex trait under directional selection. *Evolution*, 67(11), 3102–3119. <https://doi.org/10.1111/evo.12200>
- Cazelles, B., Chavez, M., Berteaux, D., Ménard, F., Vik, J. O., Jenouvrier, S., & Stenseth, N. C. (2008). Wavelet analysis of ecological time series. In *Oecologia* (Vol. 156, Number 2, pp. 287–304). <https://doi.org/10.1007/s00442-008-0993-2>
- Chesler, E. J., Wilson, S. G., Lariviere, W. R., Rodriguez-Zas, S. L., & Mogil, J. S. (2002). Identification and ranking of genetic and laboratory environment factors influencing a behavioral trait, thermal nociception, via computational analysis of a large data archive. *Neuroscience & Biobehavioral Reviews*, 26(8), 907–923. [https://doi.org/10.1016/S0149-7634\(02\)00103-3](https://doi.org/10.1016/S0149-7634(02)00103-3)
- Dark, J., & Zucker, I. (1983). *Short Photoperiods Reduce Winter Energy Requirements of the Meadow Vole, Microtus pennsylvanicus* (Vol. 31). Pergamon Press Ltd.
- Destrez, A., Costes-Thiré, M., Viart, A.-S., Prost, F., Patris, B., & Schaal, B. (2021). Male mice and cows perceive human emotional chemosignals: a preliminary study. *Animal Cognition*, 24(6), 1205–1214. <https://doi.org/10.1007/s10071-021-01511-6>
- Dewan, I., Garland, T., Hiramatsu, L., & Careau, V. (2019). I Smell a Mouse: Indirect Genetic Effects on Voluntary Wheel-Running Distance, Duration and Speed. *Behavior Genetics*, 49(1), 49–59. <https://doi.org/10.1007/s10519-018-9930-2>
- Dohm, M. R., Richardson, C. S., & Garland, T. (1994). Exercise physiology of wild and random-bred laboratory house mice and their reciprocal hybrids. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 267(4), R1098–R1108. <https://doi.org/10.1152/ajpregu.1994.267.4.R1098>
- Drickamer, L. C. (1977). Seasonal variation in litter size, bodyweight and sexual maturation in juvenile female house mice (*Mus musculus*). In *Laboratory Animals* (Vol. 11).
- Drickamer, L. C. (1990). Seasonal variation in fertility, fecundity and litter sex ratio in laboratory and wild stocks of house mice (*Mus domesticus*). *Laboratory Animal Science*, 40(3), 284–288.
- Drickamer, L. C., & Vestal, B. M. (1973). Patterns of Reproduction in a Laboratory Colony of *Peromyscus*. *Journal of Mammalogy*, 54(2), 523–528. <https://doi.org/10.2307/1379147>
- Eccard, J. A., & Herde, A. (2013). *Seasonal variation in the behaviour of a short-lived rodent*. <http://www.biomedcentral.com/1472-6785/13/43>
- Edery, I. (2000). *invited review Circadian rhythms in a nutshell*. <http://physiolgenomics.physiology.org>
- Ferguson, S. A., & Maier, K. L. (2013). A review of seasonal/circannual effects of laboratory rodent behavior. In *Physiology and Behavior* (Vol. 119, pp. 130–136). Elsevier Inc. <https://doi.org/10.1016/j.physbeh.2013.06.007>

- Foster, R. G., & Kreitzman, L. (2009). *Seasons of life: the biological rhythms that enable living things to thrive and survive*.
- Fowler, P. A. (1988). Thermoregulation in the female hedgehog, *Erinaceus europaeus*, during the breeding season. *Reproduction*, 82(1), 285–292.
<https://doi.org/10.1530/jrf.0.0820285>
- Fulford, A. J. (2014). The coefficient of cyclic variation: a novel statistic to measure the magnitude of cyclic variation. *Emerging Themes in Epidemiology*, 11(1), 15.
<https://doi.org/10.1186/1742-7622-11-15>
- Garland, T. (2003). *Selection experiments: an under-utilized tool in biomechanics and organismal biology*. <https://www.researchgate.net/publication/230627364>
- Garland, T., Kelly, S. A., Malisch, J. L., Kolb, E. M., Hannon, R. M., Keeney, B. K., Van Cleave, S. L., & Middleton, K. M. (2011). How to run far: multiple solutions and sex-specific responses to selective breeding for high voluntary activity levels. *Proceedings of the Royal Society B: Biological Sciences*, 278(1705), 574–581.
<https://doi.org/10.1098/rspb.2010.1584>
- Gillies, A. C., Ellison, G. T. H., & Skinner, J. D. (1991). The effect of seasonal food restriction on activity, metabolism and torpor in the South African hedgehog (*Atelerix frontalis*). *Journal of Zoology*, 223(1), 117–130.
<https://doi.org/10.1111/j.1469-7998.1991.tb04753.x>
- Girard, I., Swallow, J. G., Carter, P. A., Koteja, P., Rhodes, J. S., & Garland, T. (2002). Maternal-care behavior and life-history traits in house mice (*Mus domesticus*) artificially selected for high voluntary wheel-running activity. *Behavioural Processes*, 57(1), 37–50. [https://doi.org/10.1016/S0376-6357\(01\)00206-6](https://doi.org/10.1016/S0376-6357(01)00206-6)
- Goldman, B. D. (2001). Mammalian Photoperiodic System: Formal Properties and Neuroendocrine Mechanisms of Photoperiodic Time Measurement. *Journal of Biological Rhythms*, 16(4), 283–301. <https://doi.org/10.1177/074873001129001980>
- Gorman, M. R., & Zucker, I. (1995). Seasonal Adaptations of Siberian Hamsters. II. Pattern of Change in Day Length Controls Annual Testicular and Body Weight Rhythms. *Biology of Reproduction*, 53(1), 116–125.
<https://doi.org/10.1095/biolreprod53.1.116>
- Grinsted, A., Moore, J. C., & Jevrejeva, S. (2004). Application of the cross wavelet transform and wavelet coherence to geophysical time series. *Nonlinear Processes in Geophysics*, 11(5/6), 561–566. <https://doi.org/10.5194/npg-11-561-2004>
- Grolemund, G., & Wickham, H. (2011). *Journal of Statistical Software Dates and Times Made Easy with lubridate* (Vol. 40). <http://www.jstatsoft.org/>
- Gwinner, E. (1981). Circannual Systems. In *Biological Rhythms* (pp. 391–410). Springer US. https://doi.org/10.1007/978-1-4615-6552-9_21
- Gwinner, E. (1986). *Circannual rhythms: endogenous annual clocks in the organization of seasonal processes* (Vol. 18). Springer-Verlag, Zoophysiology.
- Gwinner, E., & Dittami, J. (1990). Endogenous Reproductive Rhythms in a Tropical Bird. In *New Series* (Vol. 249, Number 4971).
- Halali, S., Halali, D., Barlow, H. S., Molleman, F., Kodandaramaiah, U., Brakefield, P. M., & Brattström, O. (2021). Predictability of temporal variation in climate and the evolution of seasonal polyphenism in tropical butterfly communities. *Journal of Evolutionary Biology*, 34(9), 1362–1375. <https://doi.org/10.1111/jeb.13895>

- Hauschka, T. S., & Mirand, E. A. (1973). The “breeder: HA(ICR)” Swiss mouse, a multipurpose stock selected for fecundity. *Murphy, G. P., Pressman, D., and Mirand, E. A. (Eds.), Perspectives in Cancer Research and Treatment, Alan R. Riss, New York.*
- Helm, B., & Lincoln, G. A. (2017). Circannual rhythms anticipate the earth’s annual periodicity. In *Biological Timekeeping: Clocks, Rhythms and Behaviour* (pp. 545–569). Springer (India) Private Ltd. https://doi.org/10.1007/978-81-322-3688-7_26
- Hillis, D. A., & Garland, T. (2023). Multiple solutions at the genomic level in response to selective breeding for high locomotor activity. *Genetics*, 223(1). <https://doi.org/10.1093/genetics/iyac165>
- Hiramatsu, L., & Garland, T. (2018). Mice selectively bred for high voluntary wheel-running behavior conserve more fat despite increased exercise. *Physiology and Behavior*, 194, 1–8. <https://doi.org/10.1016/j.physbeh.2018.04.010>
- Hoffmann, K. (1982). *The Critical Photoperiod in the Djungarian Hamster Phodopus sungorus* (pp. 297–304). https://doi.org/10.1007/978-3-642-68651-1_33
- Joy, J. E., & Mrosovsky, N. (1985). Synchronization of circannual cycles: a cold spring delays the cycles of thirteen-lined ground squirrels. *Journal of Comparative Physiology A*, 156(1), 125–134. <https://doi.org/10.1007/BF00610673>
- Kay, J. C., Claghorn, G. C., Thompson, Z., Hampton, T. G., & Garland, T. (2019). Electrocardiograms of mice selectively bred for high levels of voluntary exercise: Effects of short-term exercise training and the mini-muscle phenotype. *Physiology and Behavior*, 199, 322–332. <https://doi.org/10.1016/j.physbeh.2018.11.041>
- Keeney, B. K., Meek, T. H., Middleton, K. M., Holness, L. F., & Garland, T. (2012). Sex differences in cannabinoid receptor-1 (CB1) pharmacology in mice selectively bred for high voluntary wheel-running behavior. *Pharmacology Biochemistry and Behavior*, 101(4), 528–537. <https://doi.org/10.1016/j.pbb.2012.02.017>
- Kenagy, G. J. (1973). Daily and Seasonal Patterns of Activity and Energetics in a Heteromyid Rodent Community. *Ecology*, 54(6), 1201–1219. <https://doi.org/10.2307/1934184>
- Khan, R. H., Rhodes, J. S., Girard, I. A., Schwartz, N. E., & Garland, T. (2024). Does Behavior Evolve First? Correlated Responses to Selection for Voluntary Wheel-Running Behavior in House Mice. *Ecological and Evolutionary Physiology*, 97(2), 97–117. <https://doi.org/10.1086/730153>
- Koch, L. G., & Britton, S. L. (2001). Artificial selection for intrinsic aerobic endurance running capacity in rats. *Physiological Genomics*, 5(1), 45–52. <https://doi.org/10.1152/physiolgenomics.2001.5.1.45>
- Kolb, E. M., Kelly, S. A., Middleton, K. M., Sermsakdi, L. S., Chappell, M. A., & Garland, T. (2010). Erythropoietin elevates $\dot{V}O_{2,max}$ but not voluntary wheel running in mice. *Journal of Experimental Biology*, 213(3), 510–519. <https://doi.org/10.1242/jeb.029074>
- Kolb, E. M., Rezende, E. L., Holness, L., Radtke, A., Lee, S. K., Obenaus, A., & Garland, T. (2013). Mice selectively bred for high voluntary wheel running have larger midbrains: support for the mosaic model of brain evolution. *Journal of Experimental Biology*, 216(3), 515–523. <https://doi.org/10.1242/jeb.076000>

- Kondo, N., Sekijima, T., Kondo, J., Takamatsu, N., Tohya, K., & Ohtsu, T. (2006). Circannual Control of Hibernation by HP Complex in the Brain. *Cell*, 125(1), 161–172. <https://doi.org/10.1016/j.cell.2006.03.017>
- Koteja, P., Swallow, J. G., Carter, P. A., & Garland, Jr., T. (1999). Energy Cost of Wheel Running in House Mice: Implications for Coadaptation of Locomotion and Energy Budgets. *Physiological and Biochemical Zoology*, 72(2), 238–249. <https://doi.org/10.1086/316653>
- Koteja, P., Swallow, J. G., Carter, P. A., & Garland, T. (2003). Different Effects of Intensity and Duration of Locomotor Activity on Circadian Period. *Journal of Biological Rhythms*, 18(6), 491–501. <https://doi.org/10.1177/0748730403256998>
- Krugner-Higby, L., Girard, I., Welter, J., Gendron, A., Rhodes, J. S., & Garland, T. (2006). Clostridial enteropathy in lactating outbred swiss-derived (ICR) mice. *Journal of the American Association for Laboratory Animal Science : JAALAS*, 45(6), 80–87.
- Kumar, V. (2017). Biological timekeeping: Clocks, rhythms and behaviour. In *Biological Timekeeping: Clocks, Rhythms and Behaviour*. Springer (India) Private Ltd. <https://doi.org/10.1007/978-81-322-3688-7>
- Latham, N., & Mason, G. (2004). From house mouse to mouse house: The behavioural biology of free-living *Mus musculus* and its implications in the laboratory. *Applied Animal Behaviour Science*, 86(3–4), 261–289. <https://doi.org/10.1016/j.applanim.2004.02.006>
- Lee, T. C. M., & Meng, X.-L. (2007). *Self Consistency: A General Recipe for Wavelet Estimation With Irregularly-spaced and/or Incomplete Data*.
- Lee, T., & McClintock, M. (1986). *Female rats in a laboratory display seasonal variation in fecundity*.
- Leise, T. L. (2013). Wavelet analysis of circadian and ultradian behavioral rhythms. In *Leise Journal of Circadian Rhythms* (Vol. 11, Number 5). <http://www.jcircadianrhythms.com/content/11/1/5>
- Meek, T. H., Eisenmann, J. C., & Garland, T. (2010). Western diet increases wheel running in mice selectively bred for high voluntary wheel running. *International Journal of Obesity*, 34(6), 960–969. <https://doi.org/10.1038/ijo.2010.25>
- Meijer, J. H., & Robbers, Y. (2014). Wheel running in the wild. *Proceedings of the Royal Society B: Biological Sciences*, 281(1786). <https://doi.org/10.1098/rspb.2014.0210>
- Nelson, R. J., & Demas, G. E. (1996). Seasonal Changes in Immune Function. *The Quarterly Review of Biology*, 71(4), 511–548. <https://doi.org/10.1086/419555>
- Nguyen, Q. A. T., Hillis, D., Katada, S., Harris, T., Pontrello, C., Garland, T., & Hagan-Yamanaka, S. (2020). Coadaptation of the chemosensory system with voluntary exercise behavior in mice. *PLoS ONE*, 15(11 November). <https://doi.org/10.1371/journal.pone.0241758>
- Nylin, S., & Gotthard, K. (2025). Plasticity in life-history traits. In *Annu. Rev. Entomol* (Vol. 43). www.annualreviews.org.
- Parra-Vargas, M., Bouret, S. G., Bruning, J. C., de Moura, E. G., Garland, T., Lisboa, P. C., Ozanne, S. E., Patti, M.-E., Plagemann, A., Speakman, J. R., Tena-Sempere, M., Vergely, C., Zeltser, L. M., & Jiménez-Chillarón, J. C. (2023). The long-lasting shadow of litter size in rodents: litter size is an underreported variable that strongly

- determines adult physiology. *Molecular Metabolism*, 71, 101707.
<https://doi.org/10.1016/j.molmet.2023.101707>
- Pedersen, E. J., Miller, D. L., Simpson, G. L., & Ross, N. (2019). Hierarchical generalized additive models in ecology: An introduction with mgcv. *PeerJ*, 2019(5).
<https://doi.org/10.7717/peerj.6876>
- Pengelley, E. T., Aloia, R. C., & Barnes, B. M. (1978). Circannual rhythmicity in the hibernating ground squirrel *Citellus lateralis* under constant light and hyperthermic ambient temperature. *Comparative Biochemistry and Physiology Part A: Physiology*, 61(4), 599–603. [https://doi.org/10.1016/0300-9629\(78\)90135-4](https://doi.org/10.1016/0300-9629(78)90135-4)
- Pengelley, E. T., & Asmundson, S. J. (1974). Circannual rhythmicity in hibernating mammals. In *Circannual clocks: Annual biological rhythms*, (pp. 95–160).
- Pengelley, E. T., & Fisher, K. C. (1966). *Locomotor Activity Patterns and Their Relation to Hibernation in the Golden-Mantled Ground Squirrel* (Vol. 47, Number 1).
- Perissin, L., Facchin, P., & Porro, C. A. (2003). Tonic pain response in mice: Effects of sex, season and time of day. *Life Sciences*, 72(8), 897–907.
[https://doi.org/10.1016/S0024-3205\(02\)02344-5](https://doi.org/10.1016/S0024-3205(02)02344-5)
- Pernold, K., Rullman, E., & Ulfhake, B. (2021). Major oscillations in spontaneous home-cage activity in C57BL/6 mice housed under constant conditions. *Scientific Reports*, 11(1). <https://doi.org/10.1038/s41598-021-84141-9>
- Petterborg, L. J. (1978). Effect of photoperiod on body weight in the vole, *Microtus montanus*. *Canadian Journal of Zoology*, 56(3), 431–435.
<https://doi.org/10.1139/z78-061>
- Price, T. S., Baggs, J. E., Curtis, A. M., Fitzgerald, G. A., & Hogenesch, J. B. (2008). WAVECLOCK: Wavelet analysis of circadian oscillation. *Bioinformatics*, 24(23), 2794–2795. <https://doi.org/10.1093/bioinformatics/btn521>
- R Core Team. (2025). R: A Language and Environment for Statistical Computing. In *R Foundation for Statistical Computing, Vienna, Austria*. <https://www.R-project.org/>
- Reichard, T. M., Miller, C. H., Yang, J., & Sheehan, M. J. (2025). Seasonality of the estrus cycle in laboratory mice under constant conditions. *Laboratory Animals*, 59(4), 450–463. <https://doi.org/10.1177/00236772251318772>
- Renne, U., Langhammer, M., Wytrwat, E., Dietl, G., & Bünger, L. (2003). Genetic-statistical analysis of growth in selected and unselected mouse lines. *Journal of Experimental Animal Science*, 42(4), 218–232. [https://doi.org/10.1016/S0939-8600\(03\)80004-4](https://doi.org/10.1016/S0939-8600(03)80004-4)
- Rezende, E. L., Gomes, F. R., Chappell, M. A., & Garland, T. (2009). Running behavior and its energy cost in mice selectively bred for high voluntary locomotor activity. *Physiological and Biochemical Zoology*, 82(6), 662–679.
<https://doi.org/10.1086/605917>
- Rhodes, J. S. (2005). Neurobiology of Mice Selected for High Voluntary Wheel-running Activity. *Integrative and Comparative Biology*, 45(3), 438–455.
<https://doi.org/10.1093/icb/45.3.438>
- Rimbach, R., Blanc, S., Zahariev, A., Robin, J.-P., Pillay, N., & Schradin, C. (2019). Fat content of striped mice decreased during the breeding season but not during the food-restricted dry season. *Journal of Experimental Biology*.
<https://doi.org/10.1242/jeb.208504>

- Robbers, Y., Koster, E. A. S., Krijbolder, D. I., Ruijs, A., van Berloo, S., & Meijer, J. H. (2015). Temporal behaviour profiles of *Mus musculus* in nature are affected by population activity. *Physiology and Behavior*, 139, 351–360. <https://doi.org/10.1016/j.physbeh.2014.11.020>
- Roesch, A., & Schmidbauer, H. (2014). WaveletComp: Computational Wavelet Analysis. In *CRAN: Contributed Packages*. <https://doi.org/10.32614/CRAN.package.WaveletComp>
- Sadowska, E. T., Stawski, C., Rudolf, A., Dheyongera, G., Chrzęścik, K. M., Baliga-Klimczyk, K., & Koteja, P. (2015). Evolution of basal metabolic rate in bank voles from a multidirectional selection experiment. *Proceedings of the Royal Society B: Biological Sciences*, 282(1806), 20150025. <https://doi.org/10.1098/rspb.2015.0025>
- Saré, R. M., Lemons, A., & Smith, C. B. (2021). Behavior testing in rodents: Highlighting potential confounds affecting variability and reproducibility. In *Brain Sciences* (Vol. 11, Number 4). MDPI. <https://doi.org/10.3390/brainsci11040522>
- Schwartz, & Andrews. (2013). Circannual transitions in gene expression: Lessons from seasonal adaptations. In *Current Topics in Developmental Biology* (Vol. 105, pp. 247–273). Academic Press Inc. <https://doi.org/10.1016/B978-0-12-396968-2.00009-9>
- Shephard, R. J., & Aoyagi, Y. (2009). Seasonal variations in physical activity and implications for human health. In *European Journal of Applied Physiology* (Vol. 107, Number 3, pp. 251–271). <https://doi.org/10.1007/s00421-009-1127-1>
- Small, L., Lundell, L. S., Iversen, J., Ehrlich, A. M., Dall, M., Basse, A. L., Dalbram, E., Hansen, A. N., Treebak, J. T., Barrès, R., & Zierath, J. R. (2023). Seasonal light hours modulate peripheral clocks and energy metabolism in mice. *Cell Metabolism*, 35(10), 1722–1735.e5. <https://doi.org/10.1016/j.cmet.2023.08.005>
- Sorge, R. E., Martin, L. J., Isbester, K. A., Sotocinal, S. G., Rosen, S., Tuttle, A. H., Wieskopf, J. S., Acland, E. L., Dokova, A., Kadoura, B., Leger, P., Mapplebeck, J. C. S., McPhail, M., Delaney, A., Wigerblad, G., Schumann, A. P., Quinn, T., Frasnelli, J., Svensson, C. I., ... Mogil, J. S. (2014). Olfactory exposure to males, including men, causes stress and related analgesia in rodents. *Nature Methods*, 11(6), 629–632. <https://doi.org/10.1038/nmeth.2935>
- Stearns, S. C. (1998). *The Evolution Of Life Histories*. Oxford University Press Oxford. <https://doi.org/10.1093/oso/9780198577416.001.0001>
- Stevenson, T. J., Visser, M. E., Arnold, W., Barrett, P., Biello, S., Dawson, A., Denlinger, D. L., Dominoni, D., Ebling, F. J., Elton, S., Evans, N., Ferguson, H. M., Foster, R. G., Hau, M., Haydon, D. T., Hazlerigg, D. G., Heideman, P., Hopcraft, J. G. C., Jonsson, N. N., ... Helm, B. (2015). Disrupted seasonal biology impacts health, food security and ecosystems. *Proceedings of the Royal Society B: Biological Sciences*, 282(1817), 20151453. <https://doi.org/10.1098/rspb.2015.1453>
- Suckow, M. A., & Tirado-Muñiz, N. (2023). Seasonal Variation of Laboratory Animals as a Consideration for Research Reproducibility. In *Comparative Medicine* (Vol. 73, Number 4, pp. 255–259). American Association for Laboratory Animal Science. <https://doi.org/10.30802/AALAS-CM-23-000033>
- Swallow, Carter, & Garland. (1998). Artificial Selection for Increased Wheel-Running Behavior in House Mice. In *Behavior Genetics* (Vol. 28, Number 3).

- Swallow, J., Koteja, P., Carter, P. A., & Garland, T. (1999). Artificial Selection For Increased Wheel-Running Activity In House Mice Results In Decreased Body Mass At Maturity. *Journal of Experimental Biology*, 202(18), 2513–2520. <https://doi.org/10.1242/jeb.202.18.2513>
- Swallow, J., Koteja, P., Carter, P., & Garland, T. (2001). Food consumption and body composition in mice selected for high wheel-running activity. *Journal of Comparative Physiology - B Biochemical, Systemic, and Environmental Physiology*, 171(8), 651–659. <https://doi.org/10.1007/s003600100216>
- Thompson, Z., Argueta, D., Garland, T., & DiPatrizio, N. (2017). Circulating levels of endocannabinoids respond acutely to voluntary exercise, are altered in mice selectively bred for high voluntary wheel running, and differ between the sexes. *Physiology & Behavior*, 170, 141–150. <https://doi.org/10.1016/j.physbeh.2016.11.041>
- Thompson, Z., Fonseca, I. A. T., Acosta, W., Idarraga, L., & Garland, T. (2024). Effects of food restriction on voluntary wheel-running behavior and body mass in selectively bred High Runner lines of mice. *Physiology and Behavior*, 282. <https://doi.org/10.1016/j.physbeh.2024.114582>
- Torrence, C., & Compo, G. P. (1998). A Practical Guide to Wavelet Analysis. *Bulletin of the American Meteorological Society*, 79(1), 61–78. [https://doi.org/10.1175/1520-0477\(1998\)079<0061:APGTWA>2.0.CO;2](https://doi.org/10.1175/1520-0477(1998)079<0061:APGTWA>2.0.CO;2)
- Wahlsten, D., Metten, P., Phillips, T. J., Boehm, S. L., Burkhart-Kasch, S., Dorow, J., Doerksen, S., Downing, C., Fogarty, J., Rodd-Henricks, K., Hen, R., McKinnon, C. S., Merrill, C. M., Nolte, C., Schalomon, M., Schlumbohm, J. P., Sibert, J. R., Wenger, C. D., Dudek, B. C., & Crabbe, J. C. (2003). Different data from different labs: Lessons from studies of gene–environment interaction. *Journal of Neurobiology*, 54(1), 283–311. <https://doi.org/10.1002/neu.10173>
- Weather Spark. (n.d.). *Compare the Climate and Weather in Madison and Riverside*. Retrieved November 30, 2025, from <https://weatherspark.com/compare/y/12796~1891/Comparison-of-the-Average-Weather-in-Madison-and-Riverside>
- Winters, W. (1986). Seasonal and sex influences on ketamine-induced analgesia and catalepsy in the rat; a possible role for melatonin. *Neuropharmacology*, 25(10), 1095–1101. [https://doi.org/10.1016/0028-3908\(86\)90156-5](https://doi.org/10.1016/0028-3908(86)90156-5)
- Wone, B. W. M., Madsen, P., Donovan, E. R., Labocha, M. K., Sears, M. W., Downs, C. J., Sorensen, D. A., & Hayes, J. P. (2015). A strong response to selection on mass-independent maximal metabolic rate without a correlated response in basal metabolic rate. *Heredity*, 114(4), 419–427. <https://doi.org/10.1038/hdy.2014.122>
- Wood, S. N. (2017). *Generalized Additive Models*. Chapman and Hall/CRC. <https://doi.org/10.1201/9781315370279>
- Wood, S. N., Pya, N., & Säfken, B. (2016). Smoothing Parameter and Model Selection for General Smooth Models. *Journal of the American Statistical Association*, 111(516), 1548–1563. <https://doi.org/10.1080/01621459.2016.1180986>
- Wood, S., & Scheipl, F. (2009). gamm4: Generalized Additive Mixed Models using “mgcv” and “lme4.” In *CRAN: Contributed Packages*. <https://doi.org/10.32614/CRAN.package.gamm4>

Zucker, I. (2001). *Circannual Rhythms Mammals* (pp. 509–528).
https://doi.org/10.1007/978-1-4615-1201-1_20

Tables

Table 1. Generalized additive mixed model comparison to determine heterogeneity in the cyclic spline (i.e., annual cycle) in voluntary wheel running A) distance, B) duration, and C) speed, D) body mass before wheel measurements, and for E) body mass after wheel measurements. A common base spline model (i.e., a single spline for the entire dataset) was compared to more complex models that allowed the annual cycle to differ according to sex (males vs females) linetype (i.e., control vs selected), university (Wisconsin vs Riverside) and all possible combination of the three variables. Models were compared with the Akaike Information Criterion (AIC) and adjusted R² (R-sq.(adj)). Also included are the number of parameters for the cyclic spline (# of param.) and the difference in AIC from the best model (Δ AIC). The most parsimonious model (i.e., lowest AIC) is highlighted in bold and equally preferred models (within 2 units of the lowest AIC) are highlighted in italic font. Click [here](#) to go back to main text.

Cyclic spline heterogeneity	# of param.	AIC	Δ AIC	R-sq.(adj)
A) Running distance				
common base spline	1	676985.6	190.2	0.563
university	2	676993.5	198.1	0.563
linetype	2	676835.7	40.3	0.565
sex	2	676939.4	144.0	0.563
linetype x university	4	676847.8	52.4	0.565
sex x university	4	676939.9	144.5	0.563
sex x linetype	4	676795.4	0.0	0.565
sex x linetype x university	8	676799.6	4.2	0.563
B) Running duration				
common base spline	1	429611.8	195.9	0.284
university	2	429616.5	200.6	0.285
linetype	2	429615.0	199.1	0.283
sex	2	429416.3	0.4	0.286
linetype x university	4	429616.3	200.4	0.282
<i>sex x university</i>	4	<i>429415.9</i>	<i>0.0</i>	<i>0.285</i>
sex x linetype	4	429425.3	9.4	0.282
sex x linetype x university	8	429421.0	5.1	0.285
C) Running speed				
common base spline	1	210841.2	166.8	0.564
university	2	210849.9	175.5	0.564
linetype	2	210674.4	0.0	0.568
sex	2	210848.8	174.4	0.564
linetype x university	4	210688.5	14.1	0.567
sex x university	4	210845.7	171.3	0.564
sex x linetype	4	210689.8	15.4	0.566
sex x linetype x university	8	210683.7	9.3	0.566
D) Body mass before wheel measurements				
common base spline	1	153217.8	85.6	0.664
university	2	153220.7	88.5	0.667
linetype	2	153229.4	97.2	0.666
sex	2	153191.7	59.5	0.666
linetype x university	4	153237.1	104.9	0.667
sex x university	4	153132.2	0.0	0.664
sex x linetype	4	153209.5	77.3	0.665
sex x linetype x university	8	153146.0	13.8	0.662
E) Body mass after wheel measurements				
common base spline	1	148208.2	117.3	0.685
university	2	148206	115.1	0.687
linetype	2	148209.1	118.2	0.685
sex	2	148140.9	50.0	0.685
linetype x university	4	148214	123.1	0.687
sex x university	4	148090.9	0.0	0.687
sex x linetype	4	148150.5	59.6	0.685
sex x linetype x university	8	148106.8	15.9	0.685

Table 2. Summary of the best fitting generalized additive mixed models (based on Akaike Information Criterion, see Table 1) showing nonparametric terms (estimated degrees of freedom (edf)), F statistic and p value) and amplitude for the cyclic spline (i.e., annual cycle) in voluntary wheel running behaviours and body mass. Separate models were fitted for A) distance, B) duration, and C) speed, D) body mass before wheel measurements, and for E) body mass after wheel measurements. Click [here](#) to go back to main text.

Smooth terms	edf	F	p-value	amplitude
A) Running distance: sex x linetype				
Female, control	0.974	0.487	0.0989	114.19
Female, selected	1.954	0.977	< 2e-16	865.14
Male, control	1.727	0.864	3.58E-05	334.99
Male, selected	1.977	0.988	< 2e-16	1228.79
B) Running duration: sex				
Female	1.488	0.747	0.0171	8.68
Male	1.933	0.997	< 2e-16	27.62
C) Running speed: linetype				
Control	1.653	1.101	0.00284	0.45
Selected	1.975	9.233	< 2e-16	1.82
D) Body mass before wheel measurements: sex x university				
Female, Wisconsin	1.803	3.441	2.90E-05	0.66
Female, UCR	1.051	0.696	0.119	0.05
Male, Wisconsin	1.906	7.947	< 2e-16	0.99
Male, UCR	7.45E-08	0	1	0.00
E) Body mass after wheel measurements: sex x university				
Female, Wisconsin	1.884	6.297	< 2e-16	0.74
Female, UCR	1.599	2.226	0.0021	0.29
Male, Wisconsin	1.952	16.058	< 2e-16	1.18
Male, UCR	1.674	2.853	0.0004	0.33

Table 3. Summary of the coefficient of cyclical variation (ccv) for the best fitting generalized additive mixed models (based on Akaike Information Criterion, see Table 1) showing the mean of the raw variable, standard cyclic deviation (cyclic sd) and ccv (in %) in voluntary wheel running behaviour (A distance, B duration, and C speed), body mass measured D) before and E) after six days of wheel access. A common base spline was also fitted. Click [here](#) to go back to main text.

Smooth terms	mean	Cyclic sd	ccv %
A) Running distance			
Common	8508.89	656.74	7.72
Female Control	4567.21	80.97	1.77
Female Selected	11523.42	614.14	5.33
Male, Control	3601.77	237.26	6.59
Male, Selected	9301.40	870.98	9.36
B) Running duration			
Common	464.63	14.56	3.13
Female	498.07	6.16	1.24
Male	430.56	19.55	4.54
C) Running Speed			
Common	17.39	0.94	5.41
Control	10.06	0.32	3.17
Selected	20.56	1.29	6.27
D) Body mass before wheel measurements			
Common	26.96	0.52	1.91
Female, UCR	23.96931	0.03	0.13
Female, Wisconsin	23.88121	0.47	1.96
Male, UCR	29.96978	0.00	0.00
Male, Wisconsin	30.1352	0.70	2.34
E) Body mass after wheel measurements			
Common	26.48	0.49	1.85
Female, UCR	23.68	0.20	0.86
Female, Wisconsin	23.25	0.52	2.25
Male, UCR	29.58	0.23	0.78
Male, Wisconsin	29.51	0.83	2.83

Figures

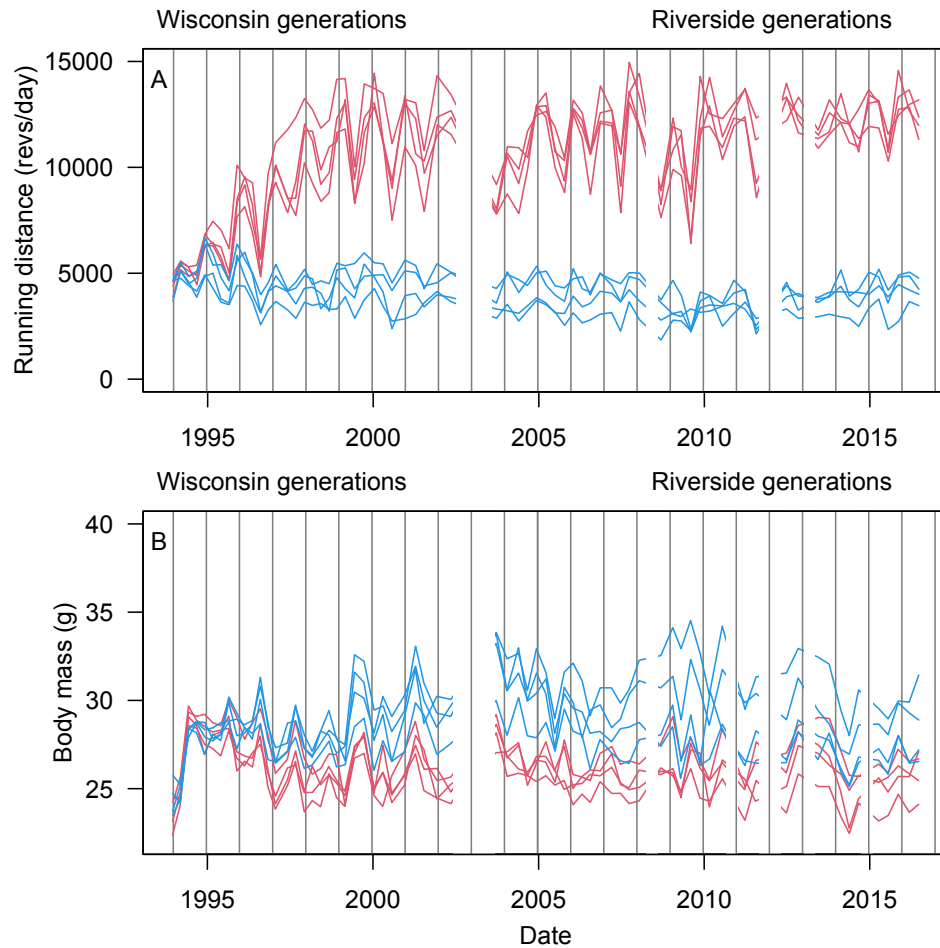


Figure 1. Unadjusted average in (A) running distance (revolutions/day) and (B) body mass (g) from each of the four selected lines (red) and control lines (blue) of laboratory mice plotted against calendar time (1996-2016). Interruptions represent missing values associated with the university change in 2002 (generations 32-35) or from excluded generations (52, 63, 64, and 67), as well as generations with missing data for body mass only (60 and 73). The data set for wheel-running distance is of 36,002 observations from 71 generations, comprised of 10,878 control and 25,124 selected mice. For body mass, the dataset consists of 35,028 observations across 69 generations including 10,567 control mice and 24,461 selected mice. Vertical gray lines indicate the winter solstice (21st of December). Click [here](#) to go back to main text.

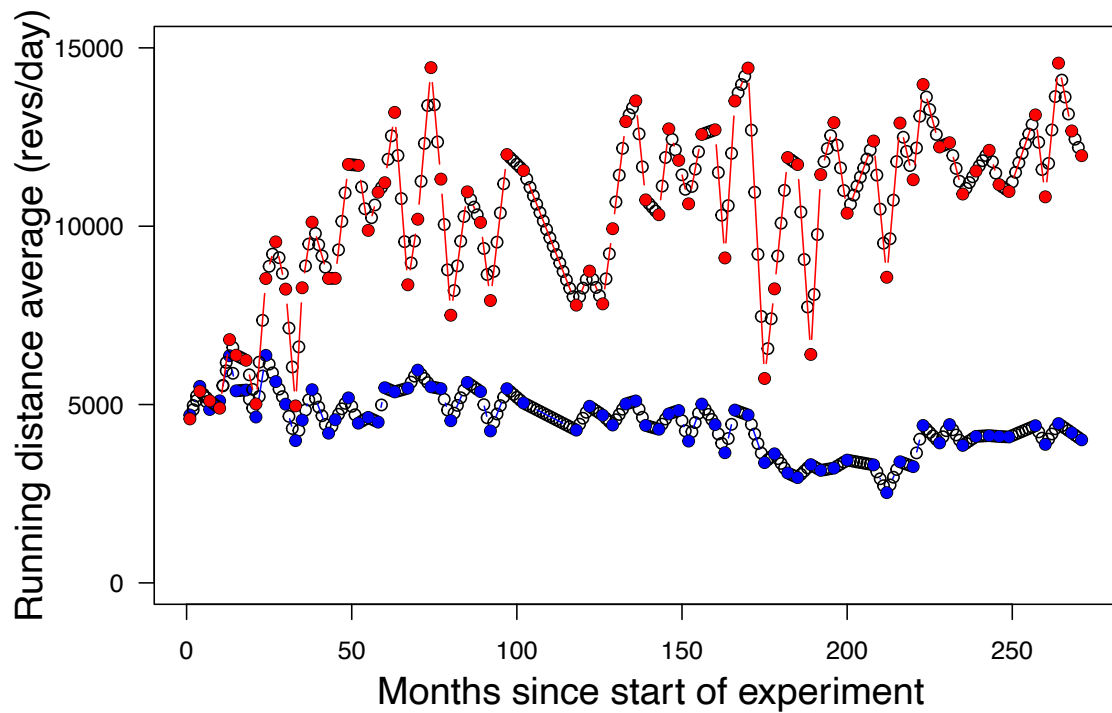


Figure 2. Wheel running (revolutions/day) generation averages in representative control line C1 (blue; 2,815 individuals) and selected line S3 (red; 6,404 individuals), plotted against months since the start of the experiment. The solid-colored dots indicate the real measured averages (69 observations, one per generation). The linearly imputed data are shown as open circles (196 observations from the `approx()` function in R). Click [here](#) to go back to main text.

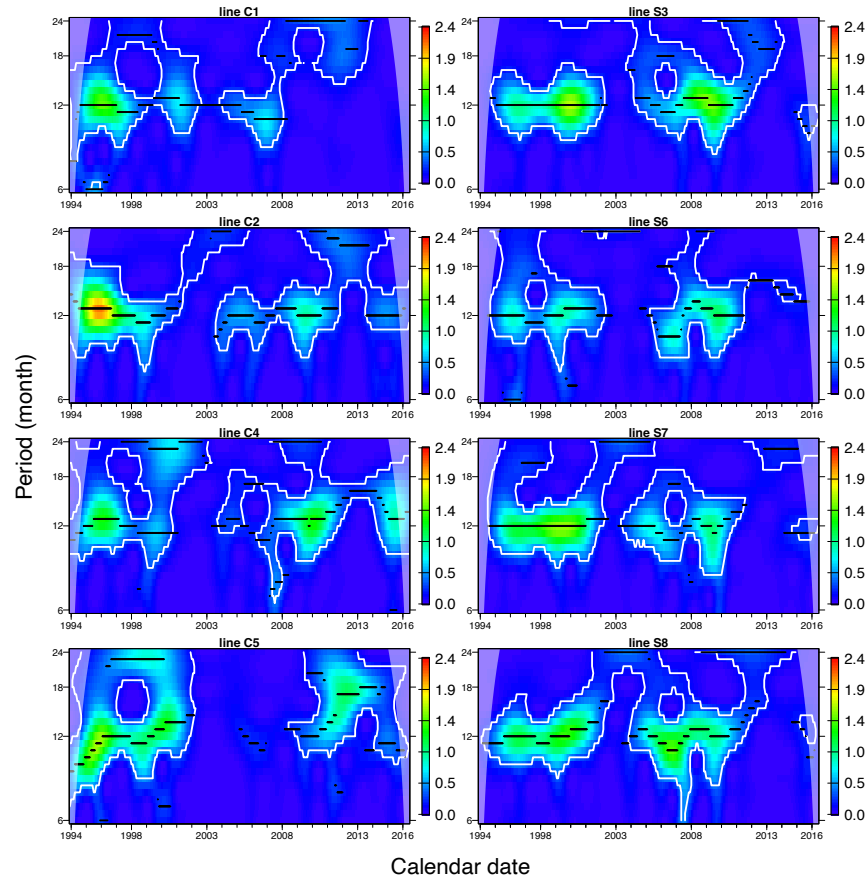


Figure 3. Wavelet power spectrum using Morlet analysis for running distance of four control (left panels) and four selected (right panels) lines of laboratory house mice. The black lines denote the main detected periods of activity in the frequency domain (the modal frequency is traced by the solid line). The vertical axis shows the Fourier periods in months and the horizontal axis shows the calendar date. Wavelet power is determined by the color of the heat map which indicates the amplitude of running distance (revs/day). Colors code from blue (low amplitude) to dark red (high amplitudes). The area within the white lines indicates that wavelet power spectra were statistically significant at the 95% confidence level. Spectral information inside the gray background is subject to an edge effect (the so-called “cone of influence”) and should be interpreted with caution. Click [here](#) to go back to main text.

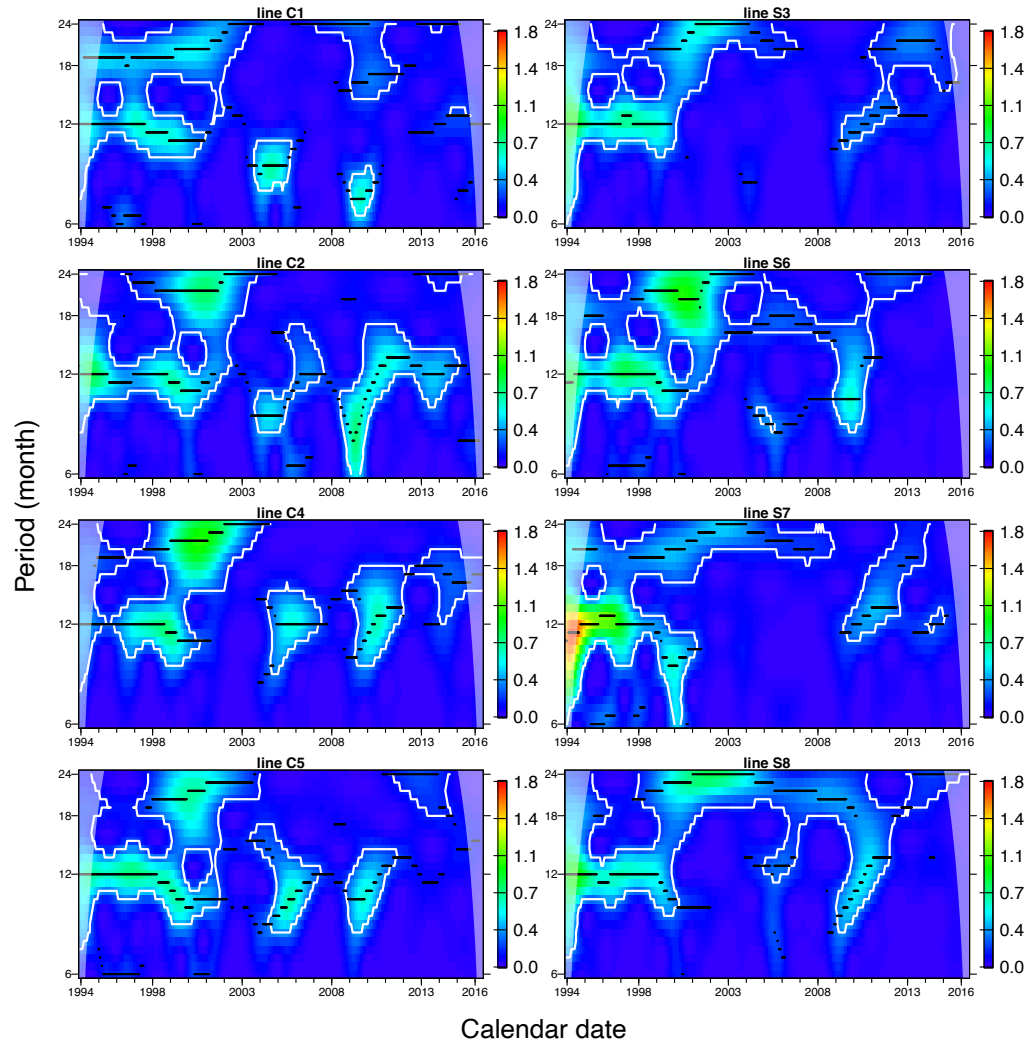


Figure 4. Wavelet power spectrum using Morlet analysis for body mass measured before six days of wheel running in four control (left panels) and four selected (right panels) lines of laboratory mice artificially selected for voluntary wheel running. See Fig. 3 caption for explanation. Click [here](#) to go back to main text.

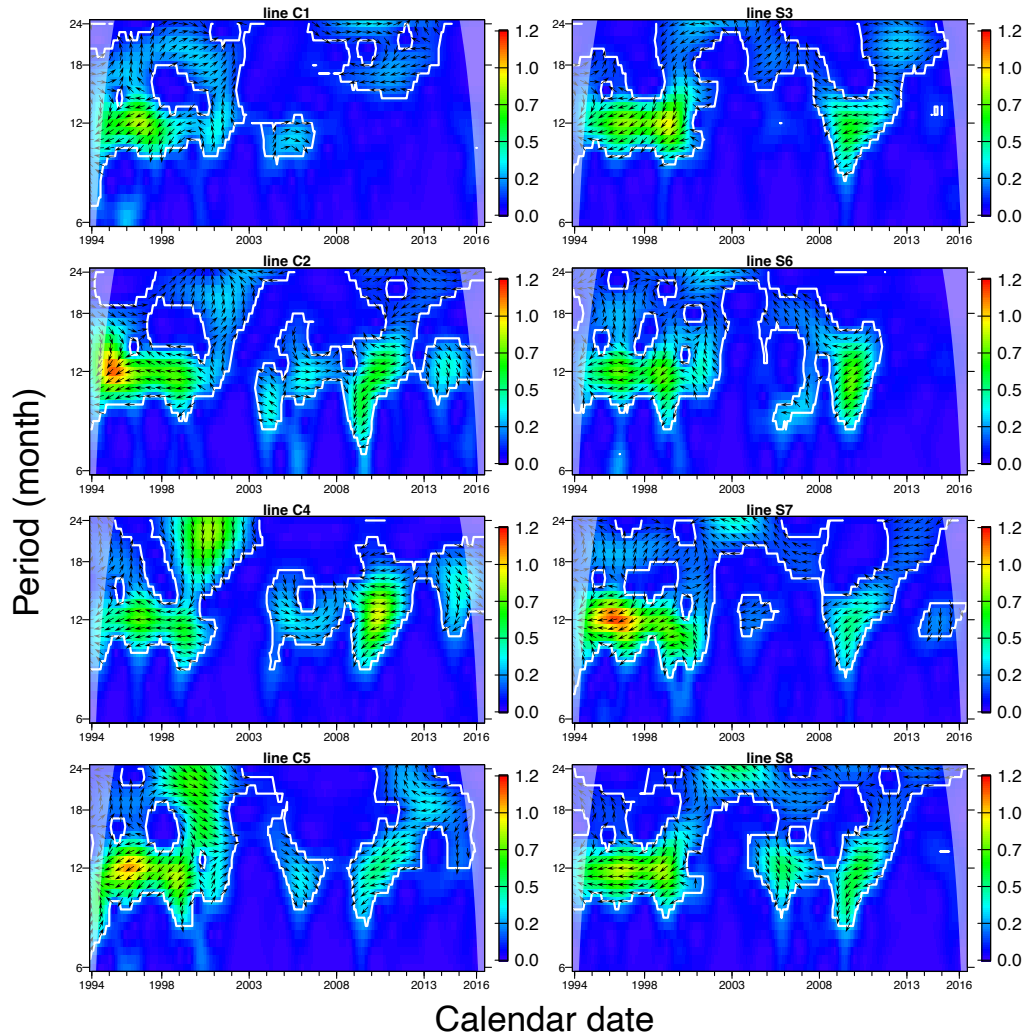


Figure 5. Wavelet coherence between running distance and body mass in 4 control (left panels) and 4 selected (right panels) lines. The horizontal axis shows time, while the vertical axis shows the period in days. The warmer the color of a region, the higher the degree of linear correlation between the pair of traits. Arrow direction refers to the phase difference between the time series. Arrows pointing to the right (left) direction mean the time series are in (out of) phase. Arrows with upward (downward) components denote that running distance leads (lags) behind the mass. The area within the white lines represents a statistically significant correlation at the 95% confidence level. Click [here](#) to go back to main text.

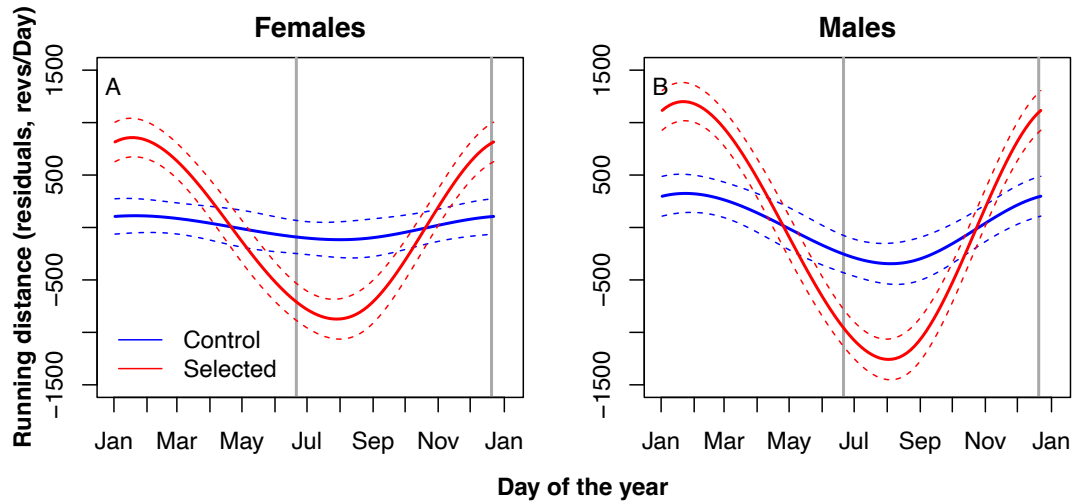


Figure 6. Estimated cyclic spline terms extracted from the most parsimonious generalized additive mixed model representing the seasonal trend over the day of the year (i.e., annual cycle) for voluntary wheel running distance for A) Females, and B) Males, in selected (red) and control lines (blue). The predicted smooth functions (lines) are shown with standard error (dotted lines). Gray lines indicate the summer and winter solstice (21st of June and December). Click [here](#) to go back to main text.

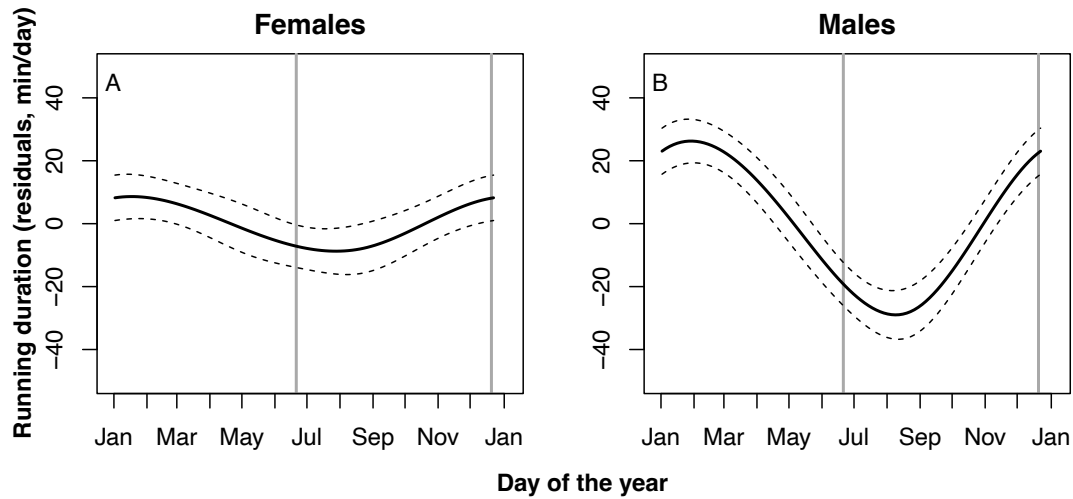


Figure 7. Estimated cyclic spline terms extracted from the generalized additive mixed model representing the seasonal trend over the day of the year (i.e., annual cycle) for voluntary wheel running duration for A) females, and B) males. The predicted smooth functions (lines) are shown with standard error (dotted lines). Gray lines indicate the summer and winter solstice (21st of June and December). Click [here](#) to go back to main text.

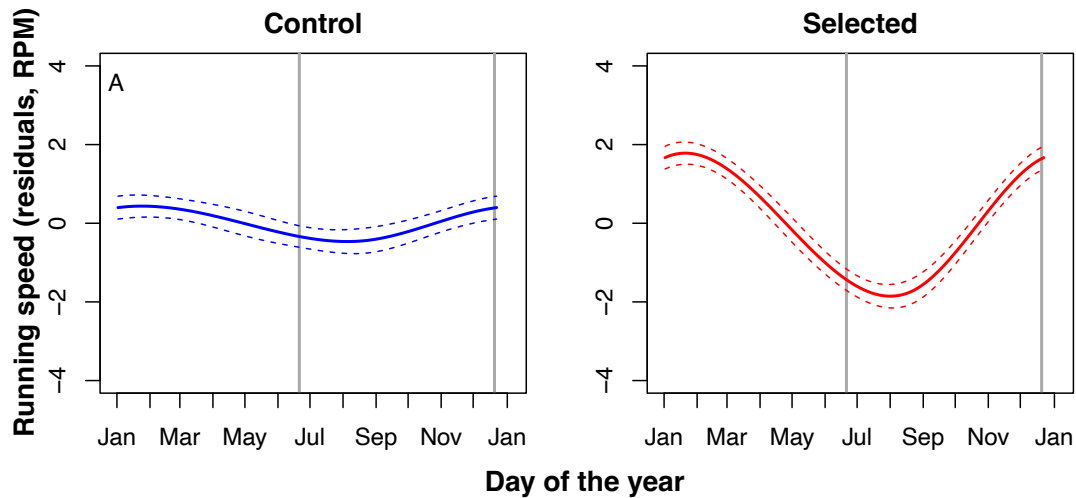


Figure 8. Estimated cyclic spline terms extracted from the most parsimonious generalized additive mixed model representing the seasonal trend over the day of the year (i.e., annual cycle) for voluntary wheel running speed for A) control and B) selected lines. The predicted smooth functions (lines) are shown with standard error (dotted lines). Gray lines indicate the summer and winter solstice (21st of June and December). Click [here](#) to go back to main text.

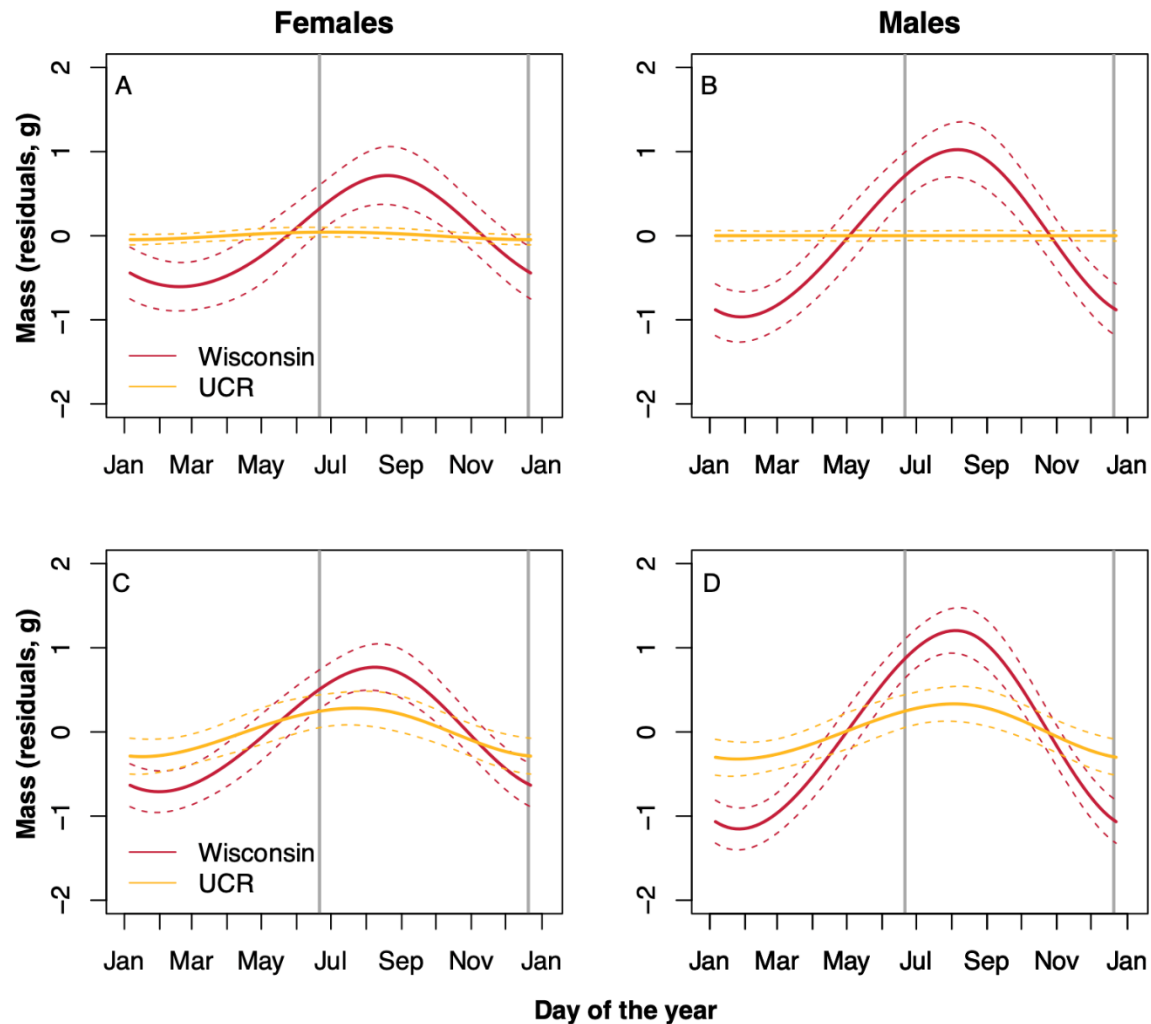


Figure 9. Estimated cyclic spline terms extracted from the most parsimonious generalized additive mixed model representing the seasonal trend over the day of the year (i.e., annual cycle) for body mass measured before (A and B) and after (C and D) wheel access in females (left panels) and male (right panels) in Wisconsin (red) and Riverside (gold). The predicted smooth functions (lines) are shown with standard error (dotted lines). Gray lines indicate the summer and winter solstice (21st of June and December). Click [here](#) to go back to main text.

Supplementary

Table S1. Summary of the second-best fitting generalized additive mixed model for running distance (see Table 1) showing nonparametric terms (estimated degrees of freedom (edf)), F statistic and p value) and amplitude for the cyclic spline (i.e., annual cycle). Click [here](#) to go back to main text.

Smooth terms	edf	F	p-value	amplitude
Running duration: sex x university				
Female, Wisconsin	1.182	0.592	0.07266	8.15
Female, UCR	5.398E-07	0	0.49004	1.50
Male, Wisconsin	1.911	0.976	< 2e-16	32.23
Male, UCR	1.941	0.975	< 2e-16	14.73

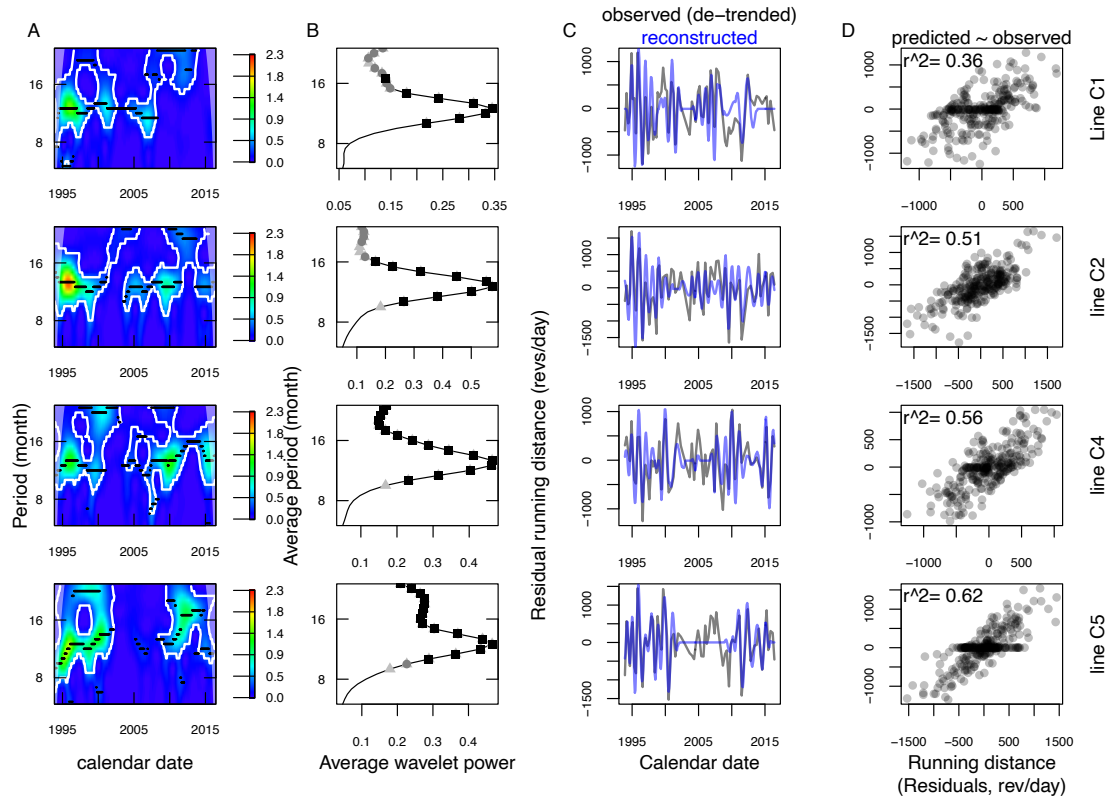


Figure S1. (A) Continuous wavelet power spectrum for wheel running of the 4 control lines. (B) Average wavelet cross-spectrum for running distance in control lines indicating average period (month) plotted against average wavelet power. (C) Residual wheel running (revs/day) in control lines plotted against calendar date. The blue solid line corresponds to the reconstructed residual data by the wavelet function and the light gray line refers to the observed (de-trended) residuals. (D) Correlation of observed wheel running residuals against predicted wheel running residuals by the wavelet function. Click [here](#) to go back to main text.

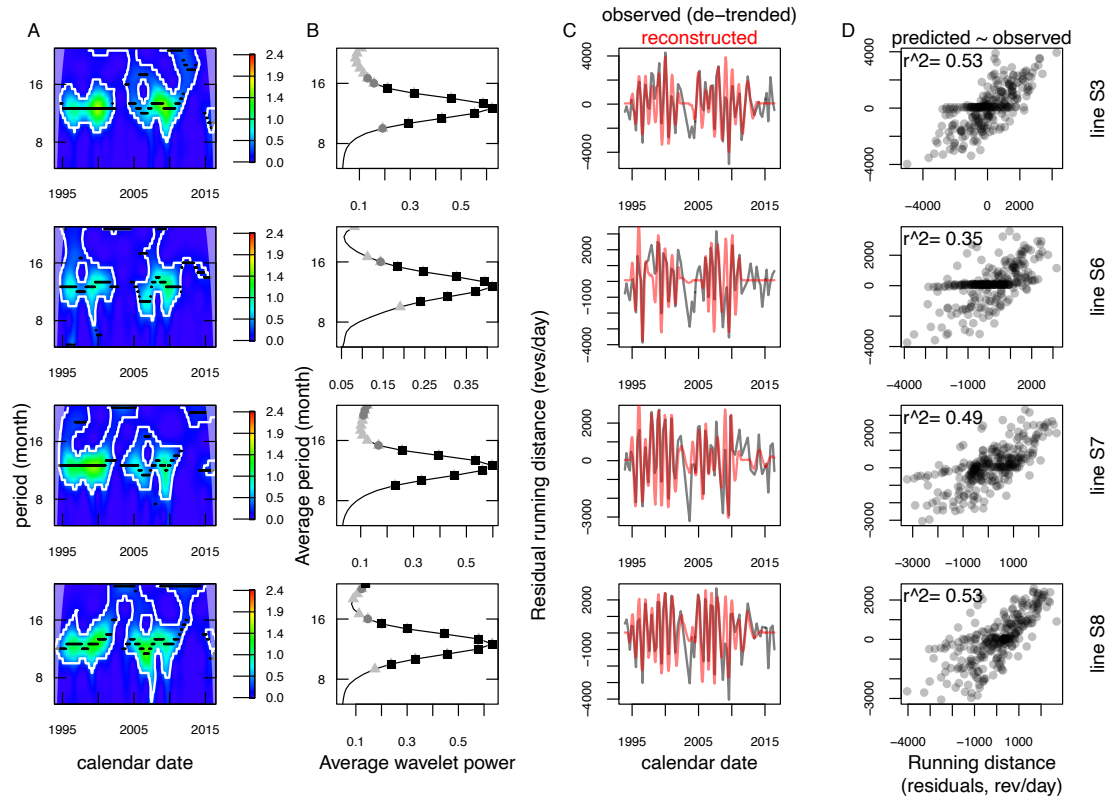


Figure S2. (A) Continuous wavelet power spectrum for wheel running of the 4 selected lines. (B) Average wavelet cross-spectrum for running distance in selected lines indicating average period (month) plotted against average wavelet power. (C) Residual wheel running (revs/day) in selected lines plotted against calendar date. The red solid line corresponds to the reconstructed residual data by the wavelet function and the light gray line refers to the observed (de-trended) residuals. (D) Correlation of observed wheel running residuals against predicted wheel running residuals by the wavelet function. Click [here](#) to go back to main text.

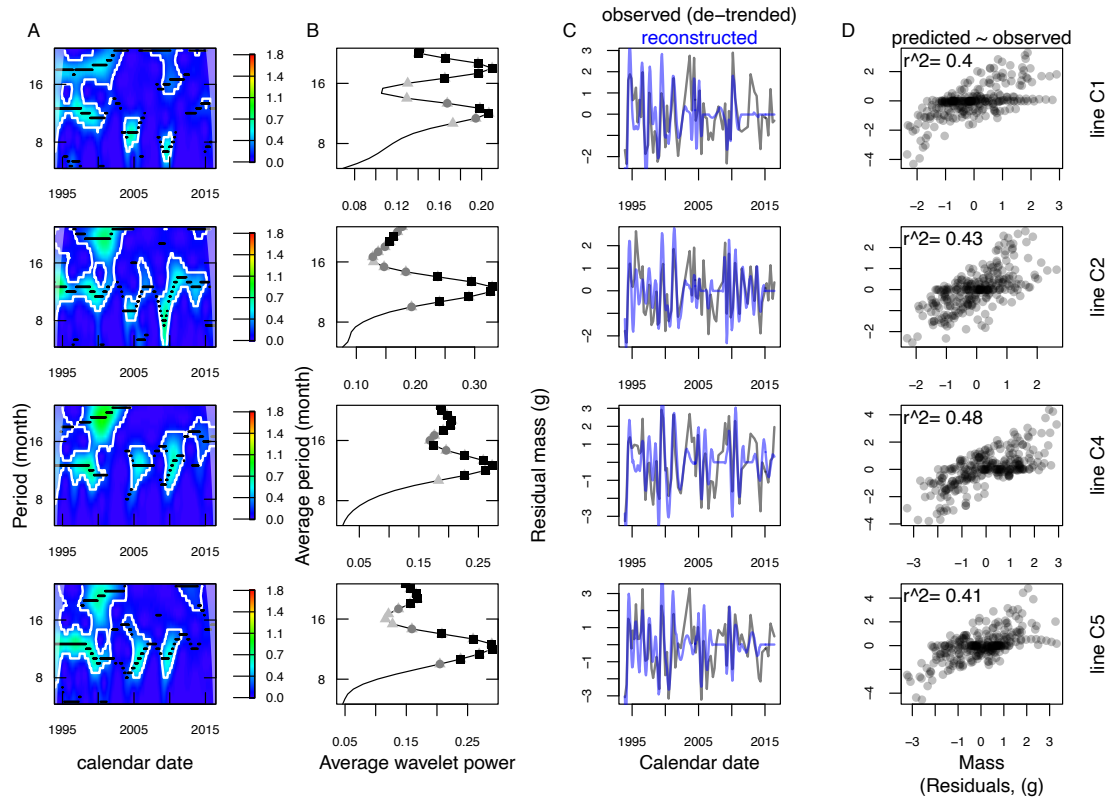


Figure S3. (A) Continuous wavelet power spectrum for body mass of the 4 control lines. (B) Average wavelet cross-spectrum for body mass in control lines indicating average period (month) plotted against average wavelet power. (C) Residual body mass (revs/day) in control lines plotted against calendar date. The blue solid line corresponds to the reconstructed residual data by the wavelet function and the light gray line refers to the observed (de-trended) residuals. (D) Correlation of observed body mass residuals against predicted body mass residuals by the wavelet function. Click [here](#) to go back to main text.

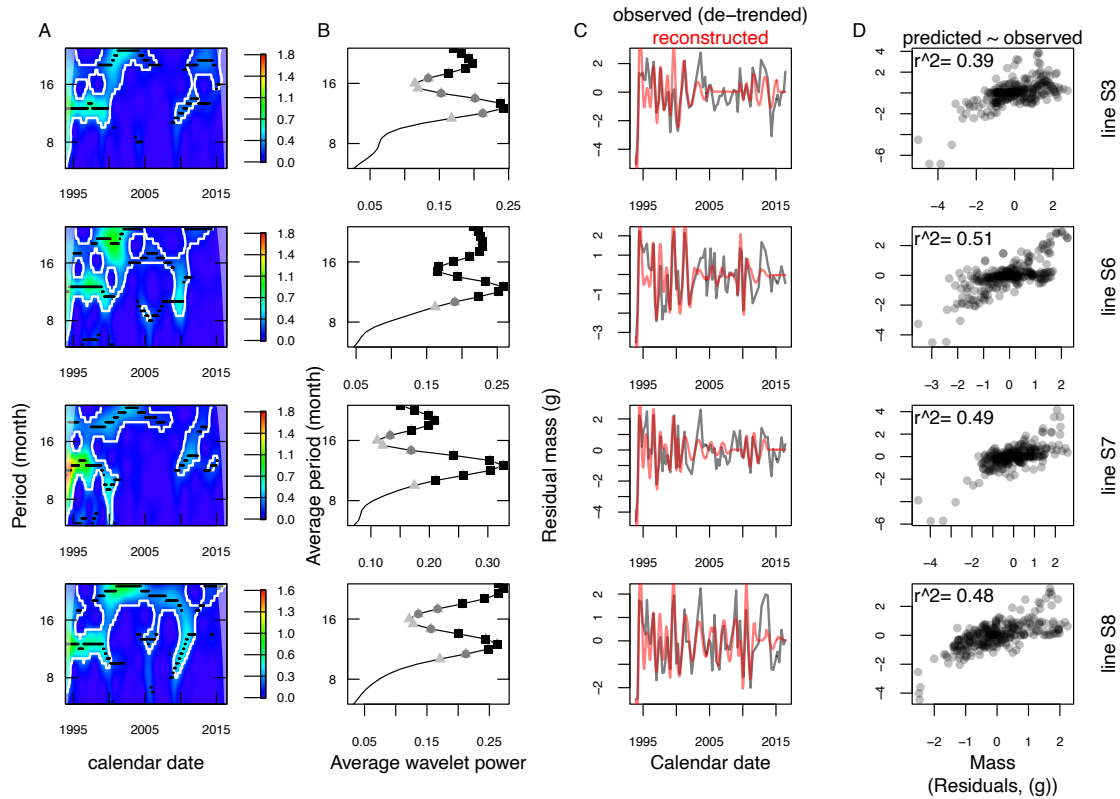


Figure S4. (A) Continuous wavelet power spectrum for body mass of the 4 selected lines. (B) Average wavelet cross-spectrum for body mass in selected lines indicating average period (month) plotted against average wavelet power. (C) Residual body mass (revs/day) in selected lines plotted against calendar date. The blue solid line corresponds to the reconstructed residual data by the wavelet function and the light gray line refers to the observed (de-trended) residuals. (D) Correlation of observed body mass residuals against predicted body mass residuals by the wavelet function. Click [here](#) to go back to main text.

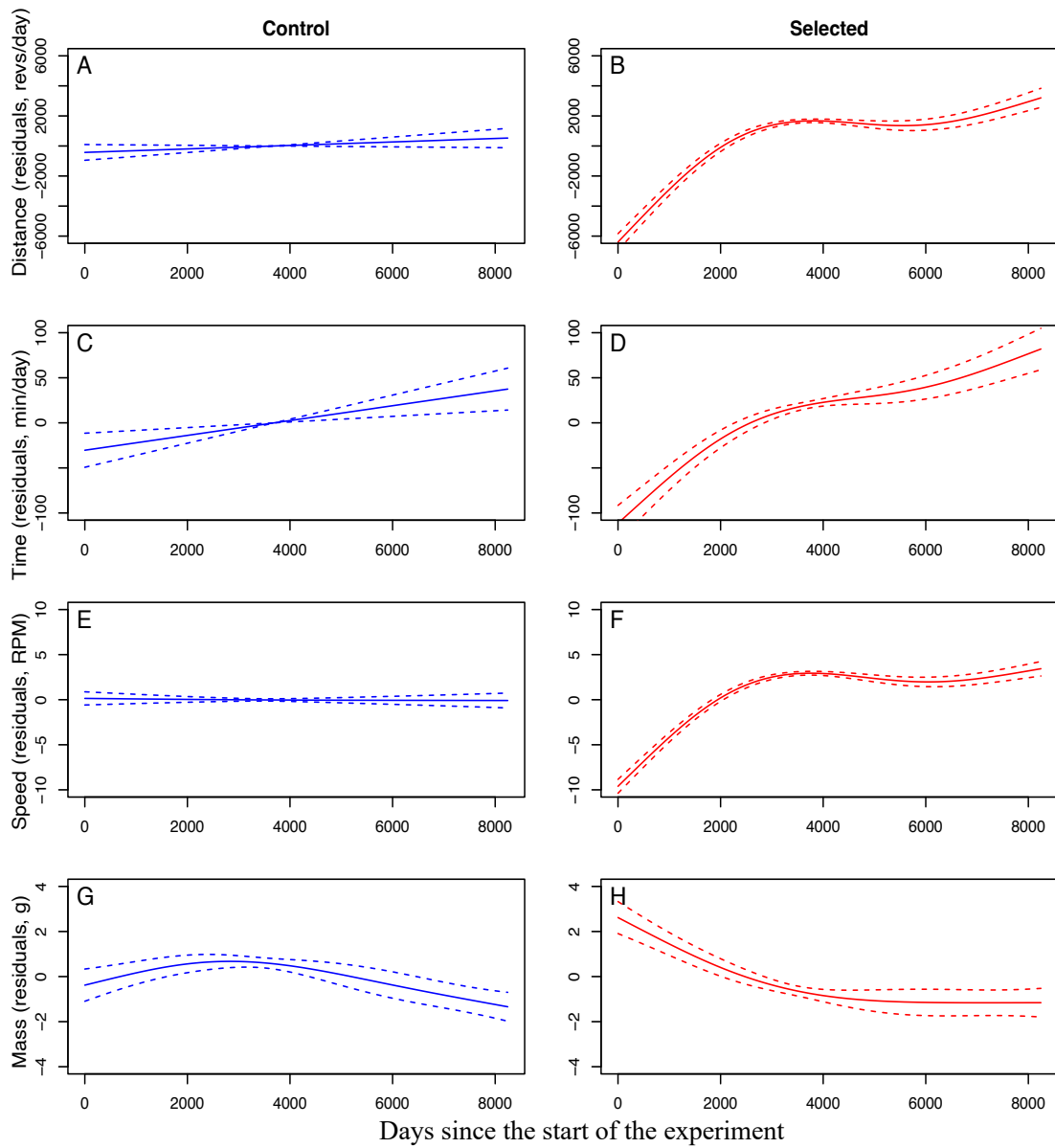


Figure S5. Estimated smooth terms from the generalized additive mixed model showing long-term trends in voluntary wheel running distance (A, B), duration (C, D), speed (E, F) and body mass (G, H) over time (days since the start of experiment) for control (left) and selected (right) lines. The predicted smooth functions (lines) are shown with 95% confidence intervals (dotted lines). Click [here](#) to go back to main text.

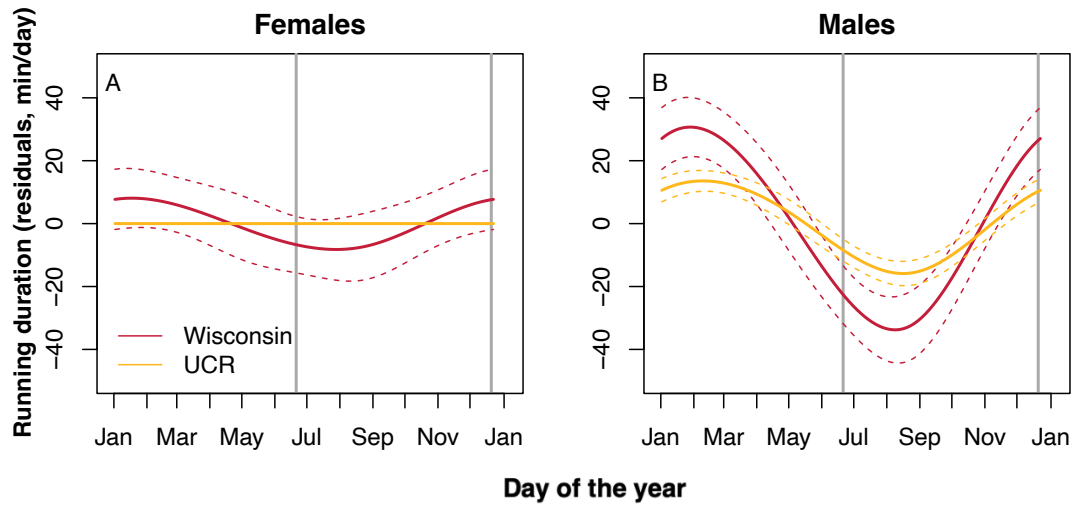


Figure S6. Estimated smooth terms from the generalized additive mixed model’s cyclic spline “Day” representing the seasonal trend over the day of the year (i.e., annual cycle) for running duration in A) females, and B) males, in Wisconsin (red) and Riverside (gold). The predicted smooth functions (lines) are shown with standard error (dotted lines). Gray lines indicate the summer and winter solstice (21st of June and December). Click [here](#) to go back to main text.