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Genetic architecture of voluntary exercise in an advanced intercross line of mice

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ABSTRACT

Exercise is essential for health, yet the amount, duration, and intensity that individuals engage in is strikingly variable, even under prescription. Our focus was to identify the locations and effects of quantitative trait loci (QTL) controlling genetic predisposition for exercise-related traits utilizing a large advanced intercross line (AIL) of mice. This AIL (G_4) population originated from a reciprocal cross between mice with genetic propensity for increased voluntary exercise (HR, selectively bred for increased wheel running) and the inbred strain C57BL/6J. After adjusting for family structure, we detected 32 significant and 13 suggestive QTL representing both daily running traits (distance, duration, average speed, and maximum speed) and the mean of these traits on days 5 and 6 (the selection criteria for HR) of a 6-day test conducted at 8 weeks of age, with many colocalizing to similar genomic regions. Additionally, 7 significant and 5 suggestive QTL were observed for the slope and intercept of a linear regression across all 6 days of running, some representing a combination of the daily traits. We also observed 2 significant and 2 suggestive QTL for body mass prior to exercise. These results, using a well-defined animal model, reinforce a genetic basis for the predisposition to engage in voluntary exercise, dissect this predisposition into daily segments across a continuous time period, and present unique QTL that may provide insight into the initiation, continuation, and temporal pattern of voluntary activity in mammals.

Key words: artificial selection, exercise physiology, Genome Reshuffling for Advanced Intercross Permutation (GRAIP), quantitative trait loci, voluntary wheel running

INTRODUCTION

According to Dickinson and colleagues (17), "Locomotion, movement through the environment, is the behavior that most dictates the morphology and physiology of animals." From an evolutionary perspective, sustained long-distance running may be a derived capacity of the genus *Homo*, originating approximately 2 million years ago, and appears to have been vital in shaping modern human physiological and anatomical architecture (e.g., 3, 9). Movement is also intimately associated with the ecology of animals and is vital for procuring food, finding mates, predator avoidance, and dispersal (e.g., 32). From a human-health perspective, substantial evidence indicates that physical inactivity is an important risk factor for a number of chronic diseases, chief of which may be obesity and cancer (30, 67; but see 69).

Despite the documented importance of exercise to health-related quality of life (2, 22, 47, 62), there remains considerable variation in human activity levels, even within a given society, sex, and age cohort, with many people remaining inactive or not exercising enough to realize the rewards (e.g., 19; see also 67). Consequently, emerging studies are now beginning to elucidate the genetic architecture underlying the predisposition for voluntary exercise, in order to better understand the nature of this important inter-individual variability.

It has been well established in both human beings and mice that the predisposition to engage in voluntary activity is heritable (e.g., 21, 41, 63), but the locations of specific genetic markers associated with this predisposition are just beginning to be elucidated in humans (e.g., 8, 16, 57) and mice (e.g., 34, 42, 46, 72). Like studies will continue to improve our understanding of the biological factors controlling individual variation in voluntary physical activity levels and, in conjunction with data reviewed by Bray et al. (4), may aid clinicians in designing more effective physical activity-based therapies with targeted dosages and intensities (see 10, 40, 54).

66 The focus of the current study was to identify the locations and magnitudes of
67 quantitative trait loci (QTL) controlling exercise-related traits utilizing a large, moderately
68 advanced intercross line (AIL) of mice. Through random intercrossing over multiple
69 generations, the production of AILs can provide a more accurate approach to map loci by
70 accumulating recombination events and providing increased mapping resolution (14). This G_4
71 population originated from a reciprocal cross between mice with genetic propensity for increased
72 voluntary exercise (high-runner line: HR) and the inbred strain C57BL/6J (B6). The HR line
73 originated from a long-term replicated artificial selection experiment for high voluntary wheel-
74 running behavior on days 5 and 6 of a 6-day wheel exposure (reviewed in 24, 64). By generation
75 16, and continuing through generation 50 and beyond, the HR lines (4 replicates) had diverged
76 from the control lines (C lines, 4 replicates) with an approximate 2.5-3.0 fold increase in total
77 revolutions/day. This was caused primarily by HR mice running faster rather than for more
78 minutes each day, but the relative importance of the two components differs between the sexes
79 (males show a significant increase in amount of time spent running) and among the four replicate
80 HR lines (see 63 and Fig. 4 in 52). These lines of mice have been the focus of numerous studies
81 characterizing the morphological, physiological, and behavioral traits that have evolved in
82 concert with high levels of voluntary activity (reviewed in 24, 64).
83 In the current study, we genotyped over 800 G_4 mice representing reciprocal crosses
84 between HR and B6 with a genome-wide SNP panel. Our primary goal was to map QTL related
85 to running traits on days 5 and 6 of the six-day exposure to wheels as this was the criterion for
86 which the HR mice were selectively bred. However, it has long been recognized that wheel
87 running activity varies temporally (60). Yet still, despite these long-standing observations, little
88 is known with regard to the mechanistic underpinnings of the initiation, continuation, trajectory,

or day-to-day variation in wheel running in rodents (39, 56). Thus, a secondary goal of the present study was to map running traits associated with the initiation, continuation, and temporal pattern of voluntary wheel running behavior across the six days of wheel access.

MATERIALS AND METHODS

G_4 creation and phenotyping. Full details of the creation and phenotyping of the G_4 population have been provided elsewhere (36) and only the pertinent features are presented here. Progenitor HR and B6 mice (n = 44, 22 males and 22 females per line) mice underwent a reciprocal breeding protocol to produce a F_1 generation. In subsequent generations (F_2, F_3, G_4), the two reciprocal cross-line populations (HR♀ X B6♂ and B6♀ X HR♂) were not mixed and were always comprised of 32 mating pairs each. From these mating pairs, no fewer than 16 unique families were represented in each reciprocal cross population. In each generation, inter-familial matings were assigned using a Latin square design to avoid inbreeding and increase the effective population size. Following the F_3 generation, a large G_4 population was produced through extra parities for extensive phenotypic and genotypic data collection. Extra parities were generated by allowing the same sets of parents to produce multiple litters.

G_4 individuals (n = 815) at 8 weeks of age were weighed (± 0.1 g) and then exposed to running wheels (model 80850, Lafayette Instruments, Lafayette, Indiana, USA; circumference = 1.1 m) for 6 days. Voluntary wheel running was recorded electronically in one-minute intervals for 23-24 hours of each of the 6 days of wheel access. Following the 6th day of wheel access, mice were weighed, sacrificed via decapitation, and tissues harvested. Throughout phenotyping, mice were provided a repeatable synthetic control diet (Research Diet D10001; 21 kcal% protein, 68 kcal% carbohydrate, 13 kcal% fat) and water *ad libitum*. All procedures were approved by

and are in accordance with guidelines set forth by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill.

From the wheel-running recordings, the following daily traits were calculated: distance (total revolutions), time spent running (cumulative 1-minute intervals in which at least one revolution was recorded), average speed (total revolutions / time spent running), and maximum speed (highest number of revolutions in any 1-minute interval within a 24 hour period). In addition to daily traits, we calculated mean values of distance, time, average speed, and maximum speed on days 5 and 6 of the 6-day test. These traits are of particular interest as the mean number of total revolutions on days 5 and 6 was the criterion for which the HR line was selectively bred (63). Further, using least-squares linear regressions, we estimated the slope and intercept for distance, time, average speed and maximum speed across the 6 days of wheel exposure. If an individual did not have trait values for all 6 days of wheel exposure, then the corresponding slope and intercept values were omitted from analyses.

Descriptive statistics. Descriptive statistics for body mass (prior to wheel access) and voluntary wheel-running traits (described above) are presented in Table 1 (for a comparison to the parental strains see 45). Partial phenotypic correlations were performed in SAS (version 9.1; SAS Institute, Cary, NC) for body mass and mean running distance, time, average speed, and maximum speed on days 5 and 6 of the 6-day exposure to running wheels (Table 2). Correlations were adjusted for factors with known phenotypic effects (see 36), parent of origin [whether a G_4 individual was descended from a progenitor (F_0) cross of HR♀ X B6♂ or B6♀ X HR♂, coded as 1 or 0 respectively], sex, and wheel freeness (the number of wheel revolutions following acceleration to a given velocity). *P* values from partial correlations were adjusted for multiple comparisons utilizing the false discovery rate procedure (12) controlling the overall

Type I error rate at 5%. For simplicity, and because mean distance (on days 5 and 6) was the selection criterion for the HR line, we chose to only present the partial correlations for a subset of the 37 traits.

Genotyping and linkage map. A total of 815 G_4 mice were genotyped for 764 single nucelotide polymorphisms (SNPs). SNPs were selected based on their relatively even spacing across the genome and their predicted informativeness based on initial genotyping of representative individuals from the F_0 parental strains (n = 12, HR; n = 1, B6) using the Mouse Diversity array (71). Specifically we used 362,000 SNPs present in the training array to identify SNPs with identical homozygous genotypes in the HR samples and informative with respect to B6. Genotyping in the G_4 was performed using the Sequenom (San Diego, CA, USA) MassARRAY system as previously described (23). Following genotyping, we confirmed that markers were fully informative by comparing F_0 HR mice (n = 32) to a subset of F_0 B6 (n = 8). We excluded any SNPs where common alleles were shared between HR and B6 F_0 parental strains. All fully informative SNPs were checked for errors in approximate Mendelian inheritance and segregation distortion. Additionally, taking into account pedigree structure and higher levels of recombination relative to a F_2 population, we estimated genotyping errors using the error detection function in Merlin (1) and dropped individual calls that were deemed extremely unlikely. The final set of SNPs (n = 530, with an average spacing of 4.7 Mb) used for QTL analyses is provided in Supplemental Table 1. A genetic map was calculated using the R/qtl package (6) for the R environment (v 2.8.1) (51) treating the G_4 population as an F_2 (Supplemental Fig. 1).

QTL analyses. In total, we evaluated 37 quantitative traits (listed in Table 1) for location and magnitude of underlying QTL. In order to appropriately account for family structure (non

158 independence of individuals) in the G_4 population, we employed the Genome Reshuffling for
159 Advanced Intercross Permutation (GRAIP) procedure (49). GRAIP uses a permutation scheme
160 to create "randomized" populations that respect family structure. Parental (F_3) genotypes were
161 first estimated using Merlin (1). GRAIP randomized populations were then created. Each
162 population was created by permuting the identities of the parents respecting sex. From each set
163 of simulated F_3 progenitors, a simulated G_4 population was then created by simulating
164 inheritance and recombination. These simulated populations respect the family structure of the
165 original population but any association between genotype and phenotype has been removed.
166 Since family structure affects the association between genotype and phenotype, locus-specific
167 and genome-wide empirical *P* values can be estimated using the distribution of *P* values for these
168 permuted maps.

169 In order to generate permuted *P* values, QTL analyses were performed for the original
170 population and the GRAIP permuted populations (n = 50,000) utilizing R/qtl. Within R/qtl, the
171 multiple imputation method (55) was employed to handle missing data, drawing 16 times from
172 possible genotypes at each missing locus. Appropriate statistical models had previously been
173 defined (36) and included parent-of-origin type, sex, and wheel freeness. Parity (order of litters
174 from individual F_3 Dams) was not included in the model as there was no statistically significant
175 effect on any wheel-running trait. When analyzing body mass, wheel freeness was excluded
176 from the model and parity was added as an additional covariate. The X chromosome was
177 analyzed in two ways. Because R/qtl is currently designed for F_2 populations, and requires the
178 identity of the parental grandmother (coded as 0, 1) to most appropriately cope with the X
179 chromosome, we analyzed the X chromosome treating it as an autosome and utilizing the same
180 additive covariates as described above. For comparison, we treated the X as a sex chromosome,

allowing R/qtl to convert the X chromosome data to an internal standard using the provided sex identifiers and by inferring the direction of the cross.

Following R/qtl mapping of the original and permuted populations, we computed locus-specific *P* values as previously described (49, 50). In brief, utilizing the output from R/qtl, locus-specific *P* values were calculated for each marker of the original data set by utilizing the value for that specific marker in each of the permuted genome maps at each locus as a null distribution. We compared the null distribution for each marker with the value for the original G_4 mapping data in order to generate locus-specific *P*-values at marker positions. *P* values were then interpolated at regular physical points on the genome, based on the known physical position of markers, and placed on a scaffold at regularly spaced sets of physical positions. Finally, we computed genome-wide, adjusted *P* values by finding the minimum possible *P* values (or highest –log *P*, LOD) from each GRAIP permuted map by generating locus-specific *P*-values for each permuted map as described above and extracting the lowest locus-specific *P*-value from each permuted map. From this set of best locus-specific *P*-values, we then generated an ordered list. Note that genome-wide GRAIP adjusted significance thresholds were generated utilizing 50,000 permutations. Therefore, for the GRAIP output, a minimum possible *P* value with 50,000 permutations is 0.00002 (1/50,000), so the maximum –log $P = 4.7$. Loci that met or exceeded 95^{th} and 90^{th} percentiles of this ordered list were deemed significant and suggestive, respectively. These percentiles are equivalent to an empirical genome-wide $P = 0.05$ and $P = 0.10$, respectively. Confidence intervals (90-95%) of QTL locations were approximated by one LOD-drop support intervals in Mb (relative to the GRAIP-permuted LOD score) (5, 43, 44). The percent variation explained by each significant and suggestive QTL was extracted by standard linear regression by fitting the imputed QTL marker genotypes, and the additive QTL effects

were expressed in phenotypic standard deviation units and as a percentage of the residual variance. In additional analyses, to test for possible covariate interactions with a QTL (i.e., the effect of the QTL varying with the covariate), we included QTL X sex and QTL X parent of origin factors in the model in a stepwise fashion. Significant interactions were identified when $LOD_{Full} - LOD_{Additive} = LOD \geq 3.0$ (55).

The production of AILs provide an effective approach to map loci, but because of the complex breeding history, the assumption of independence among individuals has been conclusively shown to be false, and several additional methods currently exist to account for family structure (33, 48, 68). Our multi-generational breeding protocol expanded the final generation by producing multiple litters from the same set of crosses. The 30 unique families were represented by 57 breeding pairs (for complete details on breeding history see 36). Each breeding pair contributed an approximately equal number of litters (mean 2, range 1-3) to the G_4 generation with a mean size of 7.5 (range 2-13). Although each individual in our testing population (G_4) was not derived from a unique pair in the breeding population (F_3), as assumed in Darvasi and Soller's (14) simulations, we maximized the number of crosses while minimizing the number of offspring resulting from each cross in an attempt to reduce the effects of family. Given the relatively short number of intercrosses and the generally well-balanced mating design used in this study, it is possible that the GRAIP-adjusted LOD scores are overly conservative for our population, and we thus in some cases present and discuss the naive or unadjusted LOD scores from the simple mapping output (i.e., Supplemental Table 2).

RESULTS

Descriptive statistics and partial phenotypic correlations are presented in Tables 1 and 2, respectively. All traits were either approximately normally distributed or slightly skewed and reasonably symmetric. In the G_4 population, mean wheel-running traits on days 5 and 6 of the 6-day test were significantly correlated with one another, while no running trait was significantly correlated with body mass after controlling for sex, parent-of-origin, and wheel freeness (Table 2).

QTL analyses. Results for all QTL analyses are presented in Table 3, Fig. 1-5, and Supplemental Table 2. In total, 41 significant ($P \leq 0.05$, LOD ≥ 3.9) and 20 suggestive ($P \leq 0.1$, LOD ≥ 3.5) QTL were observed for the voluntary wheel traits and body mass after controlling for potential family structure utilizing the GRAIP procedure. Additionally, we provide QTL detected and respective statistics for body mass and voluntary wheel-running traits from unadjusted output that were significant at the genome-wide level ($P \leq 0.05$, LOD ≥ 3.9), but did not remain significant or suggestive ($P \leq 0.1$, LOD ≥ 3.5) following the GRAIP procedure (Supplemental Table 2). Regardless of the analysis method (see MATERIALS AND METHODS), we did not observe any significant or suggestive QTL on the X chromosome.

After adjusting for the family structure in the G_4 population utilizing the GRAIP procedure, 2 significant and 2 suggestive QTL were detected for body mass on MMU5, MMU6, MMU1, and MMU16, respectively. Fig. 1 depicts both the unadjusted and the GRAIP-adjusted permuted output.

In total, GRAIP-adjusted output revealed 11 significant and 7 suggestive QTL across the 9 different running distance (revolutions/day) traits. These QTL represented both daily running distances, the mean on days 5 and 6, and the slope and intercept across all 6 days of running (Fig. 2). Running distance QTL individually accounted for 1.5-4.4% of the total phenotypic variation.

QTL on MMU7 were either significant or suggestive for running distance on all days (except day 3) and the mean on days 5 and 6. On day 3, a peak on MMU7 was not significant or suggestive in the GRAIP-permuted output (LOD = 3.0), but the unadjusted mapping output revealed a LOD score of 3.5 (Fig. 2). Analyses of total revolutions across all 6 days revealed similar results (unadjusted LOD = 6.4 at 108.9 Mb on MMU7) to those for the daily measures.

Although QTL on MMU7 exhibited a strong and consistent day-to-day pattern, additional significant and suggestive QTL were found to be unique to only days 1-3. On days 1-3, QTL were found on MMU1 (n = 3), MMU5 (n = 2), and MMU6 (n = 2) (Table 3 and Fig. 2). Thus, it appears the genetic architecture for running distance can change across time, with some QTL remaining constant while others appear only during the initial exposure to wheels. With regard to slope of wheel running distance across all 6 days, a suggestive QTL was discovered on MMU11. QTL were also discovered for the intercept of the linear regression on MMU1, MMU6, and MMU7, and the locations were close to those observed for running distance on day 1.

For time spent running (i.e., cumulative 1-minute intervals in which at least one revolution was recorded), 16 significant and 3 suggestive QTL were discovered, many of them appearing to colocalize with those observed for running distance. QTL represented daily duration values, the mean on days 5 and 6, and the slope and intercept across all 6 days (Fig. 3). QTL individually accounted for 2.2-6.6% of the total phenotypic variation for time spent running. As observed for running distance, QTL on MMU7 (significant or suggestive) exhibited a consistent pattern for running duration on all days and the mean on days 5 and 6.

Running time QTL were also observed that were inconsistent across the entire wheel-access period. As observed for running distance, significant and suggestive QTL were

discovered on days 1-3 that were not observed on days 4-6 or for the mean running duration on days 5 and 6. On days 1-3 significant QTL were found on MMU1 (n = 2), MMU5 (n = 1), MMU6 (n = 1), and MMU13 (n = 1, suggestive) (Table 3 and Fig. 3). Additionally, analysis of day 5 running duration revealed a significant QTL on MMU19 that was not observed on any other day. Significant QTL were discovered on MMU1 (slope and intercept), MMU6 (slope and intercept), MMU11 (slope only), and MMU13 (intercept only), and found in similar regions to those observed for daily traits.

Average running speed (total revolutions / time spent running) analyses revealed 4 significant and 5 suggestive QTL found on MMU2, MMU12, MMU17, and MMU14. QTL represented daily running average speed and the mean average speed on days 5 and 6 (Fig. 4). QTL individually accounted for 2.3-3.4% of the total phenotypic variation for average running speed. Daily average running speed QTL (Fig. 4) represented less of a temporal pattern, as compared to running distance (Fig. 2) or time spent running (Fig. 3), with no QTL observed on the same chromosome for more than two consecutive days. No QTL were detected for slope, but one significant QTL, on MMU12, was discovered for the intercept of the linear regression across all 6 days, but it did not appear to colocalize with any QTL observed for average speed on individual days (Table 3).

Analyses of maximum running speed (highest number of revolutions in any one-minute interval within a 24 hour period) revealed 8 significant and 3 suggestive QTL across MMU2 and MMU11. QTL represented daily maximum running speed and the mean average speed on days 5 and 6 (Fig. 5). QTL individually accounted for 1.8-4.3% of the total phenotypic variation for maximum running speed. Although not significant in the GRAIP permuted output, peaks on day 4 (MMU2) and day 5 (MMU11) each had unadjusted LOD scores of 3.4 (Fig. 5). Considering

the former, daily QTL were reasonably consistent across all days with the exception day 1 where no significant or suggestive signals were observed. Contrary to what was observed for running distance and duration, no QTL was unique to the initial wheel exposure or any single day. No QTL were detected for the slope or intercept when examining trajectory of maximum running speed across all 6 days.

Most QTL had increasing effects resulting from the HR allele, but these effects were often day-dependent (Table 3). For example, for running distance, increasing effects of the B6 allele were often observed for the initial days of wheel exposure (especially day 1), while for the final days increasing effects were noted for the HR allele. Average additive QTL effects were frequently significant and exhibited similar temporal patterns. Average dominance effects were large for most running traits examined. And, notably, in three cases we found significant dominance effects in the absence of significant additive effects: running distance on day 3, running time on day 1 (MMU7), and for the intercept of average running speed.

Separate analyses (of the QTL presented in Table 3) investigated QTL X sex and QTL X parent-of-origin factors in a stepwise fashion and revealed statistical evidence for parent-of-origin-specific QTL in three cases. Here we present unadjusted LOD scores from these analyses, as we have already demonstrated significance after accounting for family structure. First, body mass QTL on MMU6 showed a significant QTL X parent-of-origin interaction (LOD_{Full} - $LOD_{Additive}$ = 7.6). Separate analyses of the parent-of-origin types revealed unadjusted LOD scores of 0.2 for individuals descended from a progenitor cross (F_0) of HR♀ X B6♂ and 17.8 for individuals descended from B6♀ X HR♂. Second, we observed a significant QTL X parent-of-origin interaction (LOD_{Full} - $LOD_{Additive}$ = 3.4) for distance QTL on MMU1 (112.7 Mb). Separate analyses revealed a LOD of 6.9 for mice descended from HR♀ X B6♂ as compared to a

318 LOD of 1.2 for from the reciprocal cross. Lastly, a significant interaction (LOD_{Full} - $LOD_{Additive}$
319 = 3.2) was observed for the slope of time spent running (MMU11) (LOD = 0.6, HR♀ X B6♂;
320 LOD = 8.7, B6♀ X HR♂).
321
322 **DISCUSSION**
323 To date, QTL associated with mouse wheel-running activity have been mapped in either
324 second-generation intercross or backcross populations (e.g., 42, 46). Although many methods
325 exist to map individual QTL (13), the AIL approach, employed here, enables finer-mapping of
326 many QTL using a single population. By generating higher levels of recombination throughout
327 the genome, the entire genome is lengthened in terms of cM distance (compared to a F_2),
328 providing increased mapping resolution in the AIL and reductions in the confidence intervals of
329 map locations (14). In this study, the production of a G_4 population resulted in an approximate
330 threefold expansion (averaged across all chromosomes) of the genetic map relative to a new
331 standard map for the laboratory mouse (see the Revised Shifman map lengths in Table 1 of 11)
332 (comparisons are depicted in Supplemental Fig. 1). This map expansion is, as expected, less
333 than what was observed for more advanced intercrosses (e.g., 48). However, use of this
334 intermediate stage of the AIL permitted quicker access into the genetic architecture of voluntary
335 exercise, and we have maintained the AIL (now at G_9) for potential follow-up fine mapping
336 targeted at the genomic regions identified here.
337 We observed the strongest signals for wheel-running distance and duration. Our results
338 revealed a generally consistent pattern (as evidenced by overlapping confidence intervals) for
339 running distance and duration across all 6 days, with QTL primarily found in a region on
340 MMU7, with significant additive effects resulting from the HR allele. These pleiotropic effects

are reasonable given that running distance is a product of the amount of time spent running and the speed at which an individual runs. And, given the high correlation between running distance and running time, further analysis of the mean distance on days 5 and 6 was conducted with running time as an additional covariate. As expected, this analysis resulted in a reduction of the LOD score of the QTL on MMU7 (naive LOD; without time as a covariate = 4.2, with time as a covariate = 1.5). We did not observe any significant or suggestive QTL on MMU7 for average running speed or maximum running speed. This pattern is different from that previously observed by Lightfoot et al. (42) and Nehrenberg et al. (46), where significant or suggestive QTL for running speed were found to colocalize with regions for running distance.

Lightfoot et al. (42) identified 4 QTL that were deemed to be significant. These QTL represented running duration (*DUR13.1*), speed (*SPD9.1* and *SPD13.1*), and distance (*DIST13.1*) with the QTL for running speed (*SPD9.1*) accounting for the largest percent of phenotypic variance (11.3). These major QTL do not directly overlap with the QTL identified here, but direct comparisons to Lightfoot et al. (42) are difficult as they examined running values across all 21 days of wheel access, while we primarily examined daily values and mean values on days 5 and 6 of wheel access. Moreover, Lightfoot et al. (42) generated their F_2 mapping population from different mouse strains (C57L/J and C3H/HeJ) than those utilized here. A forthcoming common set of mice (the Collaborative Cross), derived from a diverse set of eight founder strains and designed for the analysis of complex traits, should, in our opinion, partially mitigate the need for comparisons of isolated mapping populations (65). However, we do feel the creation of intercross and backcross populations involving phenotype-specific strains (such as HR) will remain important.

Nehrenberg et al. (46) found little evidence of significant QTL for running time in general. Contrary to the current investigation, Nehrenberg et al. (46) employed a backcross design and an alternate replicate HR line (four currently exist). The HR line utilized in Nehrenberg et al. (46) is fixed for a Mendelian recessive allele (26) that causes an approximate 50% reduction in hindlimb muscle mass and has been mapped a to a 2.6335 Mb region between 67.453 and 70.0865 Mb on MMU11 (29). In addition to alterations in muscle mass, this replicate line exhibits a number of phenotypic differences compared to the HR line utilized here, most importantly increases in running speed (28 and references therein). However, the QTL previously detected by Nehrenberg et al. (46) for running distance and speed and the QTL observed here for distance and duration were both found in reasonably close approximation to the *tyrosinase* (*tyr*) gene (~94.6 Mb) on MMU7. This is particularly intriguing given evidence that tyrosinase can serve as a precursor for dopamine, a neurotransmitter previously demonstrated to be involved in voluntary movement and predatory aggression (53). The other prominent QTL identified by Nehrenberg et al. (46) on MMU6 (for maximum running speed) does not directly overlap with those identified here.

Although individual days generally shared some common QTL, the initial exposure (days 1 and 2) to wheels and the trajectory of running traits across the entire access period revealed some novel findings. During the initial exposure to running wheels (days 1 and 2), we have demonstrated that unique genomic regions are least partially responsible for running distance and duration as revealed by significant and suggestive QTL on MMU1, MMU5, and MMU6. In most of these cases, the B6 allele had significant additive effects, with the notable exception of the QTL detected on MMU7, where the HR allele always had an additive effect (and in most cases a significant one). These temporal differences in additivity may be illustrative

386 of variation in anxiety or fear-related behavioral differences (e.g., as might be measured by open-
387 field behavior) between HR and B6 mice.

388 Regions on MMU1 have previously been implicated in both home-cage activity (34) and
389 open-field behavior (27). Kas et al. (34) utilized a chromosome substitution strain to identify a
390 312-kb QTL interval at 80 Mb on MMU1 containing a single gene (*A830043J08Rik*) associated
391 with home-cage activity. Gene expression profiling further identified a gene (*Epha4*) outside of
392 the QTL interval as a strong candidate downstream involved in motor activity via the neuronal
393 circuitry controlling movement. Distinct from home-cage activity, but still located on MMU1,
394 loci for open-field behavior have been mapped in close proximity to 145 Mb (70), 175 Mb (31),
395 100 Mb (15), and 190 Mb (58, 59). These regions have been shown to harbor genes involved in
396 anxiety-like behavior in rodents, and human homologues have been associated with panic
397 disorder (38). Thus, based on our current findings, we preliminarily conclude that fear, or lack
398 thereof, of novel object (e.g., a running wheel), or more general anxiety resulting from novel
399 solitary housing conditions, may contribute to wheel running during initial exposure to wheels.
400 Additionally, given the results of Kas et al. (34), regions on MMU1 may play a role in the initial
401 "learning" (broadly involving neural circuitry) process involved with wheel running. Follow-up
402 investigations will be needed to elucidate a clearer picture of the regions MMU1 identified here
403 and their putative role in wheel-running behavior. It is worth noting that variation in the
404 regulation of sex hormones may also be playing important role during the initiation and
405 continuation of wheel running (see 39); however, we did not quantify estrogen / testosterone
406 levels in the current study and this may have diminished our power of QTL detection.

407 Our efforts, along with those of (46), have now led to the identification of multiple QTL
408 underlying activity-related phenotypes in the context of an artificial selection experiment for

409 increased voluntary wheel running. Although these QTL individually and collectively only
410 explain a small fraction of the phenotypic variance in activity measures, they potentially
411 represent genomic regions that have been (or currently are) under positive selection. We
412 acknowledge the difficulties in relating the importance of the current results (and those of 46) to
413 the phenotypic divergence in wheel running seen between HR as compared to control mice (e.g.,
414 see Fig. 1 in 37). First, we have utilized B6 in the creation of the G_4 as opposed to the control
415 lines derived from the Hsd:ICR strain [Harlan-Sprague-Dawley (HSD), Indianapolis, Indiana,
416 USA]. And second, we cannot rule out genetic drift as we are only examining one of the 4
417 replicate HR lines. However, given that nearly all of the allelic effects from mean running traits
418 on days 5 and 6 associated the HR allele with increased running with partial replication
419 [compared to Nehrenberg et al. (46)], we feel this provides reasonably strong evidence that at
420 least some of the identified genomic regions have been influential during the evolution of
421 voluntary wheel running in the context of this artificial selection experiment. Many adaptive
422 changes in exercise physiology, as well as motivational aspects of voluntary running, have been
423 observed in HR mice as compared to their ICR controls (see 24, 53, 64). Currently, we do not
424 know which component (motivation or ability) most accounts for variation in wheel running
425 traits or QTL identified in this mapping population. However, follow-up investigations are
426 profiling gene expression in brain and muscle tissue in a selection of G_4 mice in the hopes of
427 providing some insight into these two aspects of voluntary exercise, which may or not be
428 mutually exclusive.

429 Average dominance effects of QTL were in most cases large and appear to be playing an
430 important role in the regulation of voluntary wheel running. These findings support those of
431 previous investigations examining wheel running in F_1 populations. Dohm et al. (18) observed

net dominance in the direction high wheel running in an F_1 population resulting from wild captured house mice and ICR (the base population of HR) mice. Additionally, Nehrenberg et al. (45) observed significant heterotic inheritance of wheel running behavior in F_1 individuals from crosses of HR and C57BL/6J mice (identical to the strains utilized here). And, to our knowledge, the most comprehensive examination of heterotic inheritance of wheel running in mice was conducted by Bruell (7) and involved 4,000 mice from 13 inbred strains and 31 hybrid groups, with heterosis observed for a significant number of the hybrids.

In addition to what might initiate wheel running, we also attempted to identify genomic regions controlling temporal variation (or the trajectory) in wheel running. We mapped the slope and intercept of a linear regression for running distance, duration, average speed, and maximum speed across all 6 days of the testing period (for hypothetical examples, see Fig. 4 in 25). Here, we report the first ever, to our knowledge, QTL associated with the trajectory of running across multiple days of wheel exposure. As expected, the intercept QTL were found in similar regions to the QTL peaks identified on the initial day of exposure. However, the QTL observed for the slope of the exercise-related traits often did not coincide with locations of the individual day QTL. For example, we identified a peak on MMU11 for the slope of wheel running distance, but did not observe a peak on MMU11 for running distance on any of the individual days. Therefore, it is possible that the global trajectory of exercise behavior on longer time scales is at least partially controlled by different genomic regions than the behavior on individual days. Although further studies are needed, these regions may prove especially important given the importance of physical activity in the maintenance of weight regulation.

Previously, in this G_4 population, we reported significant effects of sex and parent-of-origin, and in some case interactions between these two effects, on voluntary wheel traits and

455 body composition (36). Formerly, we hypothesized that the mechanistic regulation of these
456 observed parent-of-origin effects may be genetic (i.e., X-linked or mtDNA variations),
457 epigenetic (i.e., genomic imprinting), or environmental (i.e., in utero environment or maternal
458 care) phenomena. Given the lack of observed QTL on the X chromosome, we can preliminarily
459 rule out direct genetic effects as an explanation for the observed parent-of-origin effects on
460 voluntary wheel-running traits. With regard to genomic imprinting, we observed QTL X parent-
461 of-origin interactions for only a small number of QTL. However, we only examined potential
462 interactions for the QTL that were initially significant utilizing additive models (Table 3).
463 Future studies will be needed to more thoroughly understand QTL X parent-of-origin interactions
464 across the entire genome, whether these potentially significant effects lie within known
465 imprinting regions, and the explanatory power of the parent-of-origin specific QTL to the %
466 phenotypic variance.

467 Results of the current investigation are an important step in continuing efforts to elucidate
468 the genetic architecture of voluntary exercise levels. The large number of QTL discovered here
469 (and by others) suggests that many genomic elements contribute to the predisposition for
470 voluntary exercise, but the identities and nature of the underlying genetic variation is not yet well
471 understood. However, as studies involving all aspects of activity (wheel running, home-cage,
472 open-field, etc.) in rodents are beginning to emerge and converge, the intricacies of such a
473 complex behavior as voluntary exercise are beginning to become clearer. And, while translation
474 from mouse to man is uncertain, given the parallels detailed in (20) we are optimistic that
475 investigations into the genetic architecture of voluntary wheel running in rodents will have
476 positive consequences for our understanding of the variation in exercise behavior in human
477 populations.

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679

680 Table 1. *Descriptive statistics for phenotypic traits measured in the G_4 population*

Trait[a]	*n*	Mean	SD	Range	Trait[a]	*n*	Mean	SD	Range
Body mass	800	26.03	4.67	16.30 – 39.30					
Revolutions					***Average speed***				
Day 1	753	8,525	3,104	287 – 23,739	Day 1	753	12.21	2.41	3.12 – 22.62
Day 2	754	8,824	2,843	379 – 16,976	Day 2	754	15.23	2.65	4.26 – 26.90
Day 3	784	8,996	3,174	848 – 22,161	Day 3	784	16.46	3.00	8.24 – 28.09
Day 4	694	9,259	3,097	537 – 22,158	Day 4	694	17.34	3.01	5.10 – 28.14
Day 5	797	10,278	3,121	2,828 – 22,053	Day 5	797	18.69	3.25	9.30 – 30.72
Day 6	769	11,000	3,621	2,287 – 24,068	Day 6	769	19.42	3.48	10.49 – 32.01
(Days 5+6)/2[b]	767	10,663	3,251	2,600 – 23,061	(Days 5+6)/2[b]	767	19.10	3.27	11.45 – 31.20
Slope (Days1-6)[c]	618	458	657	-1,747 – 2,663	Slope (Days1-6)[c]	618	1.37	0.61	-0.49 – 3.62
Intercept (Days1-6)[c]	618	8,014	3,038	-2,632 – 17,693	Intercept (Days1-6)[c]	618	11.69	2.35	3.04 – 22.54
Time					***Maximum speed***				
Day 1	753	685	186	92 – 1,164	Day 1	753	25.72	3.56	11.33 – 39.50
Day 2	754	573	146	89 – 963	Day 2	754	28.15	3.93	14.67 – 43.00
Day 3	784	539	143	103 – 994	Day 3	784	29.74	4.27	17.67 – 47.92
Day 4	694	523	130	68 – 874	Day 4	694	30.86	4.21	17.33 – 49.92
Day 5	797	545	120	174 – 922	Day 5	797	32.44	4.71	22.42 – 52.00
Day 6	769	560	132	210 – 991	Day 6	769	33.24	4.87	20.50 – 51.67
(Days 5+6)/2[b]	767	554	121	210 – 937	(Days 5+6)/2[b]	767	32.86	4.62	21.46 – 51.84
Slope (Days1-6)[c]	618	-24	29	-134 – 129	Slope (Days1-6)[c]	618	1.45	0.87	-1.09 – 4.79
Intercept (Days1-6)[c]	618	668	173	-79 – 1,087	Intercept (Days1-6)[c]	618	24.82	3.70	10.43 – 40.85

681

682 [a]Traits measured from a 6-day exposure to running wheels: body mass (g) prior to exposure to running wheels, running

683 distance (revolutions/day), time spent running (i.e., cumulative 1-minute intervals in which at least one revolution was recorded),

684 average speed (total revolutions / time spent running), and maximum speed (highest number of revolutions in any one-minute interval

685 within a 24 hour period). [b]Mean of days 5 and 6 of a 6-day exposure to running wheels; the criterion for which the HR strain was

686 selectively bred (63). [c]Slope and intercept values from a linear regression across the 6-day test. Slopes and intercepts were not

687 calculated for individuals missing one or more days of wheel-running data.

688 Table 2. *Pearson partial correlations (r) for mean voluntary-running traits from days 5 and 6 of a 6-day exposure to running wheels*

Trait	Distance	Time	Average speed	Maximum speed
Body mass	0.034	0.045	0.011	0.066
Distance		0.796*	0.753*	0.643*
Time			0.222*	0.164*
Average speed				0.877*

689

690 Pearson partial correlations (controlling for sex, parent-of-origin, and wheel freeness) for a subset of the 37 phenotypic traits presented in

691 Table 1). *$P < 0.05$ following correction for multiple comparisons utilizing the false discovery rate procedure (12).

692 Table 3. *QTL detected and respective statistics for body mass and voluntary wheel-running traits*

Trait[a]	Nearest Marker	MMU	Peak Position (Mb)	Naive LOD	GRAIP LOD[d]	CI (Mb)[e]	% Var[f]	Additive[g] ±SE	Dominance[g] ±SE
Body mass	JAX00263199	1	115.6	6.9	3.5	95-141	1.1	0.6±0.2	0.4±0.3
	JAX00127022	5	10.6	9.4	4.7*	-16	1.8	-0.6±0.2	-0.4±0.3
	JAX00139789	6	36.3	10.6	4.7*	25-40	1.0	0.6±0.2	-0.1±0.3
	JAX00415862	16	24.3	7.3	3.5	11-28	1.5	-0.7±0.2	0.4±0.3
Distance									
Day 1	JAX00240652	1	3.5	6.2	4.1*	-12	2.2	-669.3±164.1†	-141.5±226.4
	JAX00008045	1	112.7	5.9	3.9*	107-139	1.5	-492.7±153.6	208.5±227.8
	JAX00608826	6	46.8	6.4	4.4*	38-52	3.6	-761.2±146.7†	-234.1±225.0
	JAX00155508	7	108.9	7.4	4.7*	97-116	4.4	870.1±148.2†	-258.8±225.9
Day 2	JAX00009649	1	134.3	4.6	3.7	111-139	2.3	-463.3±138.4	402.3±212.0
	JAX00581735	5	50.0	4.5	3.5	48-66	2.6	375.3±147.7	794.6±205.1
	JAX00139789	6	36.3	7.3	4.7*	-60	2.8	-625.2±135.8†	-12.8±206.6
	JAX00155961	7	114.9	4.5	3.5	97-119	3.9	545.7±141.6	286.8±212.3
Day 3	JAX00582506	5	52.9	4.6	4.7*	51-59	2.9	325.6±165.1	1008.8±224.7†
Day 4	JAX00155961	7	114.9	5.7	4.7*	101-130	3.2	648.2±158.1†	537.5±238.5
Day 5	JAX00155961	7	114.9	4.1	4.2*	98-129	2.6	595.8±149.2	469.1±226.6
Day 6	JAX00155508	7	108.9	4.0	3.7	100-120	2.1	638.3±170.1	341.5±264.4
(Days 5+6)/2[b]	JAX00155508	7	108.9	4.2	4.2*	99-124	2.3	607.4±152.9	273.0±237.4
Slope (Days1-6)[c]	JAX00025338	11	24.0	5.3	3.8	20-38	3.8	172.4±38.2†	14.0±52.8
Intercept (Days1-6)[c]	JAX00240652	1	3.5	5.3	3.6	-23	3.7	-846.7±174.2†	-78.6±241.7
	JAX00008766	1	122.5	6.4	4.7*	110-136	3.9	-688.6±166.3†	518.1±249.1
	JAX00139789	6	36.3	7.3	4.4*	19-55	3.7	-774.9±160.6†	-82.1±242.0
	JAX00155743	7	112.1	5.0	3.5	102-118	3.0	664.0±162.3†	-386.7±243.7

Time									
Day 1	JAX00009797	1	136.3	11.6	4.7*	92-151	5.1	-49.4±9.0†	30.7±13.6
	JAX00645408	7	82.6	6.7	4.1*	75-86	3.8	31.7±9.4	-59.8±13.3†
Day 2	JAX00253602	1	66.2	5.8	3.9*	30-77	2.5	-30.2±8.0	10.9±11.0
	JAX00582506	5	52.9	5.9	4.7*	49-58	3.1	28.4±7.6	35.3±10.5
	JAX00139228	6	28.8	6.8	4.7*	22-42	2.3	-29.7±7.0†	-1.5±10.6
	JAX00155508	7	108.9	5.7	4.0*	99-119	2.6	31.5±7.0†	-0.2±10.8
	JAX00350930	13	15.7	4.9	3.6	-22	2.2	-28.6±7.3	7.3±10.8
Day 3	JAX00154099	7	90.0	6.4	4.7*	75-117	3.1	27.1±6.8†	-30.4±10.4
Day 4	JAX00156517	7	122.4	7.7	4.7*	91-132	4.8	39.9±6.8†	-1.12±10.0
Day 5	JAX00155508	7	108.9	6.7	4.7*	92-131	3.5	29.5±5.6†	2.3±8.5
	JAX00478815	19	46.4	5.6	4.3*	41-49	2.2	-21.8±6.6	16.8±8.7
Day 6	JAX00155508	7	108.9	8.1	4.7*	93-127	4.1	34.5±6.2†	7.0±10.0
(Days 5+6)/2[b]	JAX00155508	7	108.9	8.5	4.7*	91-129	4.3	32.3±5.6†	5.4±8.7
Slope (Days1-6) [c]	JAX00008766	1	122.5	9.4	4.7*	58-141	6.6	9.6±1.6†	-3.5±2.3
	JAX00139228	6	28.8	5.9	3.8	22-48	3.3	6.7±1.6†	3.3±2.3
	JAX00026075	11	33.9	5.5	3.6	22-37	3.5	6.4±1.6	5.0±2.3
Intercept (Days1-6)[c]	JAX00009649	1	134.3	9.9	4.7*	85-142	5.5	-46.9±9.1†	32.1±13.8
	JAX00139789	6	36.3	8.1	4.7*	-45	3.3	-41.7±9.2†	-5.2±13.8
	JAX00041702	13	10.5	6.3	4.6*	-23	2.6	-38.1±9.8	3.4±14.1
Average speed									
Day 2	JAX00436582	17	33.2	5.4	4.7*	27-47	3.0	0.6±0.1†	0.4±0.2
Day 3	JAX00496243	2	91.8	4.4	3.6	81-106	2.3	-0.3±0.2	0.7±0.2
	JAX00441944	17	52.3	4.5	3.8	29-69	2.0	0.6±0.2†	-0.01±0.22
Day 5	JAX00385288	14	79.9	3.8	3.8	68-92	2.0	0.5±0.2	0.5±0.2
Day 6	JAX00097778	2	99.0	5.1	4.2*	80-103	3.4	-0.8±0.2	0.4±0.3
	JAX00385288	14	79.9	4.1	3.7	68-92	2.3	0.6±0.2	0.6±0.3

(Days 5+6)/2[b]	JAX00097778	2	99.0	4.3	3.8	81-103	3.0	-0.7±0.2	0.4±0.2
	JAX00385288	14	79.9	4.2	3.9*	69-92	2.3	0.6±0.2	0.5±0.2
Intercept (Days1-6)[c]	JAX00037863	12	76.6	4.8	3.9*	73-81	3.4	0.1±0.1	0.9±0.2[†]
Maximum speed									
Day 2	JAX00496243	2	91.8	4.1	3.7	85-96	2.8	-0.4±0.2	1.0±0.3
Day 3	JAX00096585	2	82.8	5.3	4.2*	80-105	2.8	-0.7±0.2	0.7±0.3
	JAX00024300	11	9.9	4.7	3.9*	-13	2.8	1.0±0.2[†]	0.3±0.3
	JAX00311223	11	53.2	4.4	3.6	46-68	2.7	1.0±0.2[†]	0.1±0.3
Day 4	JAX00311223	11	53.2	5.1	4.7*	48-61	3.5	0.9±0.2[†]	0.6±0.3
Day 5	JAX00498192	2	102.8	3.9	3.5	81-115	2.6	-0.7±0.3	0.9±0.3
Day 6	JAX00496243	2	91.8	5.5	4.2*	78-114	4.3	-1.0±0.3	1.1±0.4
	JAX00311223	11	53.2	5.2	4.4*	46-62	2.5	1.0±0.2[†]	0.4±0.4
(Days 5+6)/2[b]	JAX00496243	2	91.8	4.8	4.1*	80-115	3.8	-0.8±0.3	1.1±0.4
	JAX00024300	11	9.9	4.1	4.1*	7-14	1.8	0.9±0.3	0.1±0.3
	JAX00311223	11	53.2	4.6	4.2*	45-61	2.1	0.9±0.2	-0.4±0.3

[a]Traits measured from a 6-day exposure to running wheels: body mass (g) prior to exposure to running wheels, running distance (revolutions / day), time spent running (i.e., cumulative 1-minute intervals in which at least one revolution was recorded), average speed (total revolutions / time spent running), and maximum speed (highest number of revolutions in any 1-minute interval within a 24 hour period). [b]Mean of days 5 and 6 of a 6-day exposure to running wheels. This is the criterion for which one strain (HR) was selectively bred (63). [c]Slope and intercept values from across the 6-day test. Slopes and intercepts were not calculated for individuals missing one or more days of wheel-running data. [d]LOD exceeding the 95% ($P \leq 0.05$, LOD ≥ 3.9) permutation threshold are denoted by *; other QTL exceeded the 90% ($P \leq 0.1$, LOD ≥ 3.5) threshold.

700 [e]Confidence intervals (CIs) for QTL positions were obtained using a 1.0 LOD drop in Mb (relative to the GRAIP permuted LOD score). [f]Percentage
701 of phenotypic variance accounted for by the QTL effect. [g]For additive and dominance effects: positive values indicate increasing effect of the HR
702 allele or increasing effect of the heterozygote, respectively. [†]Indicates additive and dominance effects were statistically significant at $P < 0.05$.

FIGURE LEGENDS

Fig. 1. G_4 QTL maps of body mass prior to running-wheel exposure. Red traces are the simple mapping output, and black traces are GRAIP permutation output. Genome-wide GRAIP-adjusted significance thresholds were generated utilizing 50,000 permutations. Therefore, for the GRAIP output, a minimum possible *P* value with 50,000 permutations is 0.00002 (1/50,000), so the maximum $-\log P = 4.7$. Shaded gray regions are either suggestive ($P \leq 0.1$) or significant ($P \leq 0.05$) at a genome-wide level in the GRAIP results. The black and gray lines represent the permuted 95% and 90% LOD thresholds, respectively.

Fig. 2. G_4 QTL maps of running distance (revolutions/day) on of each of 6 days of wheel access, the mean from days 5 and 6, and running trajectories across the 6-day test. Slopes were not calculated for individuals missing one or more days of wheel-running data. Red traces are the simple mapping output, and black traces are GRAIP permutation output. Shaded gray regions are either suggestive ($P \leq 0.1$) or significant ($P \leq 0.05$) at a genome-wide level in the GRAIP results. The dotted line represents the permuted 95% LOD threshold.

Fig. 3. G_4 QTL maps of time spent running (i.e., cumulative 1-minute intervals in which at least one revolution was recorded) on of each of 6 days of wheel access, the mean from days 5 and 6, and running trajectories across the 6-day test. Slopes were not calculated for individuals missing one or more days of wheel-running data. Red traces are the simple mapping output, and black traces are GRAIP permutation output. Shaded gray regions are either suggestive ($P \leq 0.1$) or significant ($P \leq 0.05$) at a genome-wide level in the GRAIP results. The dotted line represents the permuted 95% LOD threshold.

724 Fig. 4. G_4 QTL maps of average running speed (total revolutions / time spent running) on of each
725 of 6 days of wheel access, the mean from days 5 and 6, and running trajectories across the 6-day
726 test. Slopes were not calculated for individuals missing one or more days of wheel-running data.
727 Red traces are the simple mapping output, and black traces are GRAIP permutation output.
728 Shaded gray regions are either suggestive ($P \leq 0.1$) or significant ($P \leq 0.05$) at a genome-wide
729 level in the GRAIP results. The dotted line represents the permuted 95% LOD threshold.

730 Fig. 5. G_4 QTL maps of maximum running speed (highest number of revolutions in any one-
731 minute interval within a 24 hour period) on of each of 6 days of wheel access, the mean from
732 days 5 and 6, and running trajectories across the 6-day test. Slopes were not calculated for
733 individuals missing one or more days of wheel-running data. Red traces are the simple mapping
734 output, and black traces are GRAIP permutation output. Shaded gray regions are either
735 suggestive ($P \leq 0.1$) or significant ($P \leq 0.05$) at a genome-wide level in the GRAIP results. The
736 dotted line represents the permuted 95% LOD threshold.

737 **SUPPLEMENTAL MATERIAL**

738

739 **SUPPLEMENTAL FIGURE LEGENDS**

740 Supplemental Fig.1. Genetic linkage map depicting locations (cM) of markers (*n* = 530) in the
741 G_4 population. The production of the G_4 advanced intercross line increased the genetic length of
742 the entire genome by generating higher levels of recombination relative to a F_2 (see 14). For
743 comparison, we have added chromosome lengths from a new standard genetic map for the
744 laboratory mouse (gray shaded area) (11). All positions (including those from 11) were based on
745 a sex-averaged map, with the exception of the X chromosome, which was based on the female
746 map only.

747

SUPPLEMENTAL TABLES

Supplemental Table 1. *SNPs (n = 530) used in the final analyses of the G_4 population of mice with known physical (Mb) locations*

SNP	MMU	Position (Mb)	SNP	MMU	Position (Mb)
JAX00240652	1	3.46	JAX00009797	1	136.28
JAX00000321	1	7.30	JAX00268776	1	139.51
JAX00241694	1	9.99	JAX00269922	1	145.97
JAX00000760	1	13.23	JAX00010715	1	148.55
JAX00001021	1	16.68	JAX00010980	1	152.08
JAX00243650	1	20.14	JAX00011133	1	154.11
JAX00244717	1	24.81	JAX00275074	1	169.61
JAX00002001	1	29.77	JAX00012316	1	170.02
JAX00188707	1	33.66	JAX00275695	1	171.81
JAX00247128	1	36.76	JAX00276519	1	175.07
JAX00002741	1	39.61	JAX00277411	1	178.74
JAX00003014	1	43.27	JAX00278821	1	185.33
JAX00249585	1	46.33	JAX00013696	1	188.78
JAX00250156	1	50.06	JAX00280187	1	191.76
JAX00003704	1	52.50	JAX00280986	1	195.62
JAX00251429	1	55.40	JAX00090971	2	7.61
JAX00004537	1	63.62	JAX00483290	2	10.81
JAX00253602	1	66.22	JAX00091402	2	13.35
JAX00004954	1	69.19	JAX00484496	2	19.39
JAX00254795	1	72.80	JAX00484539	2	19.57
JAX00005495	1	76.41	JAX00091876	2	19.68
JAX00005735	1	79.62	JAX00092635	2	29.85
JAX00257356	1	82.62	JAX00092666	2	30.26
JAX00258190	1	89.77	JAX00092942	2	33.94
JAX00259020	1	93.04	JAX00093554	2	42.23
JAX00260131	1	98.83	JAX00093881	2	46.59
JAX00261568	1	106.63	JAX00094170	2	50.47
JAX00008045	1	112.67	JAX00094385	2	53.29
JAX00263199	1	115.56	JAX00094639	2	56.70
JAX00008766	1	122.52	JAX00094839	2	59.40
JAX00265393	1	126.39	JAX00095470	2	67.85
JAX00009649	1	134.31	JAX00095583	2	69.34

753 Supplemental Table 1...*continued*
754

SNP	MMU	Position (Mb)	SNP	MMU	Position (Mb)
JAX00493169	2	73.87	JAX00109931	3	86.29
JAX00493664	2	76.35	JAX00110107	3	88.65
JAX00096391	2	80.17	JAX00110808	3	98.33
JAX00096585	2	82.78	JAX00110851	3	99.01
JAX00097085	2	89.55	JAX00111276	3	104.69
JAX00496243	2	91.83	JAX00111864	3	112.60
JAX00097778	2	99.04	JAX00189283	3	119.29
JAX00498192	2	102.76	JAX00189293	3	125.60
JAX00098514	2	109.20	JAX00113499	3	134.56
JAX00098814	2	113.18	JAX00538751	3	136.05
JAX00500486	2	115.58	JAX00114351	3	145.92
JAX00099246	2	118.98	JAX00542768	3	154.45
JAX00501779	2	122.68	JAX00543027	3	156.02
JAX00099979	2	128.74	JAX00115604	4	6.06
JAX00100245	2	132.30	JAX00544225	4	7.08
JAX00100567	2	136.58	JAX00116659	4	20.38
JAX00100848	2	140.35	JAX00116950	4	24.42
JAX00508265	2	155.71	JAX00117341	4	29.71
JAX00509136	2	159.30	JAX00117573	4	33.02
JAX00511966	2	172.51	JAX00117972	4	38.40
JAX00103392	2	174.30	JAX00548707	4	39.69
JAX00103973	3	6.19	JAX00549337	4	44.04
JAX00104028	3	6.94	JAX00119104	4	54.22
JAX00104180	3	8.97	JAX00119212	4	55.65
JAX00515950	3	18.57	JAX00189438	4	58.47
JAX00105078	3	21.37	JAX00552983	4	64.17
JAX00105505	3	27.12	JAX00554143	4	71.10
JAX00189155	3	33.46	JAX00120481	4	73.52
JAX00520666	3	40.32	JAX00554899	4	76.13
JAX00106771	3	44.02	JAX00557140	4	88.28
JAX00107199	3	49.78	JAX00121671	4	89.43
JAX00107680	3	56.18	JAX00121710	4	89.94
JAX00524422	3	60.23	JAX00121898	4	92.50
JAX00524828	3	63.25	JAX00122676	4	102.85
JAX00108421	3	66.14	JAX00561847	4	109.15
JAX00526713	3	73.72	JAX00123647	4	116.30
JAX00109133	3	75.68	JAX00563495	4	118.55
JAX00109693	3	83.13	JAX00567938	4	135.79

755 Supplemental Table 1*...continued*
756

SNP	MMU	Position (Mb)	SNP	MMU	Position (Mb)
JAX00568742	4	139.34	JAX00139789	6	36.30
JAX00569432	4	142.03	JAX00140451	6	45.15
JAX00570195	4	147.27	JAX00608826	6	46.81
JAX00126017	4	149.26	JAX00141073	6	53.58
JAX00573023	5	7.19	JAX00612506	6	67.44
JAX00127022	5	10.64	JAX00142749	6	76.13
JAX00127317	5	15.41	JAX00615985	6	83.23
JAX00127722	5	20.81	JAX00143736	6	89.31
JAX00128228	5	28.49	JAX00617746	6	92.62
JAX00128632	5	33.91	JAX00618398	6	94.98
JAX00128815	5	36.39	JAX00619072	6	97.99
JAX00581045	5	46.89	JAX00144705	6	102.24
JAX00581735	5	49.99	JAX00621926	6	109.87
JAX00582506	5	52.91	JAX00622369	6	112.22
JAX00584541	5	65.04	JAX00623316	6	115.91
JAX00131070	5	66.45	JAX00189941	6	118.94
JAX00131182	5	67.96	JAX00624709	6	122.61
JAX00586379	5	75.10	JAX00626640	6	132.92
JAX00131790	5	76.06	JAX00189987	6	139.28
JAX00131820	5	77.23	JAX00629129	6	142.21
JAX00131888	5	78.14	JAX00630018	6	145.23
JAX00132785	5	90.09	JAX00148257	7	3.77
JAX00133006	5	93.05	JAX00148474	7	6.72
JAX00133202	5	96.86	JAX00190016	7	13.48
JAX00133397	5	99.48	JAX00149076	7	17.09
JAX00592675	5	113.20	JAX00633165	7	19.09
JAX00593521	5	116.34	JAX00149554	7	26.95
JAX00594409	5	119.65	JAX00635190	7	34.29
JAX00135190	5	123.43	JAX00635952	7	36.42
JAX00599257	5	139.84	JAX00638745	7	50.07
JAX00599877	5	142.39	JAX00641805	7	65.96
JAX00137098	5	149.15	JAX00152597	7	69.87
JAX00602977	6	10.24	JAX00643377	7	73.22
JAX00603343	6	13.27	JAX00153077	7	76.26
JAX00138460	6	18.56	JAX00190133	7	79.92
JAX00139228	6	28.82	JAX00645408	7	82.64
JAX00139316	6	29.99	JAX00645933	7	85.85
JAX00139528	6	32.81	JAX00154099	7	89.95

757 Supplemental Table 1*...continued*
758

SNP	MMU	Position (Mb)	SNP	MMU	Position (Mb)
JAX00154329	7	93.00	JAX00167703	9	4.54
JAX00155508	7	108.92	JAX00167904	9	7.24
JAX00155743	7	112.05	JAX00687899	9	25.32
JAX00155961	7	114.94	JAX00169293	9	25.85
JAX00156517	7	122.38	JAX00169301	9	25.94
JAX00156769	7	125.73	JAX00688081	9	26.77
JAX00655512	7	128.31	JAX00190451	9	29.69
JAX00157304	7	132.85	JAX00169834	9	33.05
JAX00657603	7	137.70	JAX00170132	9	37.11
JAX00658030	7	139.06	JAX00170532	9	42.46
JAX00659205	7	145.07	JAX00170819	9	46.31
JAX00190231	8	3.43	JAX00171082	9	49.80
JAX00158713	8	9.53	JAX00695061	9	56.92
JAX00190239	8	14.53	JAX00696373	9	63.51
JAX00159268	8	16.95	JAX00696900	9	66.11
JAX00159808	8	26.43	JAX00698952	9	76.24
JAX00160567	8	36.56	JAX00700236	9	83.38
JAX00666793	8	42.98	JAX00173791	9	86.06
JAX00161163	8	44.56	JAX00701802	9	92.42
JAX00667095	8	44.88	JAX00704097	9	103.02
JAX00190302	8	53.24	JAX00704581	9	105.81
JAX00162173	8	59.70	JAX00175541	9	109.55
JAX00162404	8	62.80	JAX00705853	9	112.65
JAX00190312	8	66.73	JAX00176095	9	116.96
JAX00163022	8	71.06	JAX00707462	9	118.91
JAX00163156	8	72.86	JAX00282080	10	7.51
JAX00163548	8	78.06	JAX00014851	10	10.13
JAX00673875	8	83.88	JAX00283234	10	13.52
JAX00674224	8	86.04	JAX00284586	10	20.60
JAX00190351	8	89.28	JAX00015834	10	23.31
JAX00675742	8	92.99	JAX00016105	10	26.93
JAX00165121	8	99.05	JAX00285956	10	27.00
JAX00165438	8	103.29	JAX00016116	10	27.07
JAX00678797	8	105.63	JAX00286536	10	30.62
JAX00166114	8	112.30	JAX00016388	10	30.72
JAX00166553	8	118.14	JAX00187308	10	43.69
JAX00167128	8	125.82	JAX00019034	10	66.30
JAX00683747	8	129.11	JAX00019069	10	66.76

759 Supplemental Table 1*...continued*
760

SNP	MMU	Position (Mb)	SNP	MMU	Position (Mb)
JAX00019076	10	66.86	JAX00030022	11	87.22
JAX00019077	10	66.86	JAX00318408	11	92.79
JAX00019082	10	66.95	JAX00030707	11	96.35
JAX00019083	10	66.96	JAX00031155	11	102.35
JAX00019619	10	74.11	JAX00031382	11	105.37
JAX00293914	10	81.49	JAX00031628	11	108.69
JAX00020328	10	83.79	JAX00031943	11	112.89
JAX00020403	10	84.77	JAX00032145	11	115.59
JAX00020562	10	86.91	JAX00187607	12	7.48
JAX00295678	10	89.83	JAX00325423	12	10.64
JAX00020986	10	92.59	JAX00033353	12	12.94
JAX00021324	10	97.08	JAX00327082	12	17.18
JAX00021724	10	102.46	JAX00327523	12	21.46
JAX00022058	10	106.89	JAX00329004	12	30.00
JAX00299310	10	113.81	JAX00331009	12	39.06
JAX00300375	10	119.47	JAX00035416	12	43.82
JAX00023249	10	122.82	JAX00332546	12	46.52
JAX00023839	11	3.78	JAX00036158	12	53.72
JAX00024084	11	7.05	JAX00036460	12	57.89
JAX00024300	11	9.94	JAX00335079	12	60.29
JAX00304396	11	13.51	JAX00187705	12	62.99
JAX00304853	11	16.81	JAX00037350	12	69.77
JAX00025338	11	23.97	JAX00037863	12	76.62
JAX00306858	11	30.08	JAX00339139	12	80.08
JAX00026075	11	33.90	JAX00038348	12	83.10
JAX00026291	11	36.87	JAX00340356	12	86.54
JAX00026765	11	43.20	JAX00038836	12	89.61
JAX00187495	11	46.24	JAX00341779	12	92.91
JAX00311223	11	53.24	JAX00342543	12	97.57
JAX00312699	11	56.50	JAX00345486	12	109.06
JAX00311892	11	56.50	JAX00346570	12	112.79
JAX00313044	11	61.81	JAX00348827	13	3.77
JAX00314044	11	66.20	JAX00041702	13	10.55
JAX00314703	11	69.63	JAX00350930	13	15.74
JAX00315275	11	72.94	JAX00351843	13	19.82
JAX00029177	11	75.94	JAX00352599	13	23.79
JAX00029428	11	79.30	JAX00043166	13	30.85
JAX00316531	11	82.08	JAX00353952	13	31.13

761 Supplemental Table 1…*continued*
762

SNP	MMU	Position (Mb)	SNP	MMU	Position (Mb)
JAX00354948	13	36.43	JAX00385628	14	82.64
JAX00043830	13	39.77	JAX00055542	14	86.00
JAX00356785	13	45.37	JAX00387018	14	92.60
JAX00357304	13	47.75	JAX00057997	14	119.28
JAX00044483	13	48.47	JAX00391461	14	120.36
JAX00358182	13	52.92	JAX00058152	14	121.35
JAX00358965	13	56.25	JAX00392026	15	3.52
JAX00361017	13	63.68	JAX00395686	15	23.96
JAX00045772	13	67.39	JAX00396199	15	26.49
JAX00361784	13	69.58	JAX00396735	15	30.15
JAX00046473	13	76.89	JAX00397321	15	32.39
JAX00363824	13	77.78	JAX00398163	15	37.07
JAX00047202	13	86.64	JAX00061061	15	39.43
JAX00047414	13	89.55	JAX00399798	15	45.76
JAX00366239	13	93.31	JAX00062446	15	57.94
JAX00047888	13	96.52	JAX00403855	15	66.01
JAX00048133	13	99.78	JAX00063060	15	66.11
JAX00048392	13	103.23	JAX00063396	15	70.59
JAX00048913	13	110.19	JAX00405318	15	72.18
JAX00371280	13	116.19	JAX00063956	15	78.07
JAX00372896	14	10.05	JAX00407012	15	80.08
JAX00372971	14	10.46	JAX00064382	15	83.77
JAX00373057	14	10.89	JAX00408215	15	85.97
JAX00050520	14	16.28	JAX00410365	15	94.59
JAX00050720	14	19.02	JAX00065772	15	102.33
JAX00050905	14	21.51	JAX00413022	16	6.77
JAX00051084	14	23.95	JAX00413176	16	7.55
JAX00375557	14	24.69	JAX00415862	16	24.34
JAX00052010	14	36.75	JAX00415942	16	24.89
JAX00052052	14	37.29	JAX00068044	16	32.34
JAX00378576	14	39.70	JAX00417972	16	35.48
JAX00378943	14	44.50	JAX00068339	16	36.27
JAX00052649	14	46.67	JAX00418604	16	39.65
JAX00381940	14	63.19	JAX00068876	16	43.43
JAX00382398	14	66.14	JAX00069480	16	51.50
JAX00383174	14	69.46	JAX00069872	16	56.73
JAX00054877	14	76.86	JAX00422529	16	59.78
JAX00385288	14	79.90	JAX00070376	16	63.50

763 Supplemental Table 1...*continued*
764

SNP	MMU	Position (Mb)	SNP	MMU	Position (Mb)
JAX00070865	16	70.08	JAX00084511	18	66.75
JAX00424604	16	72.92	JAX00463762	18	69.45
JAX00071217	16	74.77	JAX00464605	18	72.79
JAX00071562	16	79.36	JAX00085156	18	75.39
JAX00071974	16	84.88	JAX00465946	18	77.85
JAX00072088	16	86.42	JAX00468254	18	86.57
JAX00072361	16	90.07	JAX00086324	19	3.46
JAX00428434	16	92.97	JAX00470125	19	10.06
JAX00429186	16	96.43	JAX00087311	19	16.98
JAX00429799	17	3.97	JAX00472935	19	20.61
JAX00073232	17	6.70	JAX00473727	19	23.78
JAX00431384	17	10.41	JAX00474575	19	26.68
JAX00073820	17	14.66	JAX00088467	19	32.43
JAX00432525	17	15.50	JAX00476173	19	34.36
JAX00436582	17	33.15	JAX00089065	19	40.46
JAX00075442	17	36.68	JAX00478815	19	46.43
JAX00438327	17	40.61	JAX00479657	19	50.54
JAX00439027	17	43.15	JAX00480903	19	56.40
JAX00440286	17	47.28	JAX00709351	X	11.55
JAX00441944	17	53.15	JAX00711215	X	44.34
JAX00077328	17	62.24	JAX00711221	X	44.34
JAX00443940	17	66.24	JAX00711351	X	45.56
JAX00444142	17	67.00	JAX00179013	X	46.96
JAX00078196	17	74.18	JAX00711759	X	49.41
JAX00447544	17	79.37	JAX00712291	X	55.80
JAX00078883	17	83.33	JAX00179551	X	55.82
JAX00449090	17	86.04	JAX00179671	X	57.42
JAX00188476	17	89.63	JAX00239349	X	70.12
JAX00452266	18	13.21	JAX00180633	X	70.40
JAX00080770	18	16.70	JAX00180639	X	70.46
JAX00081229	18	22.89	JAX00180648	X	70.59
JAX00081764	18	30.02	JAX00714006	X	72.22
JAX00455751	18	33.67	JAX00715098	X	83.17
JAX00082288	18	37.04	JAX00182389	X	94.50
JAX00458347	18	46.99	JAX00182535	X	96.43
JAX00458892	18	50.23	JAX00182562	X	96.80
JAX00460030	18	56.66	JAX00182899	X	101.40
JAX00460887	18	59.82	JAX00183346	X	107.51

765 Supplemental Table 1…*continued*
766

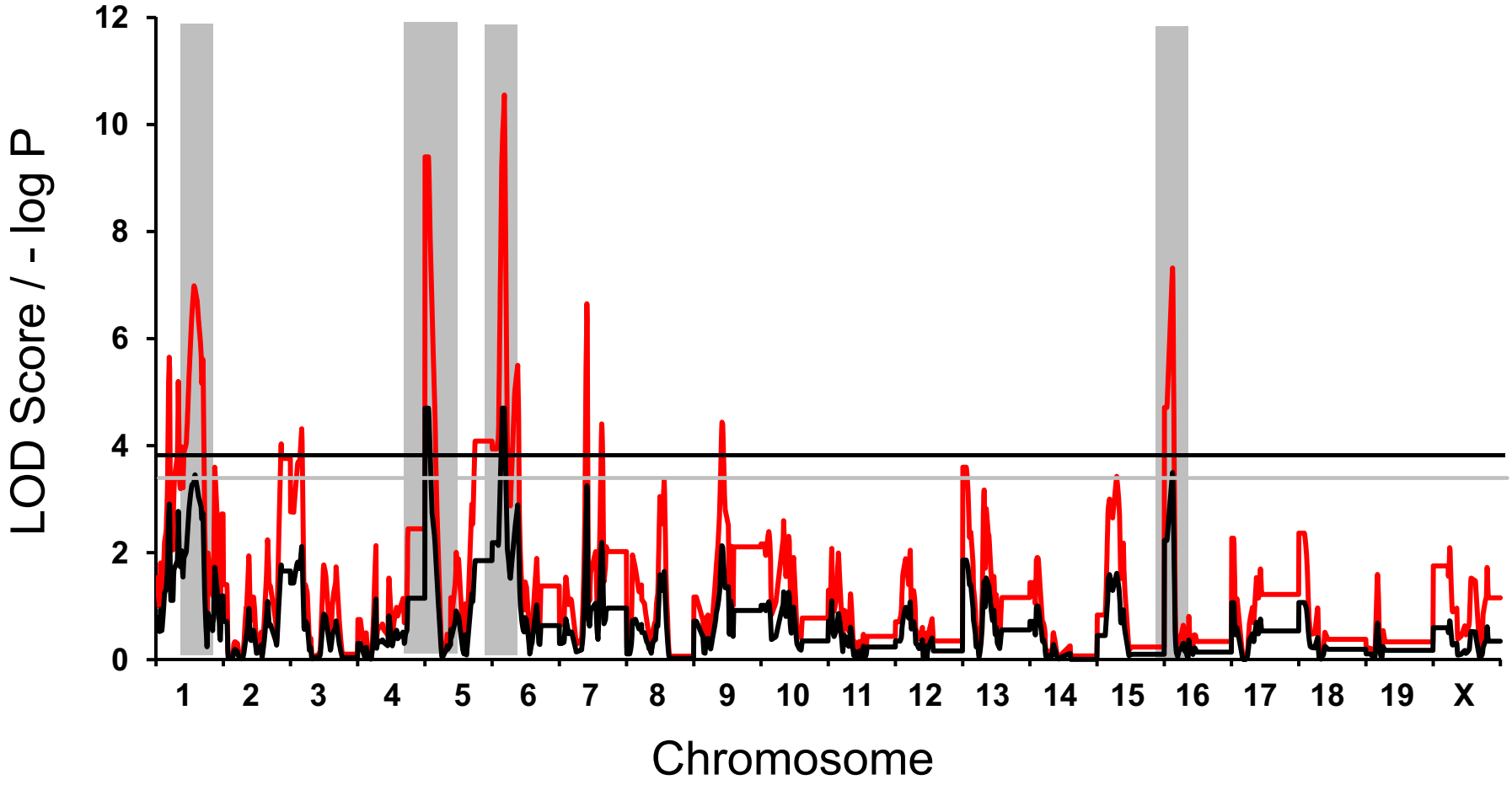
SNP	MMU	Position (Mb)
JAX00717956	X	112.23
JAX00184535	X	126.02
JAX00718909	X	126.04
JAX00185465	X	138.78
JAX00185820	X	145.90
JAX00186043	X	148.89
JAX00240371	X	154.49
JAX00722634	X	159.44
JAX00186887	X	160.42
JAX00187170	X	164.22

Supplemental Table 2. *QTL detected and respective statistics for body mass and voluntary wheel-running traits. Values represent LOD scores from simple mapping output that were significant at the genome-wide level (P ≤ 0.05, LOD ≥ 3.9), but did not remain significant or suggestive (P ≤ 0.1, LOD ≥ 3.5) following the GRAIP procedure (and hence are not depicted in Table 3 of the primary text).*

Trait[a]	Nearest Marker	MMU	Peak Position (Mb)	Naive LOD	GRAIP LOD	CI (Mb)[d]	% Var[e]	Additive[f] ±SE	Dominance[f] ±SE
Body Mass	JAX00511966	2	172.3	4.0	1.8	168-	1.2	-0.4±0.2	-0.8±0.3
	JAX00645408	7	82.6	6.7	3.2	80-84	1.4	0.4±0.2	0.9±0.3
	JAX00700236	9	83.4	4.4	2.1	79-90	0.6	0.3±0.2	0.6±0.3
Distance									
Day 1	JAX00081229	18	22.9	4.4	2.8	-31	3.0	504.5±180.2	-937.0±232.9[†]
Slope (Days1-6)[c]	JAX00008766	1	122.5	3.9	3.2	116-135	3.0	139.6±35.87	-75.1±53.1
Intercept (Days1-6)[c]	JAX00023249	10	122.8	4.5	2.9	121-	2.5	265.8±192.1	-985.1±253.6
Time									
Day 1	JAX00608826	6	46.8	5.7	3.4	26-50	2.6	-38.0±8.8[†]	-17.6±13.5
	JAX00025338	11	24.0	5.4	2.9	21-35	3.0	-34.3±10.0	-37.4±13.5
	JAX00081229	18	22.9	4.9	2.8	-28	2.6	15.7±10.7	-59.5±13.8[†]
Day 2	JAX00072088	16	86.4	4.1	2.7	82-91	3.2	-34.5±8.4[†]	-18.2±11.0
Day 3	JAX00582506	5	52.9	4.1	3.4	50-59	2.5	20.6±7.5	37.0±10.2
(Days 5+6)/2[b]	JAX00478815	19	46.4	4.2	3.0	40-49	1.3	-18.7±6.8	10.1±8.9
Slope (Days1-6)[c]	JAX00081229	18	22.9	4.2	2.8	20-28	2.3	-2.2±1.9	8.7±2.4
Intercept (Days1-6)[c]	JAX00645408	7	82.6	5.5	3.2	75-86	3.5	23.5±9.6	-55.7±13.7[†]

	JAX00026075	11	33.9	4.9	3.0	29-39	2.8	-28.4±9.7	-37.3±13.9
	JAX00081229	18	22.9	4.2	2.5	19-27	1.9	9.8±11.0	-47.7±14.2
Average speed									
Day 2	JAX00131182	5	68.0	4.1	3.3	66-78	2.8	0.1±0.1	0.9±0.2[†]
Day 3	JAX00131182	5	68.0	3.9	3.0	66-78	2.3	-0.03±0.16	0.9±0.2[†]
Slope (Days1-6) [c]	JAX00050520	14	16.3	4.3	3.4	14-18	2.8	0.09±0.03	0.17±0.05
Maximum Speed									
Day 6	JAX00131790	5	76.1	4.0	3.4	47-89	2.1	-0.8±0.2	0.8±0.3
Slope (Days1-6) [c]	JAX00025338	11	24.0	4.2	3.2	21-30	2.5	0.20±0.05	-0.01±0.07

[a]Traits measured from a 6-day exposure to running wheels: body mass (g) prior to exposure to running wheels, running distance (revolutions / day), time spent running (i.e., cumulative 1-minute intervals in which at least one revolution was recorded), average speed (total revolutions / time spent running), and maximum speed (highest number of revolutions in any 1-minute interval within a 24 hour period). [b]Mean of days 5 and 6 of a 6-day exposure to running wheels. This is the criterion for which one strain (HR) was selectively bred (63). [c]Slope and intercept values from across the 6-day test. Slopes and intercepts were not calculated for individuals missing one or more days of wheel-running data. [d]Confidence intervals (CIs) for QTL positions were obtained using a 1.0 LOD drop in Mb (relative to the Naive LOD score). [e]Percentage of phenotypic variance accounted for by the QTL effect. [f]For additive and dominance effects: positive values indicate increasing effect of the HR allele or increasing effect of the heterozygote, respectively. [†]Indicates additive and dominance effects were statistically significant at $P < 0.05$.


LOD Score / - log P
12
10
8
6
4
2
0
1
2
3
4
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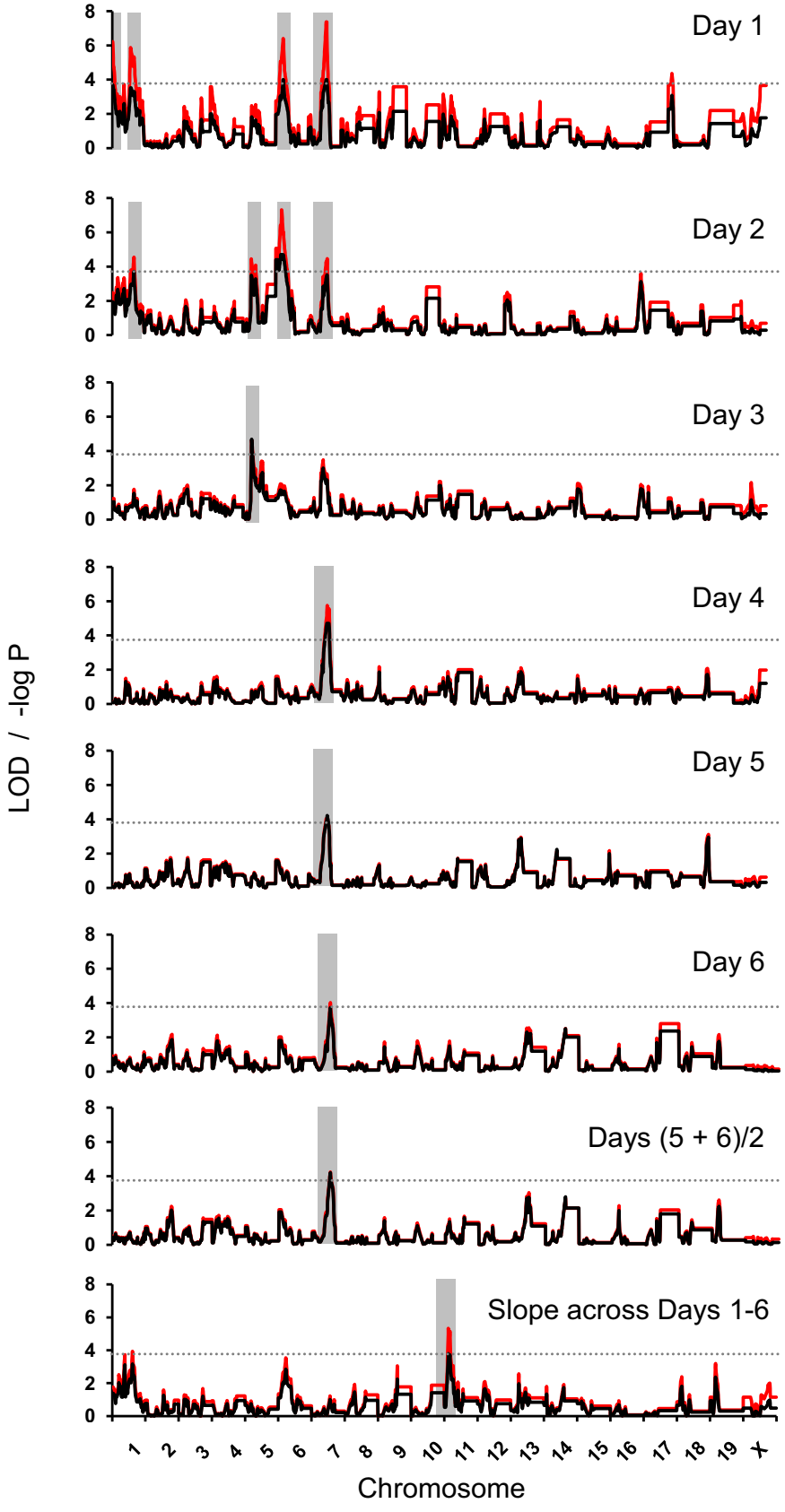
6
7
8
9
10
11
12
13
14
15
16
17
18
19
X
Chromosome


Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Days (5 + 6)/2
Slope across Days 1-6
LOD / -log P
0
2
4
6
8
1
2
3
4
5
6
7
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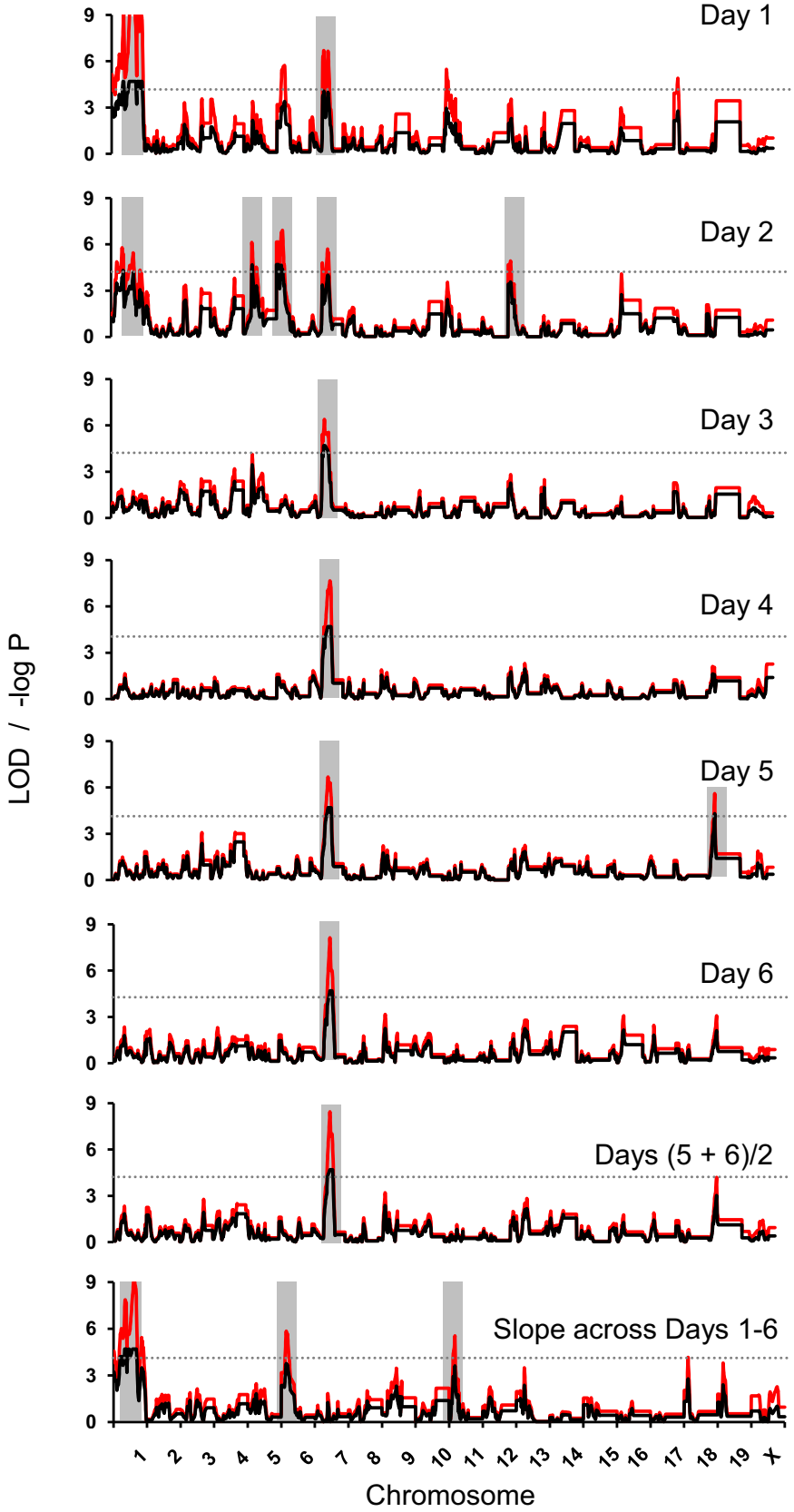
9
10
11
12
13
14
15
16
17
18
19
X
Chromosome


Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Days (5 + 6)/2
Slope across Days 1-6
LOD / -log P
0
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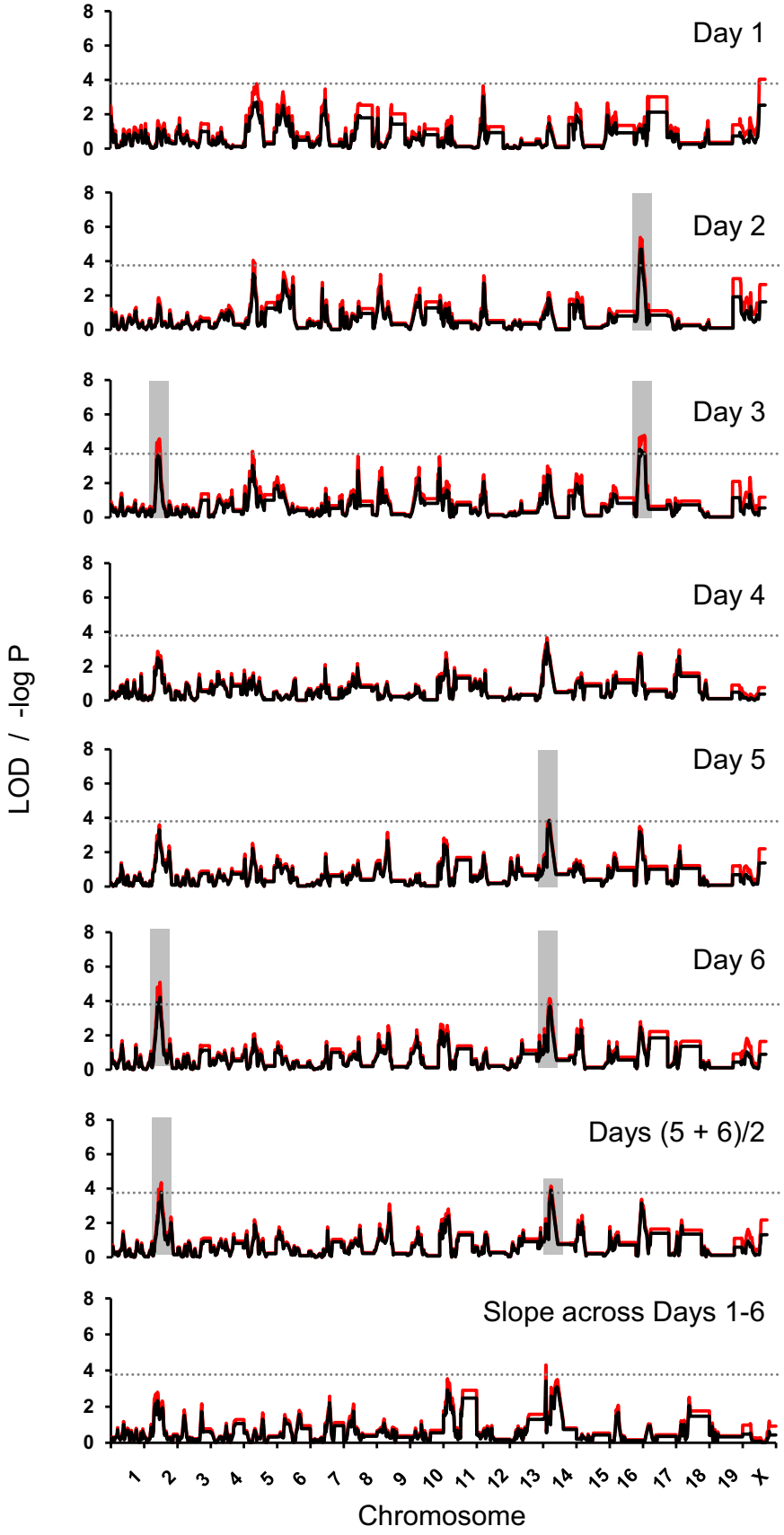
19
X
Chromosome


Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Days (5 + 6)/2
Slope across Days 1-6
LOD / -log P
0
2
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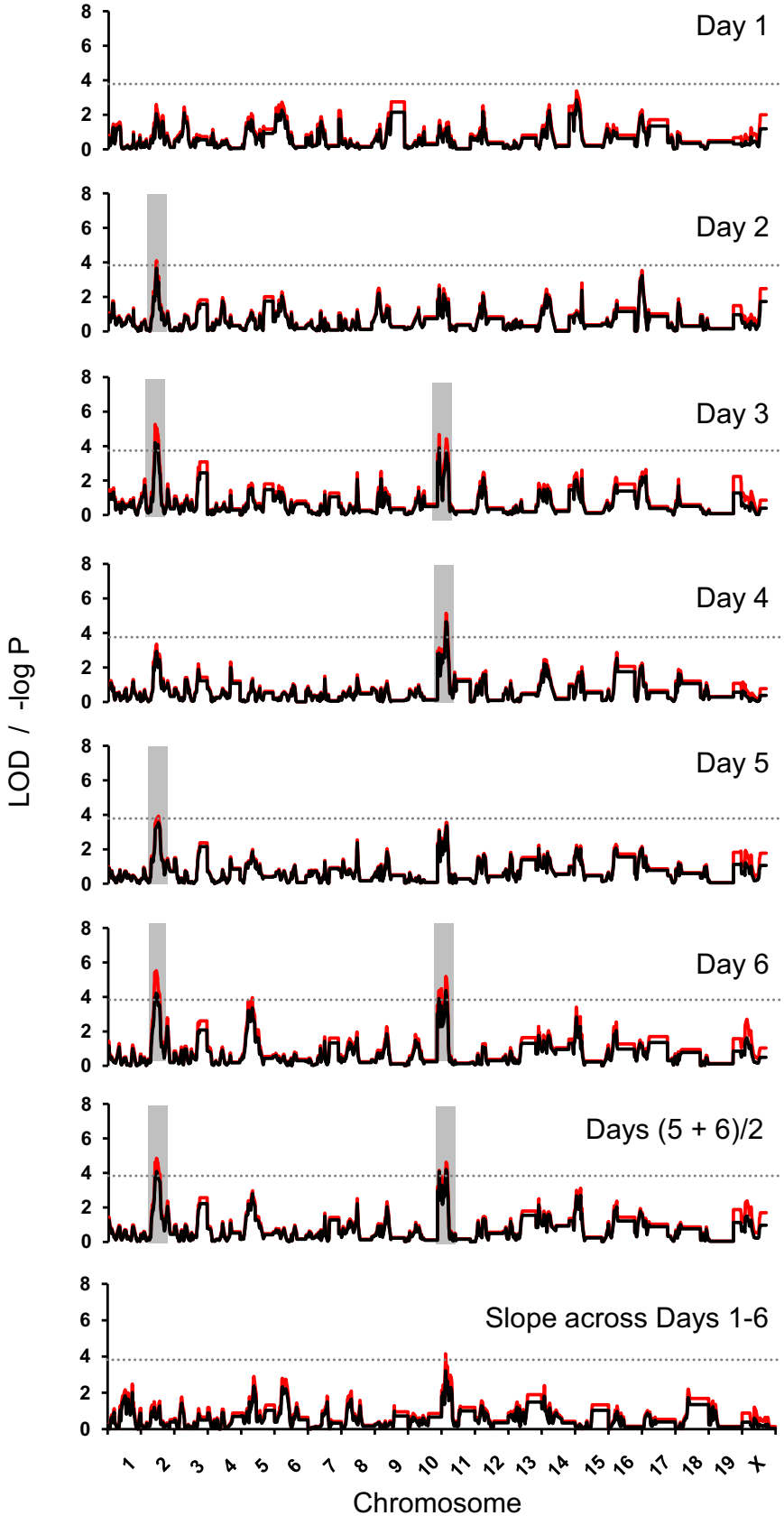
X
Chromosome


Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Days (5 + 6)/2
Slope across Days 1-6
LOD / -log P
0
2
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1
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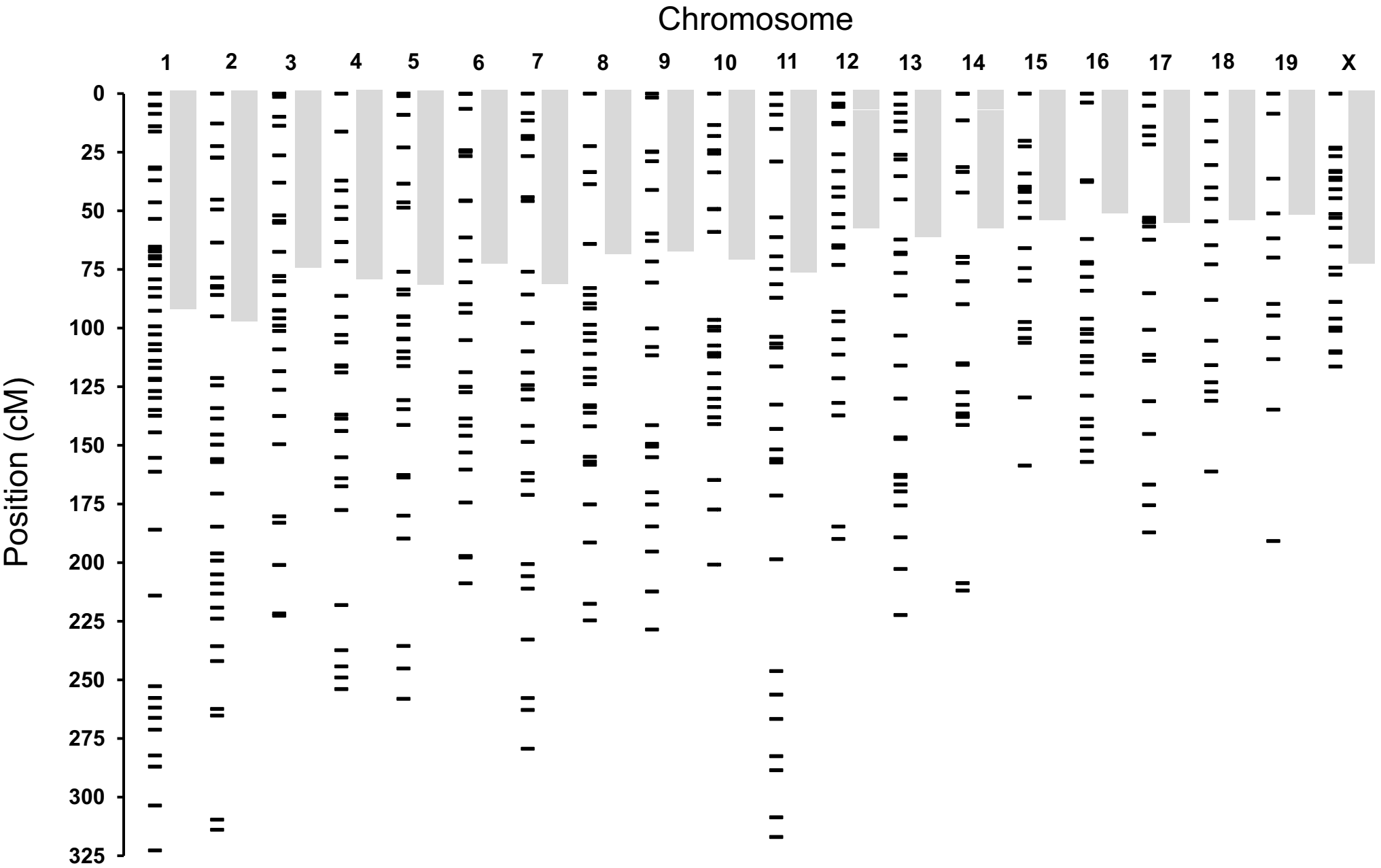
19
X
Chromosome


Chromosome
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3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
X
Position (cM)
0
25
50
75
100
125
150
175
200
225
250
275
300
325