Journal of Heredity 2010:101(1):42–53
doi:10.1093/jhered/esp066
Advance Access publication August 10, 2009

QTL Underlying Voluntary Exercise in Mice: Interactions with the "Mini Muscle" Locus and Sex

Derrick L. Nehrenberg, Shiliang Wang, Robert M. Hannon, Theodore Garland Jr, and Daniel Pomp

From the Department of Genetics, CB #7264, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7264 (Nehrenberg and Pomp); the Department of Cell and Molecular Physiology, University of North Carolina, Chapel Hill, NC 27599 (Wang and Pomp); the Department of Biology, University of California, Riverside, Riverside, CA 91521 (Hannon and Garland); and the Department of Nutrition, Carolina Center for Genome Science, University of North Carolina, Chapel Hill, NC 27599 (Pomp).

Address correspondence to Daniel Pomp at the address above, or e-mail: dpomp@unc.edu.

Abstract

Exercise improves many aspects of human health, yet many people remain inactive even when exercise is prescribed. We previously created a backcross (BC) between mice selectively bred for high levels of voluntary wheel running (VWR) and fixed for "mini muscle" (MM), a recessive mutation causing ~50% reduction in triceps surae mass. We previously showed that BC mice having the MM trait ran faster and further than mice without MM and that MM maps to chromosome 11. Here, we genotyped the BC with genome-wide single nucleotide polymorphisms to identify quantitative trait loci (QTL) controlling voluntary exercise and tissue and body mass traits and to determine whether these QTL interact with the MM locus or with sex. We detected 3 VWR QTL, representing the first voluntary exercise QTL mapped using this high running selection line, and 5 tissue mass QTL. Several interactions between trait QTL and the MM locus as well as sex were also identified. These results begin to explain the genetic architecture of VWR and further support MM as a locus having major effects, including its main effects on the muscle phenotype, its pleiotropic effects on wheel running and tissue mass traits, and through its interactions with other QTL and with sex.

Key words: *artificial selection, complex trait, genetic architecture, physical activity, running*

Exercise significantly reduces percentage of body fat (Slentz et al. 2005), reduces the incidence of diabetes (Hamman et al. 2006), and improves cardiac performance (Blumenthal et al. 2005). Despite such clear health benefits, many people remain inactive. Even when exercise is prescribed, physical activity often remains low, especially in the long term and for underserved populations (Fogelholm and Kukkonen-Harjula 2000; Fappa et al. 2008). One basic problem in designing effective physical activity-based therapies is that we know little about biological factors controlling individual variation in voluntary physical activity levels.

Several lines of evidence indicate that variation in physical activity is significantly heritable. First, in twin studies genetic factors account for as much as 72% of the variation for activity-induced energy expenditure (Joosen et al. 2005). Second, more than 214 genes and quantitative trait loci (QTL) have been mapped in humans for performance and health-related phenotypes (Bray et al. 2009), including linkages to physical activity levels (Simonen et al. 2003; Cai et al. 2006), indicating that exercise variation may be controlled primarily by many genes with individually small effects. Third, there is a large degree of variation for exercise duration and speed among mouse strains (Lightfoot et al. 2004). Fourth, several QTL for voluntary exercise have been mapped using the high active C57L/J and low active C3H/HeJ mouse strains (Leamy et al. 2008; Lightfoot et al. 2008). And last, voluntary exercise responded robustly to direct long-term selection and is associated with many correlated traits supporting high wheel running, including a lean body composition (Swallow et al. 1998, 2001).

The availability of mice selectively bred for high levels of voluntary exercise provides a unique opportunity to map genetic loci controlling exercise propensity. Voluntary wheel running (VWR) has been the target of a long-term selection experiment in which high-running (HR) mice were selectively bred from an outbred Harlan Sprague Dawley:

Institute of Cancer Research (Hsd:ICR) strain of *Mus domesticus* for wheel-running distance on days 5 + 6 of a 6-day wheel exposure. From one Hsd:ICR base population, mice were randomly assigned to 4 replicate selection (S) lines and 4 control (C) lines (Swallow et al. 1998). After 16 generations of selection, the 4 S lines ran on average 170% further than control mice. This increase in running distance was achieved mainly through an increase in running speed rather than running time (Swallow et al. 1998). Correlated traits like masses of various organs have been studied throughout the HR selection program (e.g., Swallow et al. 2005).

An unusual trait appeared in 3 of the 8 lines (1 C and 2 S), a 50% reduction in triceps surae mass that operates as a simple Mendelian recessive trait called "mini muscle" (MM; Garland et al. 2002). It was apparently lost in the C line (laboratory designated line 5), remains polymorphic in 1 S line (laboratory designated line 6), and apparently reached fixation in another S line (laboratory designated line 3) by generation 36 (Syme et al. 2005). Pleiotropic effects of MM, including increased ventricle and spleen size and increased capillarity in medial gastrocnemius muscle, have been observed and could conceivably support increased exercise capacity (Garland et al. 2002; Swallow et al. 2005; Wong et al. 2009). For example, increases in ventricle mass are often observed among endurance athletes (Maron 1986; Baggish et al. 2008). However, in the context of the HR selection experiment, HR mice having MM do not exhibit systematic differences in VWR levels compared with non-MM mice (Swallow et al. 1998, 2005; Garland et al. 2002; Houle-Leroy et al. 2003; Kelly et al. 2006; but for deviations from this trend, see Syme et al. 2005 and Gomes et al. 2009), making the impact of MM on exercise traits unclear.

To gain a better understanding of the MM mutation, we created a backcross (BC) by crossing females from S line 3 (fixed for MM; herein referred to simply as HR) with males from the C57BL/6J mouse strain (herein referred to as B6) and then crossing F_1 males back to HR parent females (Hannon et al. 2008). In the BC, the MM phenotype did not deviate from the 50:50 ratio expected for a simple Mendelian recessive trait (Hannon et al. 2008). The BC population was phenotyped for wheel-running traits according to standard selection procedures as well as for body mass and masses of triceps surae, ventricle, and spleen (Hannon et al. 2008). In the BC, mice with MM ran faster (average and maximum speed) and further than mice without MM and also had lighter body masses and heavier ventricles and spleens (Hannon et al. 2008). Female MM mice exceeded male MM mice for all running traits (Hannon et al. 2008). Subsequently, we used focused genotyping of microsatellite markers in the BC population to map the MM locus to a 2.6335 Mb gene-rich region between 67.453 and 70.0865 Mb on MMU11 (Hartmann et al. 2008).

In the current study, we have genotyped the BC population with a genome-wide single nucleotide polymorphism (SNP) panel in order to address 2 primary questions. First, what are the locations and magnitudes of effect of QTL controlling voluntary exercise and related traits in a cross using a HR line of mice? And second, do exercise QTL interact with the MM locus or with sex?

Methods

BC Creation and Phenotyping

For details regarding creation of the BC population, see Hannon et al. (2008) and Hartmann et al. (2008). Briefly, 20 B6 males were harem mated with 60 HR females. The 60 mating pairs produced 316 F_1 individuals, from which 90 males were randomly backcrossed (1 dam with 1 sire) to the MM HR female parents (mother–son and aunt–nephew matings were disallowed). At 7 weeks of age, 384 BC mice were exposed to running wheels for 6 days according to the standard selective-breeding protocol (Swallow et al. 1998), after which mice were sacrificed by CO_2, weighed, and dissected. The heart was detached and the ventricles were dissected and weighed by removing the connecting blood vessels and blotting to remove blood. Whole spleens were dissected and weighed. Right and left triceps surae muscles were dissected and weighed, including lateral and medial heads of the gastrocnemius, soleus, and plantaris. Albino, agouti, and black coat colors were recorded.

Genotyping and Linkage Map

A total of 384 BC mice and F_0 parental mice were genotyped for 154 SNPs. SNPs were initially selected based on their relatively even spacing across the genome and their predicted complete informativeness between HR and B6 F0 mice, using data from the Wellcome-CTC Mouse Strain SNP Genotype Set (http://www.well.ox.ac.uk/mouse/INBREDS). Predicted informativeness was based on genotypes from 8 mice representing lines (M16, ICR; Allan et al. 2005) derived from the same general genetic background as the Hsd:ICR population used as the base for selection of the HR lines, relative to the genotype of B6. After genotyping using matrix-assisted laser desorption ionization-time of flight mass spectrometry, we excluded any SNPs showing allele sharing among the HR and B6 F0 parents or whose BC genotypic frequencies significantly departed from the χ^2 distribution based on Mendelian expectation. The X chromosome was not genotyped due to the fact that F_1 males carried the HR X chromosome and were mated back to HR females to produce BC progeny having only HR X chromosomes. The final set of SNPs used for QTL analyses are provided in Supplementary Table 1.

QTL Analyses

QTL analyses were performed using R/qtl (Broman et al. 2003). All logarithm of the odds (LOD) significance thresholds were determined by the permutation procedure (Churchill and Doerge 1994). Loci that met or exceeded 95th and 90th percentiles were deemed significant and suggestive, respectively. Confidence intervals (CIs) for QTL positions were obtained using Bayes credible intervals (Manichaikul et al. 2006).

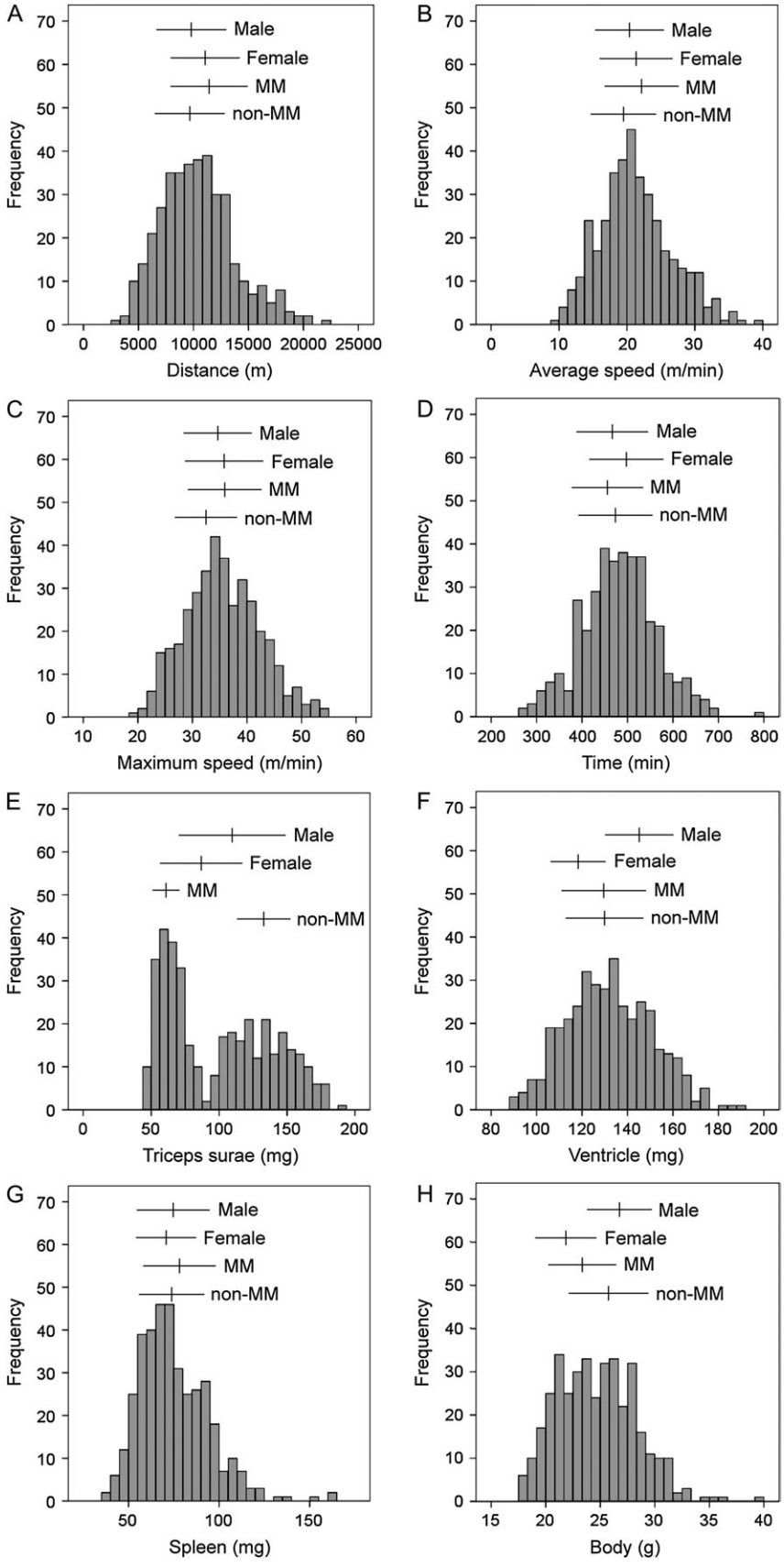
A
Male
Female
MM
non-MM
Frequency
Distance (m)
B
Average speed (m/min)
C
Maximum speed (m/min)
D
Time (min)
E
Triceps surae (mg)
F
Ventricle (mg)
G
Spleen (mg)
H
Body (g)

Table 1. Phenotypic correlations and *P* values (with Bonferroni adjustments) for measured traits

Trait	Average speed	Maximum speed	Time	Triceps surae	Ventricle	Spleen	Body
Distance	0.86***	0.78***	0.49***	−0.14	0.03[a]	−0.12	−0.07
Average speed		0.92***	0.00	−0.14[b]	0.05	−0.13	−0.09
Maximum speed			−0.01	−0.14[c]	0.09	−0.05	−0.04
Time				0.10	0.00	0.00	0.01
Triceps surae					−0.39***	−0.27[d]	0.17[e]
Ventricle						0.34***	0.30**
Spleen							0.45***

Correlations for the entire HR × B6 BC population are presented. Superscripts a–e denote significant correlations present in one or more subpopulations that were not present in the entire population.

[a] Male $P = 0.025$.

[b] Male $P = 0.011$.

[c] Male $P = 0.013$.

[d] MM $P = 0.123$, non-MM $P < 0.001$, female $P = 0.022$, male $P = 1.000$.

[e] MM $P < 0.001$, non-MM $P < 0.001$, female $P = 1.000$, male $P < 0.001$. All correlations were adjusted with appropriate fixed effects.

$^{**}P < 0.01$ and $^{***}P < 0.001$.

BC mice having the MM phenotype exhibited a range of triceps surae weights (0.0442–0.0887 g) that did not overlap with the range of triceps surae weights (0.0907–0.1932 g) of BC mice not having the MM phenotype (non-MM; Hannon et al. 2008). Therefore, we mapped the MM trait as a binary variable (0 = non-MM; 1 = MM) based on triceps surae muscle weight. For VWR traits, we calculated the average values of days 5 and 6 in the wheel because this is the criterion for which HR mice were selectively bred (Swallow et al. 1998). The following 4 VWR traits were evaluated for QTL: distance (m), average speed (m/min), maximum speed during any 1-min interval during the day 5 and 6 period (m/min), and time (number of 1-min intervals in which there was at least 1 wheel revolution recorded in a 24-h period). Four traits, which we collectively refer to as tissue mass traits, were also examined: average of left and right triceps surae mass, ventricle mass, spleen mass, and body mass (prior to tissue dissection). Fixed effects and covariates used in the analyses are summarized in Table 2. Single QTL model genome-wide scans for QTL were initially performed with and without MM as a fixed effect to determine the influence of MM.

We searched for trait QTL × MM and trait QTL × sex interactions using 2 stages of analysis to identify significant and suggestive interactions. In the first stage, we searched for significant interactions using a criterion $LOD_{Full} - LOD_{Additive} = \Delta LOD \geq 3.0$ (Sen and Churchill 2001). We then performed a second stage consisting of a search for suggestive interactions meeting 2 criteria: 1) identifying instances where QTL were present in one subpopulation but not the other (i.e., MM vs. non-MM, males vs. females) and 2) using multiple regression to calculate partial *F* statistics (R/qtl) of the VWR and tissue trait QTL × MM and QTL × sex interactions. Thus, QTL that were unique to one subpopulation and exhibited significant partial *F* statistics were considered to represent suggestive QTL interactions with MM or sex.

We performed an additional stepwise QTL analysis to further evaluate QTL × MM interactions (Manichaikul et al. 2008). Prior to stepwise QTL model selection, we imputed genotype data using the Monte Carlo algorithm (Sen and Churchill 2001). Then, for each trait, we performed the forward/backward model selection to identify the best model, which was defined as the model having the maximum penalized LOD (pLOD) score (Zeng et al. 1999). Instances where the best model included the interaction term between MM QTL and the trait QTL were deemed to confirm a significant interaction.

The effect of coat color on VWR traits was analyzed using the SAS 9.1 general linear model procedure (Cary, NC). The general linear model included sex, coat color, and batch as fixed effects, a sex × coat color interaction effect, and wheel resistance and body mass as covariates.

Results

Several analyses were performed prior to mapping VWR and tissue mass QTL and their interactions with MM or sex. We first mapped MM as a binary variable based on non-overlapping masses of triceps surae muscles (21). A genome-wide scan for the MM locus produced a single peak centered at 43.37 cM on MMU11 with a LOD of 93.56 and a 95% CI between 41.37 and 46.37 cM. Next, we examined the distributions of the measured traits. As shown in Figure 1 (and Supplementary Figure 1), all the traits

Figure 1. Distributions of traits measured in the HR × B6 BC. Displayed above the histograms are means and 2 standard deviation error bars for 2 sets of populations (MM vs. non-MM and female vs. male).

Table 2. Fixed effects and covariates included in additive[a] model QTL analyses

QTL	Populations	Fixed effects	Covariates
MM	Whole population	None	None
VWR	Whole population	Sex, batch[b], MM	Wheel resistance[b], massmean[b]
	MM versus non-MM	Sex, batch	Wheel resistance, massmean
	Female versus male	Batch, MM	Wheel resistance, massmean
Tissue mass (triceps Surae, ventricle, spleen)	Whole population	Sex, batch, MM	Massmean
	MM versus non-MM	Sex, batch	Massmean
	Female versus male	Batch, MM	Massmean
Body mass	Whole population	Sex, batch, MM	
	MM versus non-MM	Sex, batch	
	Female versus male	Batch, MM	

[a] Full model QTL analyses included appropriate interaction terms.

[b] Batch, wheel resistance, and massmean refer to wheel experimental batch, number of wheel revolutions after wheels were accelerated at a given velocity, and ([body mass prior to exercise + body mass after exercise]/2), respectively.

(except triceps surae mass) were either normally distributed or slightly skewed. We then examined correlations between traits in the whole population and within 4 subpopulations (MM, non-MM, female, and male; Table 1). In the whole population, we found, for the most part, that the VWR and tissue mass traits were correlated among themselves but not across categories. For example, triceps surae mass was correlated with ventricle mass in the full BC but not any of the VWR traits. Several subpopulations also exhibited significant correlations that were not apparent in the whole BC (Table 1). Notably, triceps surae mass was only correlated to VWR traits in the male subpopulation (Table 1).

To identify QTL controlling VWR and tissue mass traits, we initially performed single, additive QTL model genome-wide scans with and without MM in the QTL model. Note that the covariates included in each additive QTL analyses throughout the results are presented in Table 2. Without MM in the model, a QTL peak was detected at the MM locus on MMU11 for all traits except running time (Figure 2), strongly demonstrating the effect of the MM mutation on VWR and tissue mass traits. When MM was included in the model, all MMU11 QTL disappeared as expected. To account for the strong effect of the MM locus, all subsequent QTL scans included MM in the QTL model. Using the single, additive QTL model we detected 3 significant VWR QTL and 4 significant tissue mass QTL in the BC population (Figure 2 and Table 3). One significant QTL for average running speed was detected on MMU7 and 2 significant QTL for maximum running speed were detected on MMU6 and MMU7. No main effect QTL were detected for running distance or time. We also detected 4 significant QTL for tissue mass traits, including a QTL for triceps surae mass on MMU17, a QTL for ventricle mass on MMU3, and 2 QTL for spleen mass on MMU6 and MMU13 (Table 3). An additional suggestive QTL for spleen mass was detected on MMU9 (Table 3).

Our next goal was to identify significant and suggestive interactions between VWR or tissue mass QTL and MM locus or sex. Trait QTL interacting with the MM locus are presented first (Figure 3 and Table 4). In our search for significant interactions trait, using a ΔLOD interaction criterion, only the ventricle mass QTL on MMU3 was found to interact significantly with the MM QTL on MMU11 (LOD_{Full} = 8.97, ΔLOD = 2.99), because it significantly affected the ventricle mass trait among MM mice, but had little effect on non-MM mice (Figure 3A). This interaction was confirmed by the 2 QTL stepwise QTL model selection procedure (Figure 3B). The pLOD was 18.23, and the best 2 QTL model contained the interaction for ventricle mass QTL (MMU3, peak = 85.11 cM) and the MM QTL (MMU11, peak = 43.65 cM). Our second stage of trait QTL × MM QTL interaction analyses detected 5 suggestive QTL interactions (Figure 3A,C–D and Table 4). Two ventricle mass QTL on MMU2 and MMU10 interacted with the MM QTL on MMU11. These 2 suggestive QTL controlled ventricle mass in non-MM mice but exerted little effect in MM mice. One suggestive QTL for running distance QTL on MMU5 interacted with the MM QTL because it was apparently only significantly expressed in non-MM mice (Figure 3C). Two triceps surae QTL on MMU2 and MMU17 interacted with MM. Again, both of these QTL were only present to a significant degree in non-MM mice (Figure 3D).

A similar set of QTL interaction analyses detected 3 QTL × sex interactions (Table 5 and Figure 4). A suggestive QTL × sex interaction was detected between the running time QTL on MMU2 and sex because it influenced running time in males but had little to no effect in females (Figure 4A). Another suggestive interaction was detected between the triceps surae QTL on MMU2 and sex because it only influenced male triceps surae mass (Figure 4B). A highly significant QTL × sex interaction for triceps surae mass was observed on MMU11 for the interaction between the triceps surae QTL on MMU11 and sex (LOD_{Full} = 24.26, ΔLOD = 23.8; Figure 4B). This MMU11 interaction had a QTL peak located at 46.36 cM and 95% CI between 41.4 and 51 cM, which falls within the 95% CI for MM, but is located 3 cM away from the MM QTL peak.

As mutations controlling mouse coat color exert many pleiotropic effects and the location of many of these mutations are known, we examined the effects of coat color

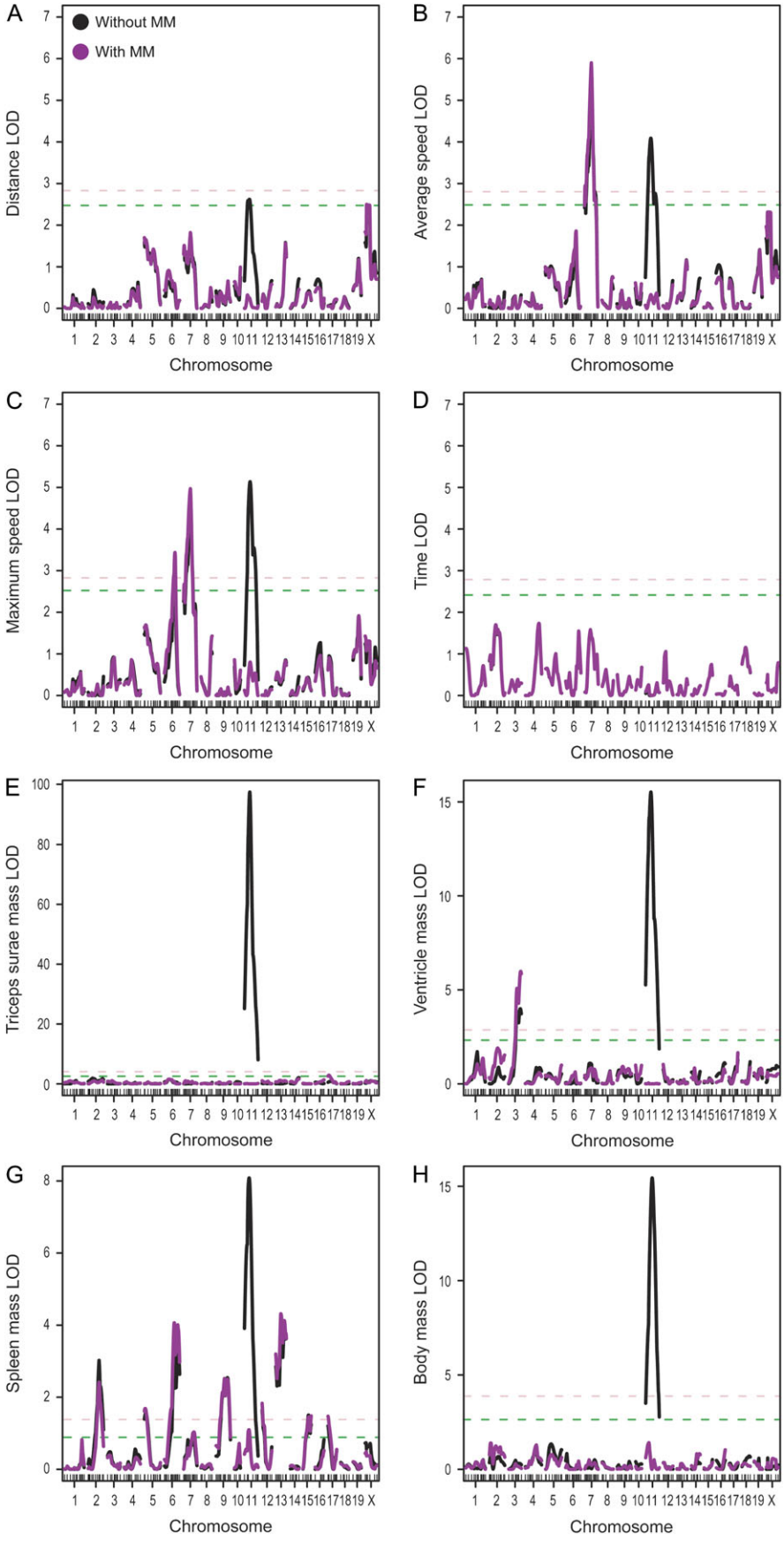
A
Without MM
With MM
Distance LOD
B
Average speed LOD
C
Maximum speed LOD
D
Time LOD
E
Triceps surae mass LOD
F
Ventricle mass LOD
G
Spleen mass LOD
H
Body mass LOD
Chromosome
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X

Table 3. Descriptive statistics for VWR and tissue mass trait QTL after accounting for the MM QTL

Trait	Chromosome	LOD[a]	Peak (cM)	95% CI (cM)	% Variance[b]
Average speed	7	5.90*	53.39	42.75–60.75	5.98
Maximum speed	6	3.43*	65.00	39.72–73.00	3.46
Maximum speed	7	4.96*	53.39	35.74–60.74	4.89
Triceps surae	17	2.87*	21.88	15.88–34.88	0.22
Ventricle	3	5.98*	90.11	63.11–93.16	2.44
Spleen	6	3.85*	60.00	53.00–89.00	3.12
Spleen	9	2.41	52.19	34.72–83.37	1.87
Spleen	13	4.08*	55.87	29.87–67.16	3.26

[a] LOD exceeding the 95% permutation threshold are denoted by *; other QTL exceeded the 90% threshold.

[b] Percentage of phenotypic variance accounted for by the QTL effect.

on VWR traits in BC mice. In the BC population, there were 41 (11%) black mice, 140 (37%) agouti mice, and 199 (52%) albino mice (data for coat color were not recorded for 4 mice), percentages that do not differ from Mendelian ratios expected for the recessive albino trait versus other coat colors in the BC ($\chi^2 = 0.327$, $P > 0.05$). Effect of sex ($P = 0.0004$, $P = 0.0425$, $P = 0.0016$, and $P = 0.0002$) and coat color ($P = 0.0132$, $P < 0.0001$, $P < 0.0001$, $P = 0.0021$) were significant, but there was no sex by coat color interaction ($P = 0.5411$, $P = 0.2222$, $P = 0.6293$, and $P = 0.7689$) on the outcome variables of running distance, average speed, maximum speed, and running time, respectively. Albino mice did not run as far as black mice ($P < 0.05$), had slower maximum speeds than agouti and black mice ($P = 0.001$, $P < 0.001$), had slower maximum speeds ($P = 0.046$, $P < 0.001$), and ran significantly more time than agouti mice ($P = 0.016$; Table 6).

Discussion

The HR × B6 BC was initially created to localize MM, a recessive mutation in HR mice showing the classic properties of a locus of major effect (Hannon et al. 2008). Using this BC, we have mapped this locus to a 2.6-Mb interval on MMU11 (Hartmann et al. 2008). One of our present goals was to identify QTL for VWR and tissue mass traits. We detected QTL on MMU11 for all traits except running time, representing pleiotropic effects of the MM locus. When MM was included as a fixed effect in the QTL model, all QTL on MMU11 disappeared, further demonstrating that MM influences VWR and tissue mass traits. We also detected 3 VWR and 5 tissue mass QTL on chromosomes other than MMU11, representing the first voluntary exercise QTL mapped using the HR lines. Our other goal was to identify QTL that interacted with MM or sex. We found that MM interacted significantly with a ventricle mass QTL on MMU3 and also showed suggestive interactions between MM and 6 VWR and tissue mass QTL. For QTL × sex interactions, we detected a robust interaction with the QTL for triceps surae mass on MMU11 (i.e., MM) as well as suggestive interactions with 2 other QTL.

These results further support MM as a locus having major effects, including its main effects on the MM phenotype, its pleiotropic effects on VWR and tissue mass traits, and through its interactions with other QTL and sex. However, it is important to consider that the expressivity of MM may not operate entirely independently from other HR alleles. In the context of HR and B6 alleles, the MM trait maintains a bimodal distribution, but we do not yet know how the MM mutation may operate in the absence of other HR alleles (e.g., if it were introgressed into non-HR genetic backgrounds). Effects of another recessive mutation, high growth (*hg*), follow a bimodal distribution when expressed in the genetic selection background (Bradford and Famula 1984) but then exhibit a normal distribution in the context of a C57BL/6J-*hg*/*hg* × CAST/EiJ F_2 cross (Horvat and Medrano 1995). And like the results we report here for exercise and MM, QTL for growth are modifiable by the *hg* mutation (Corva et al. 2001).

Only 4 QTL exceeding the 5% experiment-wise level have been previously reported for wheel-running activity (Lightfoot et al. 2008). Among these, 3 (distance, speed, and time) coincided on MMU13 (*DIST13.1*, *SPD13.1*, and *DUR13.1*); the fourth and largest QTL detected was for speed on MMU9 (*SPD9.1*). In this present study, all the QTL that exceeded the 95% threshold were related to speed (either average or maximum speed), except for the QTL detected in the male subpopulation for running time on MMU2, which to our knowledge, is the first sex-specific wheel-running QTL reported in mice. The suggestive running distance QTL we detected on MMU5 (CI = 2.21–66.20 cM) in the non-MM subpopulation falls outside the CI for another reported suggestive QTL for distance on the same chromosome (*DIST5.1*, CI = 93–109 cM; Lightfoot et al. 2008). It is not surprising that the exercise trait QTL detected in the present study do not correspond to those found in the previous study for several reasons: 1) different methods of VWR measurement, 2) laboratory-to-laboratory variation, 3) age differences, 4) differences in genetic background, and 5) differences in the genetic cross design (e.g., BC vs. F_2).

Some of the VWR QTL detected in this BC appear to be pleiotropic for other VWR traits, which is not surprising considering that running distance is the direct product of running speed and time spent running, or it could be the

Figure 2. Single genome-wide scans of HR × B6 BC phenotypes with and without MM in the whole population additive QTL model. (**A**) Running distance. (**B**) Average running speed. (**C**) Maximum running speed. (**D**) Time spent running. (**E**) Tricep surae mass. (**F**) Ventricle mass. (**G**) Spleen mass. (**H**) Body mass. The green and pink-hatched lines represent the permuted 90% and 95% LOD thresholds, respectively.

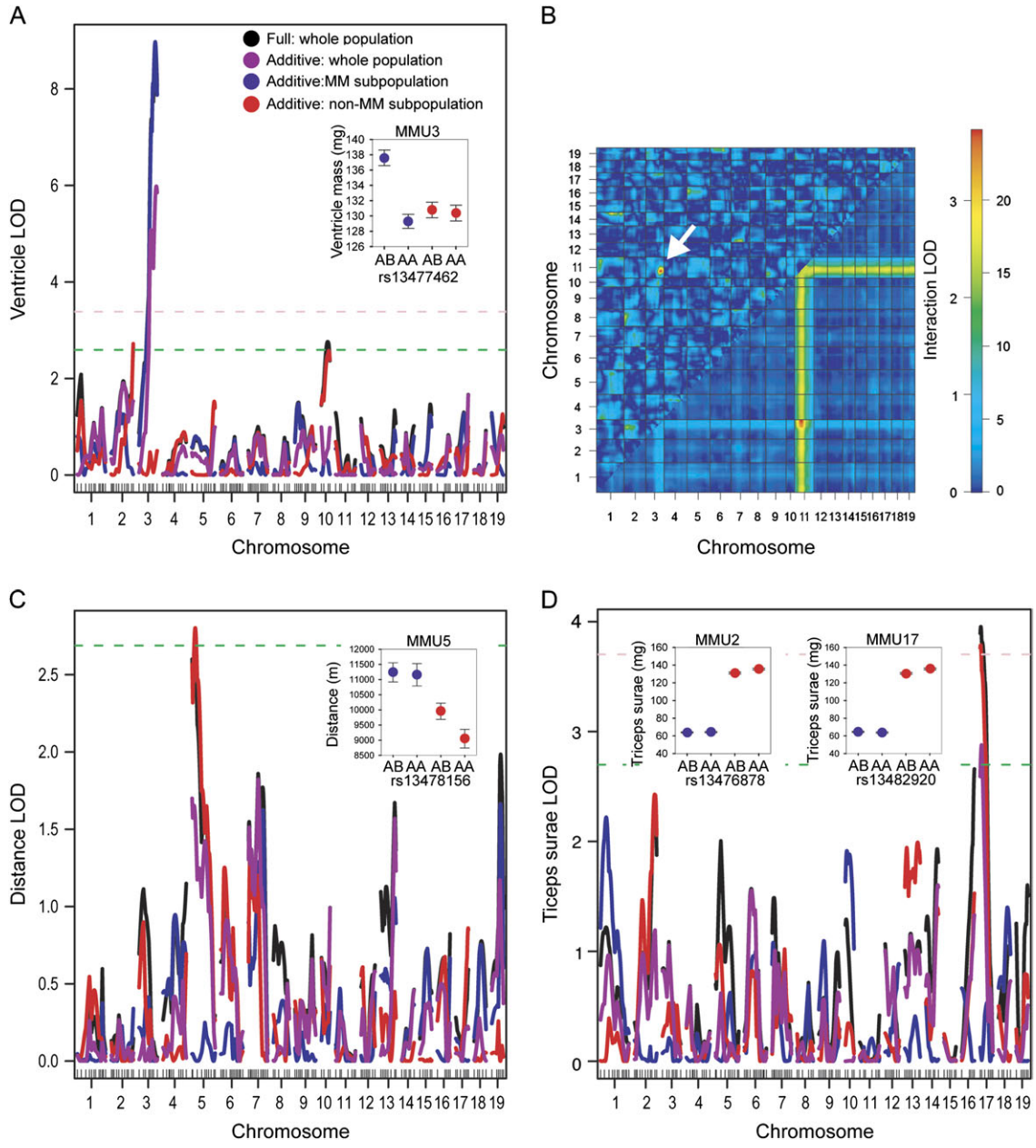

Figure 3. VWR and tissue mass trait QTL × MM QTL interactions. Two genome scans were performed for the whole population (full model including the QTL × MM interaction term and the additive model) and 2 additional scans were performed for the MM and non-MM subpopulations for trait QTL. The pink and green-hatched lines represent the permuted 95% LOD significance thresholds for the full and additive QTL models, respectively. (**A**) Ventricle mass (inset plot; the ventricle mass QTL × MM interaction on MMU3 is due to the larger effect of the rs13482920 genotype on ventricle mass in MM compared with non-MM mice). (**B**) Two QTL model stepwise QTL model heat map illustrating the interaction between the MM QTL on MMU11 and ventricle mass on MMU3. (**C**) Running distance (inset plot; the distance QTL × MM interaction on MMU5 is attributable to larger effects of the rs13478156 genotype on running distance in non-MM than MM mice). (**D**) Triceps surae mass (left and right inset plots; the triceps surae QTL × MM interactions on MMU2 and MMU17 are due to the more significant effects of the rs13476818 and rs13482920 genotypes, respectively, on decreasing triceps surae mass in non-MM compared with MM mice).

case that the traits measure similar attributes (i.e., because average speed is similar to maximum speed). Thus, the additive model QTL for maximum speed and average speed on MMU7 are likely manifested by genetic variation in the same locus. Similarly, the QTL on MMU5 for running distance and maximum speed likely represent the same underlying genetic variation, especially given that no QTL were detected for running time on MMU5. Consequently, it

Table 4. Genome-wide scans for VWR and tissue mass trait QTL in MM and non-MM subpopulations and their interaction with the MM QTL

		MM subpopulation				Non-MM subpopulation				
Trait	Chromosome	LOD[a]	Peak (cM)	CI (cM)	% Variance[b]	LOD[a]	Peak (cM)	CI (cM)	% Variance[b]	Partial *F* test
Distance	5					2.80*	16.20	2.21–66.20	4.93	0.039
Average speed	7	3.62*	53.75	30.75–75.50	7.85	3.32*	53.40	17.75–60.75	7.11	0.506
Maximum speed	5					3.28*	9.21	2.21–24.72	5.70	0.057
Maximum speed	6					2.83*	66.69	38.00–78.00	5.35	0.436
Maximum speed	7	3.48*	46.75	26.75–77.99	7.27					0.542
Triceps surae	2					2.43	89.18	29.18–98.04	0.77	0.031
Triceps surea	17					3.78*	17.98	15.88–36.88	1.17	0.037
Ventricle	2					2.72*	98.04	48.89–98.04	1.62	0.010
Ventricle	3	8.97*	85.11	74.10–92.11	4.97					<0.001
Ventricle	10					2.59	49.15	21.16–54.00	1.23	0.006
Spleen	6	3.27*	78.00	56.00–91.00	5.37					0.241
Spleen	13	3.78*	55.86	36.87–67.16	6.55					0.063

[a] LOD exceeding the 95% permutation threshold are denoted by *; other QTL exceeded the 90% threshold.

[b] Percentage of phenotypic variance accounted for by the QTL effect.

is probable that only 4 unique QTL for VWR traits were detected in the BC: 1 for running speed (maximum speed and average speed) on MMU7 in both MM and non-MM mice, 2 for maximum speed on MMU5 and MMU6 among non-MM mice, the former of which coincides with a non-MM QTL for running distance, and 1 for running time on MMU2 in the male subpopulation.

The QTL for average and maximum running speed on MMU7 are particularly noteworthy because their peaks fall ~10 Mb away from the *tyrosinase* (*tyr*) gene. Through linkage, this QTL likely explains the effects of coat color on VWR traits that were identified in this study, given that HR mice are albino, and albinism is caused by a single recessive point mutation in *tyr* (Yokoyama et al. 1990). It is also feasible to consider *tyr* as a positional candidate gene for the running speed QTL. The effect of the *tyr* locus on open-field activity is well known, and its colocalization with QTL for open-field activity (Turri et al. 2001; Henderson et al. 2004) and contextual fear (a behavioral response involving locomotor immobility; Ponder et al. 2008) have also been reported. Although a functional relationship between tyrosinase and locomotor behavior has not been established, there is accumulating evidence that tyrosinase can serve as a precursor for dopamine, a neurotransmitter central to goal-directed behavior (Cheer et al. 2007).

The predominant route for dopamine synthesis begins with the first and rate-limiting enzyme tyrosine hydroxylase, which catalyzes tyrosine to L-dihydroxyphenylalanine (L-Dopa), which is subsequently converted to dopamine by Dopa decarboxylase. Tyrosinase, a key enzyme in melanin biosynthesis that produces hair and skin pigment, also converts tyrosine to L-Dopa (Sanchez-Ferrer et al. 1995), and in the absence of tyrosine hydroxylase, tyrosinase is capable of producing dopamine in the brain and

Table 5. Genome-wide scans for VWR and tissue mass trait QTL in female and male subpopulations and their interaction with sex

		Female subpopulation				Male subpopulation				
Trait	Chromosome	LOD[a]	Peak (cM)	CI (cM)	% Variance[b]	LOD[a]	Peak (cM)	CI (cM)	% Variance[b]	Partial *F* test
Average speed	7	3.92*	53.39	33.75–64.75	7.97					0.226
Maximum speed	6					4.00*	65.00	56.00–65.00	7.80	0.107
Maximum speed	7	2.70	49.75	17.75–64.75	5.28					0.677
Time	2					3.30*	63.18	39.18–74.18	7.06	0.010
Triceps surae	1	2.99*	36.05	23.10–82.10	0.36					0.130
Triceps surae	2	3.14*	25.98	13.18–39.18	0.36					0.018
Ventricle	2	2.64	52.18	27.18–70.16	3.58					0.091
Ventricle	3	3.56*	88.11	64.10–93.16	4.84	2.69*	91.11	62.11–93.16	2.65	0.348
Spleen	6	3.04*	87.00	68.69–93.88	4.36					0.357
Spleen	13	2.92*	1.87	1.86–51.87	4.66	2.42	67.16	30.86–67.16	4.94	0.152

[a] LOD exceeding the 95% permutation threshold are denoted by *; other QTL exceeded the 90% threshold.

[b] Percentage of phenotypic variance accounted for by the QTL effect.

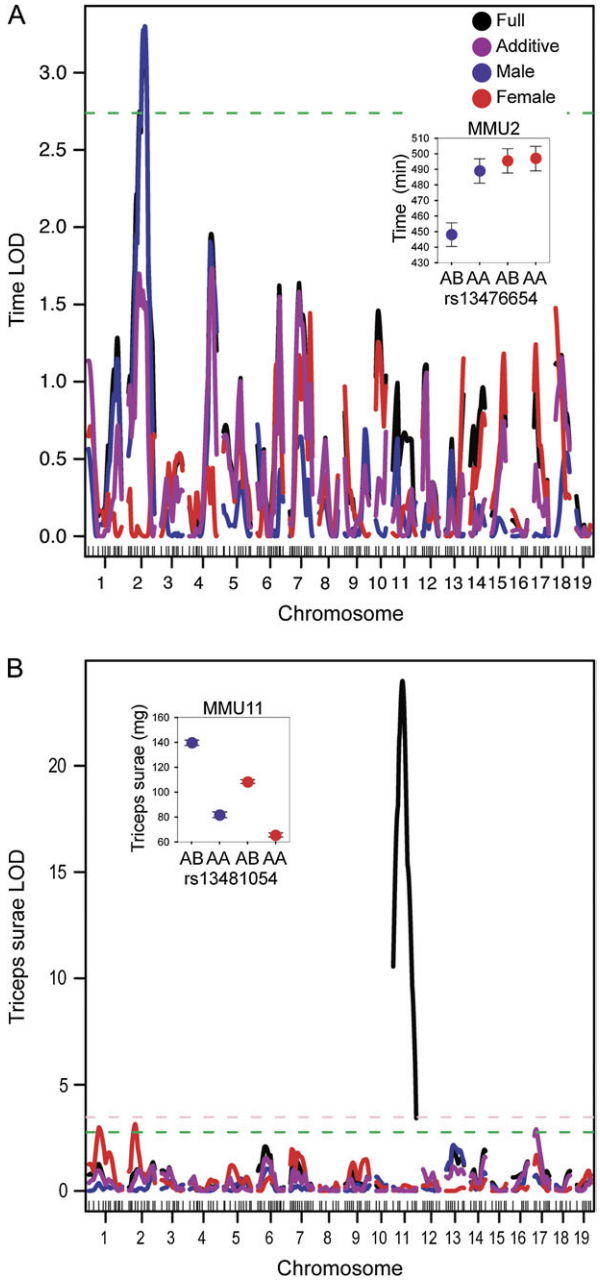

Figure 4. VWR and tissue mass trait QTL × sex interactions. Two genome scans were performed for the whole population (full model including the QTL × sex interaction term and the additive model) and 2 additional scans were performed for the female and male subpopulations. The pink and green-hatched lines represent the permuted 95% LOD significance thresholds for the full and additive QTL models, respectively. (**A**) Running time (inset plot; the time QTL × sex interaction on MMU2 is due to the significant effect of the rs13476654 genotype on increasing female running time but not male running time). (**B**) Triceps surae mass (inset plot; the triceps surae QTL × sex interaction is attributable to larger effects of the rs13482920 genotype on triceps surae mass in males compared with females).

peripheral cells of the body (Rios et al. 1999; Eisenhofer et al. 2003). Furthermore, tyrosinase is a major determinant of peripheral dopamine levels in early postnatal development (Eisenhofer et al. 2003). It remains to be determined whether tyrosinase is functionally associated with locomotor behaviors such as voluntary exercise via dopamine-related mechanisms. However, it is important to note that although *tyr* gene expression has been documented in the mouse brain (Lein et al. 2007), it remains controversial whether tyrosinase is present in the brain (Tief et al. 1998; Tribl et al. 2007). Parenthetically, we have found that female HR mice (from a different replicate of the S lines than that used here) exhibit higher levels of dopamine in ventral striatum tissue compared with other ICR-based strains (Mathes WF, Nehrenberg DL, Gordon RR, Hua K, Garland T Jr, Pomp D, unpublished data). Given this circumstantial evidence, as well as other data linking high running in HR mice to the dopamine pathway (Rhodes et al. 2005), it is tempting to speculate that the QTL for average running speed is underpinned by variation in or near the *tyr* locus. However, a large number of other genes are localized in proximity to this QTL, many of which still have unknown functions.

Our search for QTL × MM and QTL × sex interactions revealed 2 particularly interesting results. The MMU3 ventricle mass QTL × MM interaction stems from MM mice exhibiting heavier ventricles than non-MM mice even though they weigh less than non-MM mice. Enlarged ventricles have long been known to characterize athletic hearts (Maron 1986), so they could represent an advantageous, coadaptive genetic trait facilitating increased running speed in mice having the MM mutation. However, in the present BC, the lack of any significant correlation between any of the VWR traits and ventricle mass suggests that ventricle mass does not in fact directly impact running levels. The triceps surae mass MMU11 QTL interaction with sex was also particularly striking. This interaction is attributable to the fact that the MM mutation reduced tricep surae mass to a greater extent in males than females (Hannon et al. 2008). It is recognized that sex × genotype as well as sex × genotype × environment interactions play a critical evolutionary role in producing and maintaining genetic variation (Mackay 2001). Several sex × QTL interactions have been reported, including those that influence tissue mass (Rance et al. 2007), bone mineral density (Ishimori et al. 2008), and pain responses (Mogil et al. 1997). Given its magnitude and proximity to the MM locus, the triceps surae mass MMU11 QTL interaction with sex could prove beneficial for delineating the genetic architecture of MM. For example, the MM locus is located on MMU11 in a gene-rich region containing ~100 genes (Hartmann et al. 2008). Any of these genes that are known to have differential sex effects and/or expression may become higher priority candidates.

Although this study detected several VWR QTL, these QTL individually explained only a relatively small percentage of the phenotypic variance for VWR traits in BC mice, and their cumulative effects fall short of accounting for the narrow-sense heritability estimate of 0.28 for VWR in HR mice (Swallow et al. 1998). This "missing heritability" could

Table 6. Least-squares means (LSMEANs) for running trait by coat color

	Distance		Average speed		Maximum speed		Time	
Coat color	LSMEAN	SE	LSMEAN	SE	LSMEAN	SE	LSMEAN	SE
Albino	10 079	217	20.21	0.35	34.33	0.45	492.59[a]	5.30
Agouti	10 567[a]	259	22.20[a]	0.42	36.55[a]	0.53	469.36[b]	6.33
Black	11 460[a]	485	24.10[a]	0.79	39.09[a]	1.00	469.57[a,b]	11.86

LSMEANs not sharing a common superscript are significantly different ($P < 0.05$).

SE, Standard error.

be explained by several factors. First, we recently observed that HR VWR traits show dominance over B6; HR × B6 F_1 VWR traits are expressed at levels comparable to parental HR and significantly higher than the low VWR B6 parental levels (Nehrenberg et al. 2009). Although the BC design was appropriate for mapping a QTL for the MM recessive trait, it has reduced power to detect QTL in the presence of significant dominance. Second, epistatic QTL can account for significant genetic variation in wheel-running traits (Leamy et al. 2008). Third, VWR traits in HR mice could be determined by changes in ontogeny that have risen from alterations in the dynamic interplay between genetic variation and environmental variation (e.g., in utero and postnatal maternal environments) occurring over the course of HR development. In humans, the maternal gestational environment is drastically altered by exercise (Clapp 2006), and there is evidence that human mothers who continue exercising during pregnancy produce leaner offspring that also exhibit measurably better neurodevelopmental outcomes (Clapp 2000). If such dynamic phenomena contribute to the HR phenotype, then perhaps, the HR selection experiment is illustrative of an "ontogenetic selection" experiment, in which the HR phenotype materialized from selection of developmental recombinations of gene and environmental interactions supporting high levels of wheel running (Baldwin 1896; West-Eberhard 2005). Accordingly, the genome × environment × time interaction underpinning HR VWR phenotypes would amplify the complexity of VWR phenotypes to a point where studies using much larger populations, dense genetic marker sets, contrasting environmental conditions, and multiple developmental time points are required to elucidate the genetic architecture of voluntary exercise.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

Funding

The National Institute of Diabetes and Digestive and Kidney Diseases grant (DK076050) to D.P.; National Science Foundation grant (IOB-0543429) to T.G.; University of North Carolina at Chapel Hill Clinical Nutrition Research Center, The National Institute of Diabetes and Digestive and Kidney Diseases grant (DK56350); University of North Carolina Nutrition Research Institute (to S.W.).

Acknowledgments

We thank Chris Wiesen at University of North Carolina at Chapel Hill Odum Institute of Social Science for writing SAS macros used to perform the correlation analyses and John Hartmann for his efforts in extracting the DNA for this project. We are also grateful to Dr Scott A. Kelly for helpful suggestions that improved the quality of the manuscript.

References

Allan MF, Eisen EJ, Pomp D. 2005. Genomic mapping of direct and correlated responses to long-term selection for rapid growth rate in mice. Genetics. 170:1863–1877.

Baggish AL, Wang F, Weiner RB, Elinoff JM, Tournoux F, Boland A, Picard MH, Hutter AM Jr, Wood MJ. 2008. Training-specific changes in cardiac structure and function: a prospective and longitudinal assessment of competitive athletes. J Appl Physiol. 104:1121–1128.

Baldwin JM. 1896. A new factor in evolution. Am Nat. 30:441–451.

Blumenthal JA, Sherwood A, Babyak MA, Watkins LL, Waugh R, Georgiades A, Bacon SL, Hayano J, Coleman RE, Hinderliter A. 2005. Effects of exercise and stress management training on markers of cardiovascular risk in patients with ischemic heart disease: a randomized controlled trial. JAMA. 293:1626–1634.

Bradford GE, Famula TR. 1984. Evidence for a major gene for rapid postweaning growth in mice. Genet Res. 44:293–308.

Bray MS, Hagberg JM, Perusse L, Rankinen T, Roth SM, Wolfarth B, Bouchard C. 2009. The human gene map for performance and health-related fitness phenotypes: the 2006–2007 update. Med Sci Sports Exerc. 41:35–73.

Broman KW, Wu H, Sen S, Churchill GA. 2003. R/qtl: QTL mapping in experimental crosses. Bioinformatics. 19:889–890.

Cai G, Cole SA, Butte N, Bacino C, Diego V, Tan K, Goring HH, O'Rahilly S, Farooqi IS, Comuzzie AG. 2006. A quantitative trait locus on chromosome 18q for physical activity and dietary intake in Hispanic children. Obesity (Silver Spring). 14:1596–1604.

Cheer JF, Aragona BJ, Heien ML, Seipel AT, Carelli RM, Wightman RM. 2007. Coordinated accumbal dopamine release and neural activity drive goal-directed behavior. Neuron. 54:237–244.

Churchill GA, Doerge RW. 1994. Empirical threshold values for quantitative trait mapping. Genetics. 138:963–971.

Clapp JF. 2000. Exercise during pregnancy. A clinical update. Clin Sports Med. 19:273–286.

Clapp JF. 2006. Influence of endurance exercise and diet on human placental development and fetal growth. Placenta. 27:527–534.

Corva PM, Horvat S, Medrano JF. 2001. Quantitative trait loci affecting growth in high growth (hg) mice. Mamm Genome. 12:284–290.

Eisenhofer G, Tian H, Holmes C, Matsunaga J, Roffler-Tarlov S, Hearing VJ. 2003. Tyrosinase: a developmentally specific major determinant of peripheral dopamine. FASEB J. 17:1248–1255.

Fappa E, Yannakoulia M, Pitsavos C, Skoumas I, Valourdou S, Stefanadis C. 2008. Lifestyle intervention in the management of metabolic syndrome: could we improve adherence issues. Nutrition. 24:286–291.

Fogelholm M, Kukkonen-Harjula K. 2000. Does physical activity prevent weight gain; a systematic review. Obes Rev. 1:95–111.

Garland T Jr, Morgan MT, Swallow JG, Rhodes JS, Girard I, Belter JG, Carter PA. 2002. Evolution of a small-muscle polymorphism in lines of house mice selected for high activity levels. Evolution. 56:1267–1275.

Gomes FR, Rezende EL, Malisch JL, Lee SK, Rivas DA, Kelly SA, Lytle C, Yaspelkis BB III, Garland T Jr. 2009. Glycogen storage and muscle glucose transporters (GLUT-4) of mice selectively bred for high voluntary wheel running. J Exp Biol. 212:238–248.

Hamman RF, Wing RR, Edelstein SL, Lachin JM, Bray GA, Delahanty L, Hoskin M, Kriska AM, Mayer-Davis EJ, Pi-Sunyer X, et al. 2006. Effect of weight loss with lifestyle intervention on risk of diabetes. Diabetes Care. 29:2102–2107.

Hannon RM, Kelly SA, Middleton KM, Kolb EM, Pomp D, Garland T Jr. 2008. Phenotypic effects of the "mini-muscle" allele in a large HR x C57BL/6J mouse backcross. J Hered. 99:349–354.

Hartmann J, Garland T Jr, Hannon RM, Kelly SA, Munoz G, Pomp D. 2008. Fine mapping of "mini-muscle," a recessive mutation causing reduced hindlimb muscle mass in mice. J Hered. 99:679–687.

Henderson ND, Turri MG, DeFries JC, Flint J. 2004. QTL analysis of multiple behavioral measures of anxiety in mice. Behav Genet. 34:267–293.

Horvat S, Medrano JF. 1995. Interval mapping of high growth (hg), a major locus that increases weight gain in mice. Genetics. 139:1737–1748.

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

Ishimori N, Stylianou IM, Korstanje R, Marion MA, Li R, Donahue LR, Rosen CJ, Beamer WG, Paigen B, Churchill GA. 2008. Quantitative trait loci for BMD in an SM/J by NZB/BlNJ intercross population and identification of Trps1 as a probable candidate gene. J Bone Miner Res. 23:1529–1537.

Joosen AMCP, Gielen M, Vlietinck R, Westerterp KR. 2005. Genetic analysis of physical activity in twins. Am J Clin Nutr. 82:1253–1259.

Kelly SA, Czech PP, Wight JT, Blank KM, Garland T Jr. 2006. Experimental evolution and phenotypic plasticity of hindlimb bones in high-activity house mice. J Morphol. 267:360–374.

Leamy LJ, Pomp D, Lightfoot JT. 2008. An epistatic genetic basis for physical activity traits in mice. J Hered. 99:639–646.

Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, Boe AF, Boguski MS, Brockway KS, et al. 2007. Genome-wide atlas of gene expression in the adult mouse brain. Nature. 445:168–176.

Lightfoot JT, Turner MJ, Daves M, Vordermark A, Kleeberger SR. 2004. Genetic influence on daily wheel running activity level. Physiol Genomics. 19:270–276.

Lightfoot JT, Turner MJ, Pomp D, Kleeberger SR, Leamy LJ. 2008. Quantitative trait loci for physical activity traits in mice. Physiol Genomics. 32:401–408.

Mackay TF. 2001. Quantitative trait loci in Drosophila. Nat Rev Genet. 2:11–20.

Manichaikul A, Dupuis J, Sen S, Broman KW. 2006. Poor performance of bootstrap confidence intervals for the location of a quantitative trait locus. Genetics. 174:481–489.

Manichaikul A, Moon JY, Sen S, Yandell BS, Broman KW. 2008. A model selection approach for the identification of quantitative trait loci in experimental crosses, allowing epistasis. Genetics. 181:1077–1086.

Maron BJ. 1986. Structural features of the athlete heart as defined by echocardiography. J Am Coll Cardiol. 7:190–203.

Mogil JS, Richards SP, O'Toole LA, Helms ML, Mitchell SR, Kest B, Belknap JK. 1997. Identification of a sex-specific quantitative trait locus mediating nonopioid stress-induced analgesia in female mice. J Neurosci. 17:7995–8002.

Nehrenberg DL, Hua K, Estrada-Smith D, Garland T Jr, Pomp D. 2009. Forthcoming. 2009. Voluntary exercise and its effects on body composition depend on genetic selection history. Obesity (Silver Spring). 17:1402–1409.

Ponder C, Huded C, Munoz M, Gulden F, Gilliam T, Palmer A. 2008. Rapid selection response for contextual fear conditioning in a cross between C57BL/6J and A/J: behavioral, QTL and gene expression analysis. Behav Genet. 38:277–291.

Rance KA, Hambly C, Dalgleish G, Fustin JM, Bunger L, Speakman JR. 2007. Quantitative trait Loci for regional adiposity in mouse lines divergently selected for food intake. Obesity (Silver Spring). 15:2994–3004.

Rhodes JS, Gammie SC, Garland T Jr. 2005. Neurobiology of mice selected for voluntary wheel-running activity. Integr Comp Biol. 45:438–455.

Rios M, Habecker B, Sasaoka T, Eisenhofer G, Tian H, Landis S, Chikaraishi D, Roffler-Tarlov S. 1999. Catecholamine synthesis is mediated by tyrosinase in the absence of tyrosine hydroxylase. J Neurosci. 19:3519–3526.

Sanchez-Ferrer A, Rodriguez-Lopez JN, Garcia-Canovas F, Garcia-Carmona F. 1995. Tyrosinase: a comprehensive review of its mechanism. Biochim Biophys Acta. 1247:1–11.

Sen S, Churchill GA. 2001. A statistical framework for quantitative trait mapping. Genetics. 159:371–387.

Simonen RL, Rankinen T, Perusse L, Rice T, Rao DC, Chagnon Y, Bouchard C. 2003. Genome-wide linkage scan for physical activity levels in the Quebec Family study. Med Sci Sports Exerc. 35:1355–1359.

Slentz CA, Aiken LB, Houmard JA, Bales CW, Johnson JL, Tanner CJ, Duscha BD, Kraus WE. 2005. Inactivity, exercise, and visceral fat. STRRIDE: a randomized, controlled study of exercise intensity and amount. J Appl Physiol. 99:1613–1618.

Swallow JG, Carter PA, Garland T Jr. 1998. Artificial selection for increased wheel-running behavior in house mice. Behav Genet. 28:227–237.

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

Swallow JG, Rhodes JS, Garland T Jr. 2005. Phenotypic and evolutionary plasticity of organ masses in response to voluntary exercise in house mice. Integr Comp Biol. 45:426–437.

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

Tief K, Schmidt A, Beermann F. 1998. New evidence for presence of tyrosinase in substantia nigra, forebrain and midbrain. Brain Res Mol Brain Res. 53:307–310.

Tribl F, Arzberger T, Riederer P, Gerlach M. 2007. Tyrosinase is not detected in human catecholaminergic neurons by immunohistochemistry and Western blot analysis. J Neural Transm Suppl. 72:51–55.

Turri MG, Henderson ND, DeFries JC, Flint J. 2001. Quantitative trait locus mapping in laboratory mice derived from a replicated selection experiment for open-field activity. Genetics. 158:1217–1226.

West-Eberhard MJ. 2005. Developmental plasticity and the origin of species differences. Proc Natl Acad Sci USA. 102(Suppl 1):6543–6549.

Wong LE, Garland T Jr, Rowan S, Hepple RT. 2009. Forthcoming. 2009. Anatomic capillarization is elevated in medial gastrocnemius muscle of mighty mini mice. J Appl Physiol. 106:1660–1667.

Yokoyama T, Silversides DW, Waymire KG, Kwon BS, Takeuchi T, Overbeek PA. 1990. Conserved cysteine to serine mutation in tyrosinase is responsible for the classical albino mutation in laboratory mice. Nucl Acids Res. 18:7293–7298.

Zeng ZB, Kao CH, Basten CJ. 1999. Estimating the genetic architecture of quantitative traits. Genet Res. 74:279–289.

Received March 27, 2009; Revised June 29, 2009; Accepted July 8, 2009

Corresponding Editor: Roger H. Reeves