Phenotypic Variation in Strength Among Eleven Inbred Strains of Rats (44324)

B. J. BIESIADECKI, P. H. BRAND,[1] P. J. METTING, L. G. KOCH, AND S. L. BRITTON
Department of Physiology and Molecular Medicine, Medical College of Ohio, Toledo, Ohio 43614-5804

Abstract. **As a first step toward the long-range goal of identifying the genes that determine strength, we subjected 11 inbred strains of rats to three tests of muscular strength. The tests consisted of measuring (1) the force exerted by the rat as it was pulled by the base of the tail off a grid on the pan of a top-loading electronic balance (scale test); (2) the length of time the rat hung from a 2.5-mm-diameter U-shaped wire (wire-hanging test); and (3) the length of time the rat hung from a vertically oriented grid consisting of 4-mm-diameter rods (grid-hanging test). Six rats of each gender from each strain were tested at 12 weeks of age, once/day for 5 consecutive days. For the two tests that required use of all four limbs (the scale and grid-hanging tests), one strain performed best (DA). In contrast, on the test that required primarily the use of the front limbs (wire-hanging test), the DA was the lowest performing strain and the F344 rats the best. This differential ranking suggests that the tests selected for variance in the morphological distribution of strength among the strains. There was a 1.5- to 5.2-fold divergence observed between the males of the highest and lowest strains on the scale test and grid hanging tests. This large divergence provides the opportunity to search for intermediate phenotypes and quantitative trait loci that contribute to the different performances of the strains on these strength tests.**
[P.S.E.B.M. 1998, Vol 219]

A long-term goal of our laboratory is to define the genetic basis for the variation in physical performance within species of mammals. Strength is a major component of physical performance. Muscular strength may be defined as the maximum force a muscle or muscle group can generate (1). Numerous studies have explored the mechanisms of muscular contraction at the organismal, cellular, and molecular levels. However, a complete understanding of the physiological basis of strength requires knowledge of the genes whose expression creates the mechanisms producing muscular strength. Recent developments in technology have made identification of these genes feasible (2).

Inbred genetic models have proven to be useful substrates for determining the differences between individuals that are a result of the allelic variation in complex traits such as hypertension (3). Development of an inbred strain involves brother-sister mating for at least 20 generations producing strains that are 97.5% homozygous at all genetic loci (2). The development of an inbred strain with the largest yield of genes causative of a given phenotype requires selective breeding for the extremes of the phenotype from a widely outbred parental stock (4). The subsequent selective breeding and inbreeding required to produce contrasting homozygous strains may require in excess of 10 years to complete in the rat. However, once homozygosity has been achieved by inbreeding, cosegregation analysis and genomic scanning provide powerful tools for identification of the allelic variations responsible for the selected differences in the phenotype.

An alternative approach is to use existing strains of rats that have been inbred for the purpose of producing homozygosity, without selection for any trait. Due to the effect of genetic drift on the assortment of genes during the process of inbreeding, wide variations between inbred strains will occur for many traits. Inbred strains exhibiting wide variation for a given trait, analogous to the variation observed between individuals, can serve as models to explore the genetic determinants of this variation. The goal of this work was to determine to what extent variation exists between 11

[1] To whom requests for reprints should be addressed at Department of Physiology and Molecular Medicine, Medical College of Ohio, 3035 Arlington Avenue, Toledo, OH 43614-5804. E-mail: pbrand@mco.edu
This work was supported by a grant from the Medical College of Ohio Foundation.

Received April 13, 1998. [P.S.E.B.M. 1998, Vol 219]
Accepted July 2, 1998.

0037-9727/98/2192-0126$10.50/0

inbred strains of rats for three operationally defined tests of strength. Our results demonstrated that significant variation exists between strains in each of these tests. The magnitude of the differences suggests that we have identified strains that can be utilized to explore the genetic basis of variation in strength.

Materials and Methods

Animals. Seven strains of rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and were received at 7–8 weeks of age. Four strains were obtained from the colonies maintained by John Rapp at the Medical College of Ohio (Toledo, OH). The seven strains obtained from Harlan Sprague-Dawley were the ACI/SegHsd, AUG/OlaHsd, BUF/NHsd, COP/Hsd, DA/OlaHsd, F344/NHsd, and PVG/OlaHsd; those obtained from John Rapp were the LEW, WKY, SR/Jr, and MNS. All animals were housed two rats per cage; only rats of the same sex, age, and strain were housed together. Animals were housed on a 12:12 light:dark cycle with the light cycle occurring from 6 AM to 6 PM. Food and water were available *ad libitum.*

Experimental Protocol. At 13 weeks of age, six male and six female rats from each of the 11 inbred strains were subjected to tests that measured (1) the force exerted by the rat as it resisted being pulled by the tail off a horizontal grid attached to the pan of a top-loading electronic balance (scale test); (2) the length of time the rat hung from a U-shaped wire (wire-hanging test); and (3) the length of time the rat hung from a vertically oriented grid (grid-hanging test). All rats were tested daily for 5 consecutive days between 9 AM and noon. Tests occurred in the order of scale test, wire-hanging test, and grid-hanging test. The order in which the animals were tested was random from day to day but was consistent for all three tests on any given day.

Scale Test. The scale test we developed was a modification of that reported by Bertelli and Mira (5). The apparatus consisted of a horizontal set of rubber-coated parallel bars (0.4 cm in diameter, 38.5 cm long, 22 cm wide, and spaced 15 mm apart) that was weighted to obtain a total weight of 3.161 kg and placed on an electronic top-loading balance (model BP 6100; Sartorius, Edgewood, NY). This weight was sufficient to prevent the rats from pulling the parallel bars off the balance pan during the test. To begin the test, the balance was tared to zero. The rat was grasped at the base of the tail and placed on the grid with the long axis of the rat's body perpendicular to the axis of the bars. When all four of the rat's paws were grasping the grid, the rat was pulled backwards by the tail at a 45° angle with a constant and steady motion. The absolute value of the negative weight recorded at the point at which the rat released its grip on the grid was taken as the measure of strength by this test. The scale data were transmitted at 10 Hz to a Personal Computer (Northgate 386-20-1; Northgate Computer Systems, Plymouth, MN) *via* an RS232 output port from the balance using Labtech Notebook (Labtech Notebook 7.2.0; Laboratory Technologies Corp., Wilmington, MA). Data were stored as Microsoft Excel files for off-line analysis. Each rat was tested twice per day with a 5-sec interval between tests.

Wire-Hanging Test. For the wire-hanging test, a modification of the method described by Molinengo and Orsetti was employed (6). This apparatus consisted of a 0.25-cm-diameter U-shaped wire (32 cm long and 11 cm wide) that was suspended 50 cm above a padded box in a manner that allowed the wire to swing freely back and forth. To begin the test, the rat was grasped by the skin on the back of the neck, causing the rat to extend its forelegs. The rat was allowed to grasp the wire at the lowest point with its forepaws, and then released. The time from when the rat was released until it fell to the padded box located 50 cm below the bar was recorded as the measurement of strength by the wire-hanging test. If the rat failed to grasp the wire and fell immediately without attempting to hang, it received a second trial. If the rat failed to grasp the wire the second time, a score of zero was recorded. Less than 1% of the wire-hanging tests resulted in a score of zero. Each rat was tested on the wire-hanging test once per day.

Grid-Hanging Test. This test evaluated how long a rat could maintain itself on a vertical grid using all four limbs. The grid consisted of the same type of rubber-coated parallel bars used in the scale test. The rat was placed on the grid, with the grid in the horizontal position. After a 2-min acclimation period, the grid was rotated to the vertical position over 4 sec. With the grid vertical, the initial orientation of the rat was head upward. The time from when the grid achieved the vertical position until the rat fell into a padded box was recorded as the measure of strength by this test. In preliminary trials, rats hung for 7–11 min. In order to shorten the grid-hanging time, weights were attached to the base of each rat's tail just before performing the grid-hanging test. The amount of weight applied was equal to 30% of body weight in the females and 15% in males, based on the rat's weight on the first day of testing. Weights of this magnitude resulted in hanging times of about 3 min. Such weighting of the rats presumably increased the strength component and decreased the endurance component of the test. Each rat was tested on the grid-hanging test once per day.

Data Analyses. Raw data collected for the scale test (grams of force) and wire-hanging test (min hung before falling) were divided by body weight in kilograms to normalize for differences in body weight between males and females. Similarly, the data from the grid-hanging test were normalized by dividing the time hung on the grid by the sum of the body weight and the weight attached to the tail in kilograms. For each rat, the best performance out of the five daily trials was taken as the measure most closely associated with the genetic component of strength. The reason for estimating the genetic component of strength from the rat's best performance, rather than from the average of all performances, for example, as two origins: 1) The environment can have an infinite negative influence upon performance (i.e., on a given day a detrimental environment can take the strength performance of any rat to zero). For example, factors such as subtle differences in housing or daily handling could cause a genetically superior rat to perform below its maximal ability on a given day. 2) On the other hand, the environment can have only a finite positive influence upon the expression of any trait that is determined primarily by genetic factors. That is, environmental influences cannot cause a rat to perform above its genetically determined upper limit of ability. Thus the rat's best performance comes closest to the genetically determined upper limit of its ability. A measure that approaches the genetically determined limit of performance was most suitable for our goal, which was to determine if there was inter-strain variation in strength between inbred strains that is of great enough magnitude to provide suitable substrates for identifying genes contributing to strength.

The mean value of the best performances was calculated for each strain and used for further analysis. For each test the significance of differences in performance between the lowest performing strain and the other strains was determined with the Dunnett test. Also, for each strain, differences in performance between males and females were evaluated using the Student's *t* test for nonpaired data at the significance level of $P < 0.05$. Data are presented as the mean ± one standard error of the mean.

Results

Scale Test. Figures 1A and 1B show the means of the best performance on the scale test for the females and males respectively in all 11 strains. In the females, the best performing strain was the DA, and the lowest performing strain was the SR/Jr. The performances of these two strains were not statistically different from one another (DA: 2528 ± 205 g of force/kg body weight; SR/Jr: 1690 ± 395 g of force/kg body weight; Dunnett, $P = 0.424$). In the males, the DA also exhibited the highest performance (3087 ± 631 g of force/kg body weight), which was 2.3-fold greater than and significantly different from that of the lowest strain, the WKY (1339 ± 64 g of force/kg body weight; $P = 0.004$).

Table I compares the mean values for the best performance on the scale test for females versus males. For the WKY strain, the maximal force exerted on the scale (per kg body weight) by the females was significantly greater than the force exerted by the males ($P = 0.002$). None of the other strains exhibited significant gender differences in strength as measured by the scale test.

Wire-Hanging Test. Figures 2A and 2B show the best performance of each strain on the wire-hanging test. In the females, the F344 hung onto the wire for the longest time (13.9 ± 2.70 min/kg body weight), whereas the BUF hung on the wire for the shortest time (1.14 ± 0.65 min/kg body weight). This 12.2-fold difference in performance was significant ($P = 0.032$). In the males, the F344 strain was also the highest performing strain. The average wire-hanging time in male F344 rats was 3.97 ± 1.06 min/kg

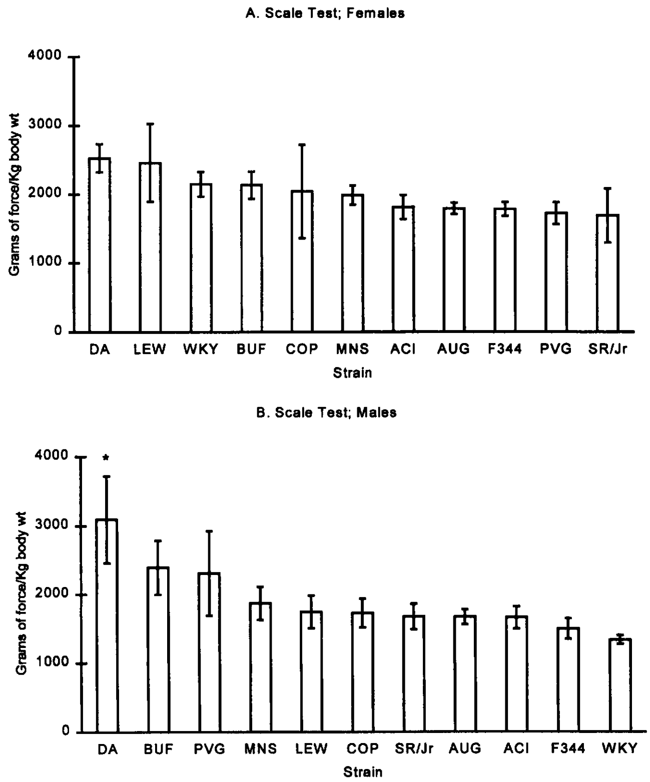

Figure 1. Scale test: The mean value of each animal's best performance for each strain, in g of force/(kg body weight) ± one standard error of the mean for: A) females, B) males. Asterisks denote strains that were statistically different from the lowest strain by Dunnett analysis ($P < 0.05$). There was no difference in strength in females across the strains as assessed by the scale test, but in the males, the DA rats performed significantly greater than the WKY.

body weight, which was over five times longer than the DA males who exhibited the lowest performance (0.73 ± 0.14 min/kg body weight; $P = 0.006$).

Table I compares the mean values of the best performances on the wire-hanging test for females versus males. The performance of the females in the F344, SR/Jr, and WKY strains was significantly greater than that of the males ($P < 0.05$).

Grid-Hanging Test. Figures 3A and 3B show the mean of the best performance on the grid-hanging test. Among the females, the WKY strain hung on the grid the longest (34.7 ± 8.90 min/kg body weight + tail weight), whereas the COP strain hung for the shortest time (6.32 ± 1.46 min/kg body weight + tail weight), a significant 5.5-fold difference ($P = 0.028$). In the males, the DA exhibited the highest performance hanging from the grid for 22.4 ± 5.01 min/(kg body weight + tail weight), whereas the SR/Jr exhibited the lowest performance hanging for 4.28 ± 0.64 min/(kg body weight + tail weight), a significant 5.2-fold difference in strength ($P < 0.0001$).

Table I compares the mean values of the best performances on the grid-hanging test for females versus males. A significant difference between the males and females was observed in the BUF, LEW, MNS, SR/JR, and WKY strains

Table I. Comparison of Females and Males for All Eleven Strains in Three Tests of Strength

Strain	Scale test force/kg body wt.		Wire-hanging test min/kg body wt.		Grid-hanging test min/(kg body wt. + tail wt.)	
	Female	Male	Female	Male	Female	Male
ACI	1811 ± 177	1662 ± 163	7.02 ± 4.98	3.23 ± 1.27	14.4 ± 3.06	10.5 ± 2.80
AUG	1790 ± 84.0	1670 ± 110	11.6 ± 4.69	1.72 ± 0.402	31.1 ± 14.8	13.4 ± 2.04
BUF	2133 ± 200	2389 ± 397	1.14 ± 0.647	1.21 ± 0.537	7.30 ± 0.894*	4.69 ± 0.55
COP	2042 ± 682	1726 ± 208	3.86 ± 1.17	1.64 ± 0.707	6.32 ± 1.46	5.65 ± 1.93
DA	2528 ± 205	3087 ± 631	4.68 ± 3.14	0.730 ± 0.138	16.4 ± 1.57	22.4 ± 5.01
F344	1782 ± 278	1498 ± 148	13.9 ± 2.70*	3.97 ± 1.06	11.7 ± 2.59	6.65 ± 0.948
LEW	2458 ± 569	1742 ± 237	5.93 ± 2.62	0.828 ± 0.156	17.9 ± 4.32*	4.61 ± 0.511
MNS	1985 ± 140	1867 ± 240	4.55 ± 1.54	1.04 ± 0.367	11.3 ± 1.16*	7.03 ± 1.05
PVG	1721 ± 161	2306 ± 616	9.06 ± 4.27	1.55 ± 0.479	27.4 ± 11.0	14.4 ± 6.45
SR/Jr	1690 ± 395	1674 ± 184	4.90 ± 0.515*	0.792 ± 0.302	10.9 ± 1.96*	4.28 ± 0.640
WKY	2146 ± 181*	1339 ± 64.5	9.20 ± 1.76*	2.46 ± 0.526	34.7 ± 8.90*	7.82 ± 1.72

Note. The mean of each animal's best trial ± one standard error of the mean (SEM) is shown for the scale test in grams of force/kg body weight, the wire-hanging test in min/kg body weight, and the grid-hanging test in min/(kg body weight + tail weight). Asterisks indicate a significant difference in performance between females and males (Student's *t* test, $P < 0.05$).

($P < 0.05$). In each case, the females hung on the grid longer than the males.

Discussion

The purpose of this study was to measure the variations in strength that occur in various inbred strains of conscious, intact rats. Strength was evaluated by measuring the force each rat exerted as it was pulled off an electronic balance, and by measuring the time each rat could hang from a wire and from a grid. The differences in performance on these tests could be due to differences in such intermediate phenotypes as cross-sectional area of skeletal muscle, neural supply, anatomical differences, vascularity, or variations in muscle fiber type. We chose to use tests involving strength in conscious, intact rats because our ultimate goal was to determine the genetic basis of variation in strength occurring under normal conditions. Performance on "strength" tests in intact animals may be influenced by factors other than muscular strength, such as coordination, motivation, reaction to aversive stimuli, or pain tolerance. However, there are data supporting the hypothesis that increased strength expressed in the intact animal is related to increased strength of isolated muscles as assessed by direct tests. For example, in response to resistance training, there are increases in both the weight an intact rat can lift and the maximum tetanic tension developed by the same rat's muscle in an isolated muscle preparation (7, 8). These results support our use of the intact animal to quantify strength in order to search for differences in strength between inbred strains. Our data demonstrated that there is a 1.5- to 5.2-fold divergence observed in strength between the DA and F344 for both genders in the scale test and the grid-hanging test. This degree of divergence provides a model that can be used as a starting point to begin the evaluation of the genes causative of differences in strength in the intact animal.

Simple additive models of heredity plus environment have been used to estimate the genetic contribution to variance in human strength. Studies of monozygotic and dizygotic twins have suggested that some determinant of the variation in muscular strength is genetically based. Using these methods, Komi estimated that 97.8% of the variation in muscular strength is genetically determined in human males (9). More recent studies estimate that the genetic component of strength accounts for about 40%–50% of the total phenotypic variation in strength (10). In rats that were selectively bred for muscle fiber composition, it was suggested that 29% of the variation observed in fast twitch muscle fiber composition (11) and 17% of the variation observed in slow twitch muscle fiber composition is determined genetically (12). These studies provide evidence that genetic variance accounts for a substantial portion of the variation in strength between individuals.

In several strains, we observed differences in performance on our tests between males and females. In humans, males perform better than females on strength tests, even if strength data are normalized by body weight or if human males and females of similar body size and composition are studied (1, 13). In our study, because the females' body weights were about 33% less than the males', we normalized the data per kg of body weight (including added tail weights for the grid-hanging test). In every strain in which there was a significant difference between males and females, the females performed at a higher level than the males. The females did better than the males on the grid- and wire-hanging tests even when data were not normalized for body weight differences. When the data were not normalized for body weight, males performed better only on the scale test. Furthermore, normalization by body weight did not change the strain ranking and divergence between the high- and low-performing strains for each strength test. We are unaware of other studies comparing strength in female and male rats. Wolf *et al.* (14) demonstrated that female mice had greater grip strength than males as measured with an automated grip strength meter.

Our intention was to devise tests of muscular strength in the intact animal, although, as noted above, factors other than strength may influence the results of tests with conscious animals. If generalized muscular strength were the major factor determining the response of the rats on all of the tests, the same strains should have exhibited the highest and lowest performance on all three tests. This was not the case. On the scale and grid-hanging tests, the DA males hung 2.3- to 5.2-times longer than males of the lowest strains, SR/Jr and WKY. On the wire-hanging test, the ranking was reversed, with F344 males (which were not statistically different from the lowest strains on the other tests) performing best (Figures 1B, 2B, and 3B). Whereas the grid-hanging and scale tests require effort by all four limbs, the wire-hanging test requires effort primarily by the front limbs. These results suggest that strength of specific muscle groups is distributed differently among the 11 strains of rat. To further exploit these strain differences as models for studying the genetic basis of strength, in future studies intermediate phenotypes related to muscular strength should be examined for differences between the highest and lowest performing strains.

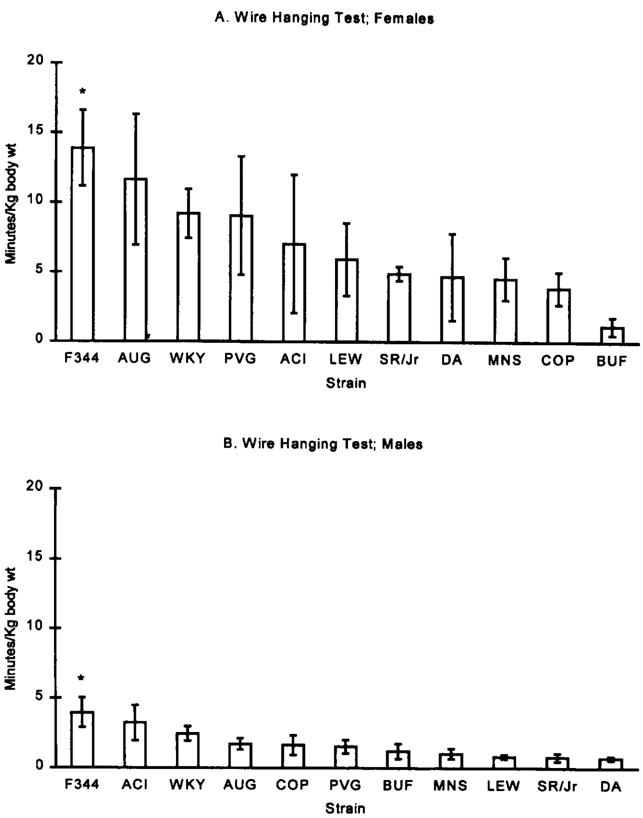

Figure 2. Wire-hanging test: The mean value of each animal's best trial for each strain, in min/(kg body weight) ± one standard error of the mean for: A) females, B) males. Asterisks denote strains that were statistically different from the lowest strain by Dunnett analysis ($P < 0.05$). In both the females and the males the performance of the F344 rats on the wire-hanging test was significantly greater than that of the BUF and DA, respectively.

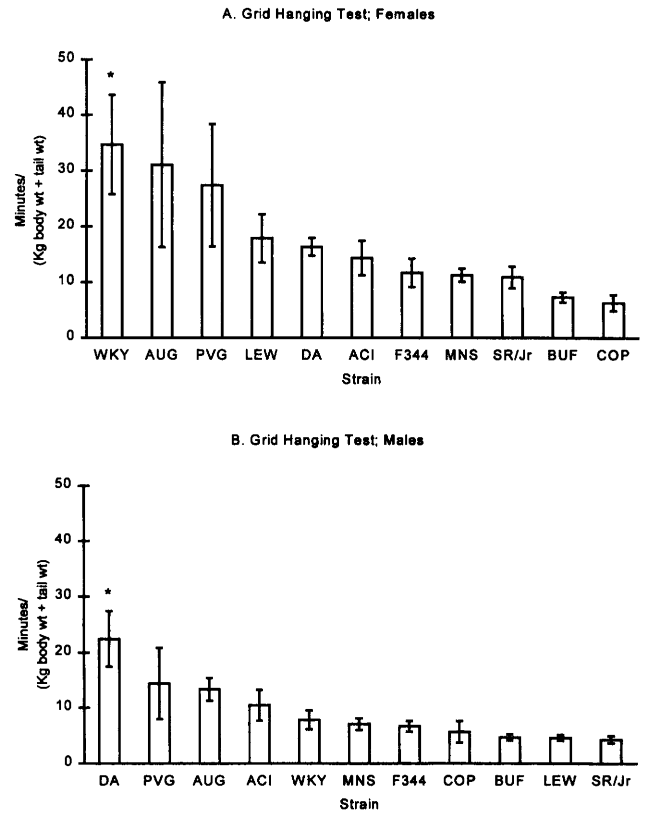

Figure 3. Grid-hanging test: The mean value of each animal's best trial for each strain, in min/(kg body weight + tail weight) ± one standard error of the mean for: A) females, B) males. Asterisks denote strains that were statistically different from the lowest strain by Dunnett analysis ($P < 0.05$). In the females the WKY rats performed significantly greater than the COP rats on the grid-hanging test, whereas in the males, the DA rats performed significantly greater than the SR/Jr rats.

The identification of genes responsible for a given phenotype by comparison of inbred strains that differ widely in expression of that phenotype, is based upon two related and apparently immutable facets of biology: 1) genes are causative of traits and not vice versa; and 2) in a segregating F_2 population, genes causative of a given trait (such as strength performance) will remain associated with that trait, and other genes will segregate randomly relative to the trait. It is cosegregation of trait and gene that underlies the ability to determine genetic causation. The path and criteria to identify the genetic basis of a complex trait in inbred rat models has been developed and is described more fully by John Rapp (3).

The application of this method with inbred strains such as DA and F344, which differ widely in performance on two tests of strength, allows a beginning to be made along the path that will ultimately lead to determining the genes responsible for expression of muscular strength in the intact animal.

The authors acknowledge the excellent secretarial assistance of Judy Suleski.

1. Costill DL, Wilmore J. Physiology of Exercise and Sport. Champaign IL: Human Kinetics Publishing, 1994.
2. Falconer DS, Mackay TFC. Introduction to Quantitative Genetics. Essex, England: Addison Wesley Longman, Ltd., 4th ed., 1996.
3. Rapp JP. The search for the genetic basis of blood pressure variation in rats. In: Laragh JH, Brenner BM, Eds. Hypertension: Pathophysiology, Diagnosis, and Management. New York: Raven Press, Ltd., Chap. 76, pp1289–1300, 1995.
4. Nicholas FW. Veterinary Genetics. New York: Oxford University Press, 1987.
5. Bertelli J, Mira J. The grasping test: A simple behavioral method for objective quantitative assessment of peripheral nerve regeneration in the rat. J Neurosci Methods **58:**151–155, 1995.
6. Molinengo L, Orsetti M. Drug action on the ''Grasping'' reflex and on swimming endurance: An attempt to characterize experimentally antidepressant drugs. Neuropharmacology **15:**256–260, 1976.
7. Gardiner PF, Hibl B, Simpson DR, Roy R, Edgerton VR. Effects of a mild weight-lifting program on the progress of glucocorticoid-induced atrophy in rat hindlimb muscles. Pflugers Arch **385:**147–153, 1980.
8. Exner GH, Staudte HW, Pette D. Isometric training of rats: Effects upon fast and slow muscle and modification by an anabolic hormone (Nandrolone Decanoate). Pflugers Arch **35:**1–14, 1973.
9. Komi P, Karlsson J. Physical performance, skeletal muscle enzyme activities, and fiber types in monozygous and dizygous twins of both sexes. Acta Physiol Scand **S462:**1–43, 1979.
10. Bouchard C, Lortie S, Boulay M, Marcotte M, Thibault M. Genetic effects in human skeletal muscle fiber type distribution and enzyme activities. Can J Physiol Pharmacol **64:**1245–1251, 1985.
11. Suwa M, Nakamura T, Katsuta S. Heredity of muscle fiber composition and correlated response of the synergistic muscle in rats. Am J Physiol **271:**R432–R436, 1996.
12. Nakamura T, Masui S, Wada M, Katoh H, Mikami H, Katsuta S. Heredity of muscle fiber composition estimated from a selection experiment in rats. Eur J Appl Physiol **66**(1):85–89, 1993.
13. McArdle WD, Katch FI, Katch VL. Exercise Physiology: Energy, Nutrition, and Human Performance. Baltimore, MD: Williams and Wilkins, 4th ed., pp421–426, 1996.
14. Wolf E, Wanke R, Schenck E, Hermanns W, Brem G. Effects of growth hormone overproduction on grip strength of transgenic mice. Eur J Endocrinol **133:**735–740, 1995.