Artificial selection for intrinsic aerobic endurance running capacity in rats

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Koch, Lauren Gerard, and Steven L. Britton. Artificial selection for intrinsic aerobic endurance running capacity in rats. Physiol Genomics 5: 45–52, 2001.—Artificial selection for intrinsic aerobic endurance running capacity was started using genetically heterogeneous N:NIH stock of rats as a founder population (n = 168). Selection for low and high capacity was based upon distance run to exhaustion on a motorized treadmill using a velocity-ramped running protocol. The starting velocity was 10 m/min and was increased by 1 m/min every 2 min (slope was constant at 15°). At each generation, within-family selection was practiced using 13 families for both the low and high lines. A rotational breeding paradigm maintained the coefficient of inbreeding at less than 1% per generation. On average the founder population ran to exhaustion in 355 ± 11 m. Six generations of selection produced lines that differed in running capacity by 171%, with most of the change occurring in the high line. At generation 6 the low line ran 310 ± 8 m and the high line 839 ± 21 m at exhaustion. Selection for running capacity produced changes in body weight as a correlated trait. By generation 6, the low-line females were 20% heavier than the high-line females, and the low-line males were 16% heavier than the high-line males.

The capacity of an organism to utilize oxygen in an aerobic endurance event is a trait that encompasses a vast array of biochemical and physiological traits (23, 30). Traits of this complexity are termed multifactorial to emphasize their determination by multiple genetic and environmental factors (13). Twin studies suggest a substantial genetic component to aerobic endurance capacity and two types of genetic substrates seem to be evident. First, there is a complement of genes that determine intrinsic exercise capacity in the untrained state (4, 24). On top of intrinsic capacity is apparently another set of genes that dictate the adaptational response to exercise (3, 5). Thus the trait of aerobic capacity can be considered the result of expression of both intrinsic and adaptational genes as they interact with the environment.

Given such complexity, animal models in which both genetic and environmental variation approach mimics can be of substantial value for determining the genes causative of variation in physical capacity (9, 32). In theory, divergent artificial selection for a complex trait produces somewhat ideal genetic models, because contrasting allelic variation is concentrated at the extremes from one generation to the next. Selection is possible if sufficient additive genetic variance exists in a population for that trait (13). Based on Fisher’s 1930 Theorem of Natural Selection (14), traits peripherally associated with evolutionary fitness, such as morphology and complex physiology, demonstrate more additive genetic variance because of less pressure from natural selection (29). Operation of Fisher’s Theorem is presumably demonstrated by the success of artificial selection in producing several widely studied rat models of complex traits such as spontaneously hypertensive rats (SHR) (33), Dahl salt-sensitive rats (25), and alcohol-sensitive rats (10).

Because of the importance of aerobic capacity in both health and disease (6), we developed a plan to create rat genetic models of intrinsic aerobic endurance treadmill running capacity in rats. As a first approach in a small-scale study (26), we devised a treadmill running protocol that yielded a significant response to selection for low and high capacity in Sprague-Dawley rats. Three generations of divergent selective breeding for running capacity produced low and high lines that differed by 70%. Because we had apparently defined a complex aerobic phenotype with substantial response to selection, we undertook a large-scale selective breeding program to develop low and high lines that would diverge widely for intrinsic aerobic capacity. We report here progress across six generations of artificial selective breeding for intrinsic (i.e., untrained) treadmill endurance running capacity.1

METHODS

Founder population. The starting population was 96 male and 96 female genetically heterogeneous rats (N:NIH stock) obtained from a colony maintained at the National Institutes of Health (17). Each rat in the founder population was of different parentage, so selection was not among brothers and sisters, which broadens the genetic variance (18). The rats were 4–5 wk old upon arrival at our institution and were housed two per cage. Each rat was provided food (Ralston

1 We propose the following nomenclature for these outbred stocks: 1) LCR for the “low-capacity runners” and 2) HCR for the “high-capacity runners.”
Purina, diet 5001) and water ad libitum and placed on a 12:12-h light-dark cycle with the light cycle coinciding with daytime. All procedures were carried out with approval by our Institutional Animal Care and Use Committee and were conducted in accordance with the “Guiding Principles in the Care and Use of Animals” as approved by the Council of the American Physiological Society. The protocol for estimation of aerobic running capacity required 2 wk and was started when the rats were 10 wk old (26).

Estimation of endurance running capacity. The first week consisted of introducing each rat to the treadmill (model Exer-4; Columbus Instruments, Columbus, OH) for gradually increasing duration each day. The goal of the first week was to expose each rat to sufficient treadmill education so that they could run for 5 min at a speed of 10 m/min on a 15° slope. This amount of exposure to treadmill running is likely below that required to produce a significant change in aerobic capacity (1, 11).

The first 2 days of introduction to treadmill running consisted of simply placing the rat on the belt that was moving at a velocity of 10 m/min (15° slope) and picking the rat up and moving it forward if it started to slide off the back of the belt. During introduction days 3–5, the belt speed was gradually increased up to 15 m/min, and failure to run caused the rats to slide off of the moving belt and onto a 15 × 15-cm electric shock grid that delivered 1.2 mA of current at 3 Hz. The rats were left on the grid for about 1.5 s and then moved forward onto the moving belt. This process was repeated until the rats learned to run to avoid the mild shock. The ability to achieve this minimal level of running at least once constitutes the threshold performance necessary for inclusion in evaluation for maximal capacity the following week.

Rats not achieving this minimal running ability were dropped from further testing.

During the second week, each rat was evaluated for maximal endurance running capacity on five consecutive days. Each daily endurance trial was performed at a constant slope of 15° with the starting velocity at 10 m/min. Treadmill velocity was increased by 1 m/min every 2 min and each rat was run until exhausted. Exhaustion was operationally defined as the third time a rat could no longer keep pace with the speed of the treadmill and remained on the shock grid for 2 s rather than run. At the moment of exhaustion, the current to the grid was stopped and the rat was removed from the treadmill and weighed.

Figure 1 displays the nomogram for the relationship between distance run, time, and speed for the ramped running protocol. The general idea was to have a test of treadmill running that mostly estimated aerobic endurance capacity over a reasonable duration. This goal was achieved satisfactorily as demonstrated on Fig. 1 by the placement of the average capacity for rats in the founder population and after six generations of high line selection. The founder population ran to exhaustion by 355 m, which converts into a time of about 22 min and a speed of 20 m/min. The sixth generation of selection in the high line produced a population that was exhausted on average after running 839 m (time = 42 min and speed = 30 m/min).

For each of the five trials, the total distance run (in meters) was used as the estimate of aerobic endurance capacity. The single best daily run of five trials for each rat was considered the trial most closely associated with the heritable component of endurance running capacity. All estimates of capacity reported here are based upon the single best day of running for each rat.

Selective breeding. Using the criterion of single best day, the 13 lowest and 13 highest capacity rats of each sex were selected from the founder population and randomly paired for mating. The resultant offspring from each of the 26 families were weaned 28 days after birth. At 10 wk of age the offspring were introduced to the treadmill and subsequently tested for capacity as described above.

At each subsequent generation, within-family selection from 13 mating pairs was practiced because it decreases the rate of inbreeding to yield retention of genetic variation and thus increases the overall response to selection (13). In practice, one female and one male offspring were selected from each family and became parents for the next generation. The prearranged schedule of matings followed a simple sequence, one female and one male offspring were selected from the founder population and randomly paired for mating. The resultant offspring from each of the 26 families were weaned 28 days after birth. At 10 wk of age the offspring were introduced to the treadmill and subsequently tested for capacity as described above.

Analysis of data. The average response for a given generation is the difference for the trait between the mean of the population from which the parents were selected and the
Table 1. Prearranged schedule of matings: a simple sequence based on assigned family number

<table>
<thead>
<tr>
<th>Family Number</th>
<th>Founder F×M = family</th>
<th>Generation 1 F×M = family</th>
<th>Generation 2 F×M = family</th>
<th>Generation 3...n F×M = family</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1×1 = 1</td>
<td>1×2 = 1</td>
<td>1×3 = 1</td>
<td>1×4 = 1</td>
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<tr>
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<td>2×2 = 2</td>
<td>2×3 = 2</td>
<td>2×4 = 2</td>
<td>2×5 = 2</td>
</tr>
<tr>
<td>3</td>
<td>3×3 = 9</td>
<td>3×4 = 3</td>
<td>3×5 = 3</td>
<td>3×6 = 3</td>
</tr>
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<td>Continue</td>
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<td>Continue</td>
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<tr>
<td>13</td>
<td>13×13 = 13</td>
<td>13×1 = 13</td>
<td>13×2 = 13</td>
<td>13×3 = 13</td>
</tr>
</tbody>
</table>

At each subsequent generation, within-family selection from 13 mating pairs was practiced because it decreases the rate of inbreeding to yield retention of genetic variation and thus increases the overall response to selection (15). In practice, one female and one male offspring were selected from each family and became parents for the next generation. The prearranged schedule of matings followed a simple sequence based on assigned family number (1 to 13, F = female, M = male). When the rotation has completed one entire cycle (i.e., generation 13), the 1×1, 2×2, etc., matings will be skipped to avoid sibling matings (12).

RESULTS

Figure 2 shows the one best day performance for each rat of the founder population ranked from the lowest to highest capacity by sex. Eighty-eight of 96 females and 80 of 96 males passed the threshold test (total = 168 rats) required for inclusion in the population to be tested for running capacity. On average, the entire founder population ran to exhaustion in 355 ± 11 m with the females running significantly further than the males. The founder females ran an average for 380 ± 15 m, and the males ran on average for 327 ± 16 m. The 13 lowest founder females averaged 205 ± 7 m and the lowest 13 males averaged 167 ± 7 m run to exhaustion, and these were randomly paired and mated to produce 13 families for the first generation of the low line (Table 2). The 13 highest founder females averaged 633 ± 26 m and the 13 highest males averaged 541 ± 21 m run to exhaustion, and these were randomly paired and mated to produce 13 families for the first generation of the high line (Table 3). This represents a 3.1-fold difference for the females (633/205) and a 3.2-fold difference for the males (541/167) between the low and high parents selected from the founder population. Tables 2 and 3 provide average data for the low and high lines for distance, time, and speed at exhaustion for both sexes for the founder population parents and at each of the six generations.

Figure 3 depicts the emerging differences in aerobic running capacity across the first six generations of selection for the females in the form of histograms. It is obvious from inspection that the high line responded more strongly to selection and has greater variation relative to the low line. The female founder population recorded an initial capacity of 380 ± 15 m, and the high-line females improved to 912 ± 32 m on average by generation 6 (140% increase). The low-line females
decreased their average running capacity to 321 ± 13 m by generation 6 (−18%). Figure 4 shows that a similar pattern of response to selection occurred for the males. The founder male population ran on average for 327 ± 16 m to exhaustion, and the high-line males increased running capacity to 756 ± 26 m (131%). The low-line males decreased running capacity to 295 ± 8 m (−11%) as a consequence of six generations of selection.

Figure 5 shows the mean response to selection for females and males combined at each generation for the low and high-capacity lines. At generation 6 the low line averaged 310 ± 8 m and the high line averaged 839 ± 21 m, representing an overall divergent response to selection of 171% (529 m). On average, regression estimates unadjusted for body weight showed that the low line decreased running capacity by 13.4 m/generation (\(y = -13.4x + 377, r = 0.187, P < 0.001\))

and the high line increased running capacity by 56.7 m/generation (\(y = 56.7x + 479, r = 0.360, P < 0.001\)).

Selection for running capacity also produced differences in body weight as a correlated trait in both sexes. In general, the low line became increasingly heavier and the high line increasingly lighter at each generation (Fig. 6). By generation 6 the low-line females weighed 204 ± 4 g and the high-line females weighed 170 ± 2 g (20% difference). By generation 6 the low-line males weighed 291 ± 4 g and the high-line males weighed 251 ± 3 g (16% difference). Because of the changes in body weight, the responses to selection shown in Fig. 5 were also fitted to a linear model using both generation and body weight as predictors of distance run to exhaustion. The adjusted model estimated that body weight accounted for a statistically significant part of the variation in distance run to exhaustion for both the low and high lines. Body weight accounted

Table 3. Summary of high-line females and males for each generation

<table>
<thead>
<tr>
<th>Distance to Exhaustion, m</th>
<th>Time to Exhaustion, min</th>
<th>Speed at Exhaustion, m/min</th>
<th>Body Wt. g</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Founder Parents</td>
<td>633 ± 25.7</td>
<td>34.7 ± 0.93</td>
<td>26.7 ± 0.49</td>
<td>189 ± 4.6</td>
</tr>
<tr>
<td>G1</td>
<td>565 ± 29.5</td>
<td>31.5 ± 1.14</td>
<td>25.3 ± 0.57</td>
<td>181 ± 2.7</td>
</tr>
<tr>
<td>G2</td>
<td>652 ± 35.5</td>
<td>34.7 ± 1.26</td>
<td>26.9 ± 0.63</td>
<td>175 ± 2.3</td>
</tr>
<tr>
<td>G3</td>
<td>722 ± 27.0</td>
<td>37.3 ± 0.97</td>
<td>28.2 ± 0.49</td>
<td>175 ± 2.0</td>
</tr>
<tr>
<td>G4</td>
<td>784 ± 33.0</td>
<td>39.5 ± 1.11</td>
<td>29.2 ± 0.55</td>
<td>176 ± 1.9</td>
</tr>
<tr>
<td>G5</td>
<td>813 ± 34.0</td>
<td>40.4 ± 1.13</td>
<td>29.7 ± 0.57</td>
<td>172 ± 1.8</td>
</tr>
<tr>
<td>G6</td>
<td>912 ± 31.8</td>
<td>43.7 ± 1.00</td>
<td>31.4 ± 0.50</td>
<td>170 ± 1.6</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Founder Parents</td>
<td>541 ± 20.9</td>
<td>31.1 ± 0.81</td>
<td>25.1 ± 0.42</td>
<td>275 ± 10.9</td>
</tr>
<tr>
<td>G1</td>
<td>513 ± 33.6</td>
<td>29.0 ± 1.41</td>
<td>24.0 ± 0.70</td>
<td>265 ± 3.7</td>
</tr>
<tr>
<td>G2</td>
<td>495 ± 23.5</td>
<td>28.7 ± 0.96</td>
<td>23.8 ± 0.48</td>
<td>270 ± 3.4</td>
</tr>
<tr>
<td>G3</td>
<td>630 ± 25.3</td>
<td>34.1 ± 0.91</td>
<td>26.5 ± 0.46</td>
<td>258 ± 3.5</td>
</tr>
<tr>
<td>G4</td>
<td>611 ± 24.2</td>
<td>33.5 ± 0.88</td>
<td>26.3 ± 0.44</td>
<td>261 ± 3.2</td>
</tr>
<tr>
<td>G5</td>
<td>662 ± 24.2</td>
<td>35.3 ± 0.88</td>
<td>27.1 ± 0.44</td>
<td>253 ± 3.0</td>
</tr>
<tr>
<td>G6</td>
<td>756 ± 26.4</td>
<td>39.2 ± 0.86</td>
<td>28.6 ± 0.54</td>
<td>251 ± 3.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of rats.

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for a decrease of 0.54 m/g body wt for the low line \( (y = -12.2x_1 - 0.54x_2 + 500, r = 0.311, P < 0.001) \) and a decrease of 1.8 m/g body wt for the high line \( (y = 52.0x_1 - 1.8x_2 + 891, r = 0.490, P < 0.001) \).

**DISCUSSION**

Artificial divergent selection can yield somewhat ideal genetic models, because contrasting alleles ultimately become fixed at loci controlling the selected genotypes. Our long-term plan is to continue selection until the responses plateau and then create inbred strains of both low and high aerobic endurance capacity rats that can be made available for study. Aerobic endurance capacity, like other complex physiological traits such as height, blood pressure, coordination, and strength, is an example of a quantitative trait that demonstrates continuous variation. The inherent complexity of quantitative traits amplifies the benefits of animal genetic models in which genetic and environmental variations approach minimum values. An inbred strain is defined as one that has been exclusively sister-brother mated for at least 20 generations (31). Each generation of inbreeding increases the probability of attaining homozygosity at any given genetic locus, and by 20 generations ~97.5% of the loci are homozygous. Thus the major value of inbred strains emanates from their close genetic uniformity that facilitates genotyping, phenotyping, and the opportunity for multiple investigators to evaluate the same genetic substrate repeatedly; this type of uniformity cannot be approached in human studies.

The availability of N:NIH rats from the Animal Resource Center of the National Institutes of Health for use as a founder population was of major benefit. This stock of rats originated from the intentional crossbreeding of eight inbred strains (ACI, BN, BUF, F344, M520, MR, WKY, and WN) by Hansen and Spuhler in 1979 (17). The crossbreeding produced a population that has relatively wide genetic heterogeneity and is thus somewhat ideal as a starting population for arti-
ficial selection. To maintain genetic heterogeneity across the generations, we used within-family selection and rotational breeding for the 13 families in each line. With equal representation of one male and one female offspring from each family, the rate of inbreeding per generation (DF) can be estimated as: 

\[ DF = \frac{1}{4N} \]

where 

\[ N = \text{number of individual parents for each line} \] 

(12). Because we had 26 parents/generation for each line, the rate of inbreeding was \(0.96\%/\text{generation}\).

We used a running protocol with a starting velocity of 10 m/min that was ramped up in velocity 1 m every 2 min. The choice of parameters for this protocol was based upon four factors. First, it has been demonstrated that oxygen consumption increases linearly as a function of running velocity over a wide range in rats. Gleeson and Baldwin (15) measured oxygen consumption in untrained rats that averaged 48 ml kg\(^{-1}\) min\(^{-1}\) when running at 11 m/min and increased linearly to 86 ml kg\(^{-1}\) min\(^{-1}\) at a velocity of 27 m/min (0% grade). Brooks and White (7) reported a significant association \((r = 0.83)\) between oxygen consumption and running velocity (range 5 to 43.1 ml kg\(^{-1}\) min\(^{-1}\)) in untrained rats tested at a slope of 15%. Second, we presumed that selection would produce rats with greater capacity, and we wanted to avoid having a test that became exceedingly long to perform at future generations; the ramped nature of the test precludes this as a possibility. Third, the “average” unselected and untrained rat can complete this protocol in about 20–22 min. This duration assures that aerobic capacity comprises a major component of the test (8, 27). Fourth, employing one grade (15°) throughout the run and only changing velocity simplifies the actual assessment of each rat.

Joyner (23) developed a model of endurance running capacity that is the product of three physiological variables: 1) the maximal rate at which oxygen and nutrient substrates can be utilized to produce energy in the form of ATP \((\dot{V}O_2_{max}, \text{ml O}_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1})\), 2) the per-
cent of $\dot{V}_{O_2\text{max}}$ at the threshold for lactate release ($\%\dot{V}_{O_2\text{max}}$ at LT), and 3) the efficiency of running (REE, km·min$^{-1}$·$\dot{V}_{O_2\text{ max}}^{-1}$). Our overall working hypothesis is that allelic differences for each of these three complex intermediate phenotypes of the Joyner model will largely account for the magnitude of the difference in aerobic running capacity between the low and high selected lines. Knowledge that originates from the seminal work of A. V. Hill (20–22) supports the contention that the ability of the heart to deliver oxygen is the predominant singular factor that limits maximal aerobic capacity (8, 27). From this we predict that artificial selection based upon the ramped protocol will concentrate genes associated with both maximal aerobic power and enhanced cardiac function in the high line.

The distance run (in meters) at exhaustion was used as the estimate of aerobic endurance capacity and thus breeding value. The single best trial of five was used, because it was deemed the best indicator of capacity determined by intrinsic genetic composition (9). This idea of estimating the genetic component from the one best day of performance, rather than the average for all trials, for example, has two origins. First, the environment can have an infinite negative influence upon capacity by reducing the distance run to zero. Factors such as subtle differences in housing or daily handling could cause a genetically superior rat to perform below its normal on a given day. Second, the environment can have only a finite positive influence upon any test of capacity for a given genotype. An extension of this logic is that the estimate of breeding value (distance run) has more error for the low line relative to the high line. Although selecting on the one best day may reduce environmental error, it simultaneously created the possibility that a component of selection may be related to short-term adaptation (2). Indeed, for the five consecutive days of testing, on average, the low-line rats ran best on day 2.9 ± 0.05 and the high-line rats ran best on day 3.67 ± 0.05 ($P < 0.005$). Although this may represent a minor component of aerobic biochemical adaptation for the high line, other factors such as differences in behavioral responses to running may also be involved.

A change in an unselected trait produced by selection for another trait is termed a correlated response. Selection produced differences in body weight as a correlated trait in both sexes (Fig. 6). In general, the low line became increasingly heavier and the high line increasingly lighter at each generation. Across the six generations there were small but significant correlations between body weight and distance run for both sexes in each line (low-line females, $r = -0.306$, $P < 0.001$; low-line males, $r = -0.141$, $P < 0.009$; high-line females, $r = -0.150$, $P < 0.001$; and high-line males, $r = -0.225$, $P > 0.001$). The genetic component of a phenotypic correlation is produced by pleiotropy, which is the property of an allele to simultaneously effect two or more traits. As such, the low and high lines might also serve as contrasting models to determine genes that influence body weight and composition.

It is obvious from inspection that the high line responded more strongly to selection (+484 m) compared with the low line (~45 m) across the six generations. Selection for the low trait character is often of lesser magnitude relative to the high for two primary reasons (13). First, selection can be lower simply because the low trait approaches zero as a limit. Second, it is possible that natural selection has constrained variation in aerobic capacity at the low end because a minimal threshold capacity may be associated with evolutionary fitness. In contrast, more genetic variation may exist for high aerobic capacity because this trait has been under less evolutionary pressure (29). We imposed a threshold test for inclusion in the study that excluded rats either unwilling or unable to run for a minimum of 5 min at 10 m/min (15° grade) by the end of the education week. Our idea was to select the low line for low running capacity, not zero running capacity. Thus we likely imposed some amount of selection for cooperation and willingness to run. Swallow and colleagues (32) have selectively bred mice for low and high willingness to engage in voluntary wheel running; these contrasting lines will be of value to address directly factors related to motivation for aerobic exercise.

Response to artificial selection in the first and subsequent generations originates from four primary sources (13): 1) increased frequency of genes causative of the change in phenotype on which selection is based (this is, of course the usual goal of selection); 2) unpredictable and subtle changes in the environment (the use of divergent selection for low and high directions (i.e., two-way selection) for the trait, as employed here, serves as a control for environmental shifts across generations); 3) sampling errors in estimating the generation means; and 4) random changes in gene frequency (i.e., random genetic drift). Variation from sampling errors and genetic drift are reduced by an increase in the number of animals in the founder population and the number of families maintained at each generation. On the other hand, the contribution from genetic drift can only be estimated by maintaining replicate selected lines. Because the scale of any breeding program is ultimately resource limited, the maintenance of replicate lines operates to decrease fractionally the size of each line to 1/(n + 1) compared with no replicate lines ($n =$ number of replicate lines) (13). Our decision was to reduce sampling error and contribution from genetic drift by employing all resources for development of only one low and one high line. The tradeoff was that we have no direct estimate of the contribution of genetic drift.

Despite a substantial differential response after six generations of selection, we cannot predict how many generations will be required to reach the selection limit because: 1) the outcome is determined by the summed influence of individual genes segregating in the founder population, and these are obviously not known beforehand; and 2) mutations are constantly producing new allelic variation, and the rate and character of such mutations are not easily determined (13, 16, 19).
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REFERENCES


