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Genetic variation and correlations between genotype and locomotor physiology in outbred laboratory house mice (*Mus domesticus*)

Patrick A. Carter [a,*], Theodore Garland, Jr. [a], Michael R. Dohm [b], Jack P. Hayes [c]

[a] *Department of Zoology, 430 Lincoln Drive, University of Wisconsin, Madison, WI 53706-1381, USA*
[b] *Department of Biology, University of Hawaii at Hilo, Hilo, HI 96720-4091, USA*
[c] *Department of Biology, University of Nevada, Reno, NV 89557-0015, USA*

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Abstract

Laboratory strains of house mice (*Mus domesticus*) are increasingly used as model organisms in evolutionary physiology, so information on levels of genetic variation is important. For example, are levels of genetic variation comparable to those found in populations of wild house mice? We studied allozymes to estimate genetic variation in outbred Hsd:ICR mice, which have been used in several studies with evolutionary emphasis. The physiological significance of allozyme variation remains obscure. Several workers have reported relationships between multi-locus heterozygosity and metabolic traits, but endotherms have not been studied. Therefore, we also measured mice for basal metabolic rate (BMR), maximal oxygen consumption during forced treadmill exercise ($\dot{V}O_2$max), and 12 other traits related to locomotor physiology, before genotyping them for 10 allozyme loci. Four of these loci were polymorphic, all were in Hardy–Weinberg equilibrium, and inbreeding coefficients were not significantly different from zero. Average heterozygosities were 11%, similar to values reported for wild populations of house mice. Fourteen percent of the associations between single-locus genotype and physiological traits were statistically significant. Multi-locus heterozygosity was not significantly related to $\dot{V}O_2$max, but was positively correlated with BMR, a result opposite to the negative correlation between standard metabolic rate and heterozygosity reported in many ectotherms. Therefore, the proposed mechanisms for the effect of multi-locus heterozygosity on metabolic rate in ectotherms may not apply to endotherms.

Keywords: Allozymes; Evolution; Genotype; Genetic variation; Heterozygosity; Basal metabolic rate, (VO_2max); Locomotion; House mice, *Mus domesticus*

1. Introduction

Many soluble proteins, including some enzymes, show allelic variation within natural populations. Initial studies revealed much more allelic variation in enzymes (allozymes) than had been expected [22,34]. Since those initial studies of *Drosophila* and humans, allozyme variation has been reported for more than 1000 species [37,41]. This variation has been used to study the genetic structure of, evolutionary relationships among, and evolutionary processes within, both natural and domestic populations of animals (e.g. [2,13,30], and references therein). Estimates of genetic variation within and among laboratory strains of house mice (*Mus domesticus*) are important because laboratory mice are increasingly used as model systems in physiological genetics. For example, strains of laboratory mice have been used to examine constraints on nutrient uptake and metabolic rate [20,21], the evolution of basal metabolic rate (BMR) and organ masses [33], behavioral and physiological responses to cold exposure [35,36], and genetic correlations between physiological traits [10]. A question that arises is whether levels of genetic variation found in non-inbred laboratory strains are comparable to those found in wild populations.

* Corresponding author. Present address: Department of Zoology, Washington State University, Pullman, WA 99164-4236, USA. Tel.: +1-509-3351447; fax: +1-509-3353184.
E-mail address: pacarter@wsu.edu (P. A. Carter)

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Consequently, we compare allozyme variation previously measured in wild house mice (review in Ref. [55]) to allozyme variation in the outbred Hsd:ICR strain of laboratory house mice, which has been used in numerous studies of physiological genetics and evolutionary physiology (e.g. [10,11,14,15,26,43,45,52,53]).

Many allozyme polymorphisms have been analyzed for differences in biochemical function, and significant kinetic variation has been reported for most, but not all, polymorphic enzymatic loci studied (review in Ref. [38]). A second question that we address is whether allozyme variation in Hsd:ICR mice appears to have physiological consequences. Identification of correlations between single-locus or multi-locus genotype and physiological traits is usually the first step in studies of the effect of allozyme variation on physiological function [37]. Single-locus studies have tended to focus on physiological traits related to the function of the enzyme produced by that locus; for example, differences in K_{cat} and K_m among lactate dehydrogenase allozymes affect blood chemistry and swimming performance in the teleost fish *Fundulus heteroclitus* [42]. Multi-locus studies have tended to focus on traits directly or indirectly related to metabolism, such as resting or standard metabolic rate, growth rate, and/or body size. Negative correlations between resting metabolic rate and multi-locus heterozygosity have been observed in clams, mussels, snails, rainbow trout, and tiger salamanders (review in Ref. [38]). In addition, one study on tiger salamanders demonstrated a positive correlation between active oxygen consumption and multi-locus heterozygosity [39].

Few studies of possible relationships between metabolic rate and allozyme variation have used vertebrates, and none of them have examined birds or mammals (endotherms). As a test of generality, we examined the relationships between multi-locus heterozygosity and both BMR and maximal oxygen consumption during forced exercise ($\dot{V}O_2max$) in Hsd:ICR mice. As part of a larger quantitative genetic analysis of locomotor performance and metabolism, 12 additional traits were measured [9,11]: forced maximal sprint running speed, maximal swimming endurance, tail length, body mass, blood hematocrit and hemoglobin concentration, thyroid hormone levels (T3 and T4), serum total cholesterol, and masses of heart, liver, and right triceps surae muscle. We also tested these traits for correlations with multi-locus heterozygosity, and for differences among single-locus allozyme genotypes.

2. Materials and methods

2.1. Mouse husbandry

The outbred Hsd:ICR mice used herein were originally developed from Swiss–Webster albino house mice in the early 1950's [24]. The ICR strains are now maintained by various commercial suppliers, including 10 different closed colonies at Harlan Sprague Dawley facilities throughout the United States [9]; our mice were purchased from the Indianapolis, IN facility (room 202, Barrier A). The ICR strain is random-bred, was genetically heterogeneous in studies conducted 18 and 25 years ago [24,44], and has served as a model system for numerous quantitative genetic analyses (e.g. [5,10,43,48]). Husbandry and breeding design of the mice we studied are described elsewhere [9,10,26]. Briefly, breeder mice ('parents') were purchased from Harlan Sprague Dawley in five separate batches. They were weaned at 19 days of age and immediately shipped to our laboratory (arrival dates were 30 Nov. 1988, 28 Dec. 1988, 22 Feb. 1989, 30 Aug. 1989, 13 Dec. 1989). Each male was later mated with 4 or 5 females; their offspring as well as the parents were studied.

2.2. Phenotypic measures

Forced maximal sprint running speed, maximal swimming endurance, $\dot{V}O_2max$ and BMR were measured as described elsewhere [9,10,26].

Heparinized microcapillary tubes (length: 75 mm, i.d. 1.1–1.2 mm) were used to take blood samples from the sub-orbital sinus. Tubes were centrifuged for 6.5 min in a Clay–Adams microfuge (Autocrit Ultra 3). Hematocrit (Hct) was determined immediately following centrifugation. For measurement of hemoglobin concentration ([Hb]), 25 μl blood samples (drawn from an additional heparinized microcapillary tube) were added to 5 ml of Drabkin's reagent. Concentration of cyanmethemoglobin was determined at 540 nm with a Beckman spectrophotometer ([3], Sigma Technical Bulletin No. 525) and human hemoglobin standard (Sigma Catalog No. 525-18). Hct and [Hb] were determined in duplicate, and means were analyzed.

After blood sampling, mice were sacrificed by cervical dislocation. Following a midventral incision, the heart was lifted with forceps and the ventricles were cut free from the atria and major blood vessels. The ventricles were blotted and any coagulated blood was removed. The gall bladder was excised before removing the liver for weighing; the liver was cut into two pieces to facilitate subsequent analyses. Finally, the right triceps surae (which includes the lateral and medial heads of the gastrocnemius muscle, the soleus, and the plantaris [also known as the flexor digitorum superficialis]) was removed by cutting the muscle from the lateral condyle of the tibia and medial condyle of the fibia, followed by cutting the Achilles' tendon approximately midway between its origin and the muscle's insertion. Wet mass of tissues was recorded to the nearest 0.1 mg on an electronic balance; tissues were then frozen on dry ice, and stored at $-80°C$.

After determination of Hct, the microcapillary tubes were broken and the serum (some coagulation was evident despite the heparin coating of the microcapillary tubes) was blown into microcentrifuge tubes. These samples were frozen on dry ice and later stored at −80°C. Total serum cholesterol was determined using a Sigma Cholesterol Assay (No. 352). This enzymatic method is a modification of Allain et al. [1], involving the hydrolysis of cholesterol esters, the oxidation of cholesterol, and the spectrophotometric measurement of a reaction product at 500 nm. Duplicate 10 μl serum samples from each mouse were assayed at room temperature for total cholesterol in sets of 10 mice. Overall, 17 assays for total cholesterol were performed. Each assay was calibrated using Sigma calibrators (100, 200, and 400 mg dl^{-1}). In addition, duplicate samples of a normal control were also run with the assay. Samples were incubated with the diagnostic reagent for 12–18 min before absorbance readings were taken at 500 nm. All absorbances were recorded within 30 min as specified by the assay procedure. The mean intra-assay coefficient of variation was 4.65% ($\pm$0.55 S.E.).

Analyses of serum concentration of thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4), were performed by J. Armstrong of the Radionucleotide Laboratory, School of Veterinarian Medicine, University of Wisconsin, Madison (as in Ref. [45]). Assays involved Coat-a-Count Total T_3 and Total T_4 kits (Diagnostic Products, Los Angeles, CA) and were run in duplicate (unless sample volume was too small) at room temperature for 16 h.

2.3. Electrophoresis

During July 1993, liver tissue which had been stored at −80°C was thawed and homogenized in approximately 3 volumes of ice cold 0.09 M Tris, 0.001 M DTT, pH 7.0 buffer in a glass-on-glass grinder. Homogenates were centrifuged at full speed for 10 min in a Bel Art mini-centrifuge at 4°C to remove cellular debris. Supernatants were stored at −80°C for up to 20 days before electrophoresis. Cellulose acetate electrophoresis was performed following methods modified from Carter et al. [4] and Hebert and Beaton [27]. Cellulose acetate plates were soaked for 20 min in a 0.01 M Tris–glycine pH 8.5 buffer. Liver homogenates were thawed and electrophoresed on the pre-soaked plates using a 0.1 M Tris–glycine pH 8.5 electrode buffer for 30 to 35 min at 200 V and approximately 4 mA/gel. Plates were stained for lactate dehydrogenase (Ldh), malate dehydrogenase (Mdh, sometimes called Mor), glutamate-oxaloactate transferase (Got), malic enzyme (Me, sometimes called Mod), phosphoglucose isomerase (Gpi, sometimes called Pgi), phosphoglucose mutase (Pgm), and 6-phosphogluconate dehydrogenase (6Pgd), following Carter et al. [4] and Hebert and Beaton [27]. These loci were chosen because they occur in primary metabolic pathways (glycolysis and the TCA cycle) and have been examined in a variety of other taxa [38].

2.4. Statistics

Most variables were transformed to improve normality and/or homoscedasticity in relation to covariates (e.g. body mass). Maximal sprint running speed, maximal swimming endurance, $\dot{V}O_2$max, all tissue masses, body mass, T3 and T4 hormone levels, and cholesterol were $\log_{10}$ transformed; tail length and blood hemoglobin were cubed. Some decisions as to best transformations were modified after inspection of residuals from the multiple regression equations (see next paragraph).

We controlled statistically for the effects of variation in body mass, sex, age, measurement block (mice were measured in separate groups at different times), generation, and other relevant covariates using ordinary least-squares multiple regression. We used a stepwise algorithm, with entry and removal levels set at $P = 0.05$ and $P = 0.10$, respectively, to determine the best multiple regression equation for each variable [9,10]. Sex, measurement block, and whether an individual was a parent (i.e. a breeder obtained from Harlan Sprague Dawley) or an offspring born in our laboratory were scored as 0–1 dummy variables. The product of the parent and sex dummy variables was also used to check for interactions. For sprint speed, we also used dummy variables coding for observer; for swimming endurance we used water temperature as a potential covariate (see Ref. [10]). For cholesterol and thyroid hormone levels, we used amount of time the sample was frozen as a covariate. For time, age at measurement, fast length, and freeze time, orthogonal polynomials (e.g. [Z-transformed age]^2) were also used to allow for non-linear associations with the dependent variable. For regressions in which the squared term was statistically significant, but the first-order term was not included in the final stepwise model, we forced the first-order variable into the equation prior to computing residuals [40]. All residuals showed a reasonable approximation to normality.

The computed residuals from the multiple regression equations were then used for all subsequent analyses. Oneway ANOVA was used to test for differences in trait residuals among the three genotypes at each polymorphic locus. Pearson product–moment correlations were calculated to test for significant associations between trait residuals and multi-locus heterozygosity (the total number of loci heterozygous in an individual; range = 0 to 4). All statistical analyses were performed using SPSS/PC+ version 5.0 on an IBM-compatible personal computer.

Most studies attempting to detect significant single-gene effects on quantitative traits have used ordinary least-squares approaches. Under certain conditions of polygenic inheritance and natural or artificial selection, this statistical approach can find spuriously significant effects of single genes when no effect actually exists [29]. Our use of ordinary least-squares on both parents and offspring is, nevertheless, justifiable in the present case for the following reasons. Firstly, in previous quantitative genetic studies using maximum likelihood methods with the same individual mice, we estimated both narrow-sense and broad-sense heritabilities for all of the traits under consideration herein ([9,10], Dohm et al. unpublished). In addition to genetic effects, the breeding design we used incorporated a cross-fostering scheme which allowed us to test for postnatal common environmental effects, an additional component of family resemblance. Narrow-sense heritabilities for all traits were less than about 0.4; the modal narrow-sense heritability was about 0.15 ([9,10] Dohm et al. unpublished). Furthermore, we did not detect large contributions from common environmental effects to total phenotypic variance for any trait (i.e. most estimates indicated an effect less than 10%). Thus, the chief causes of family resemblance (narrow-sense heritability for resemblance between parents and offspring; narrow-sense heritability plus common environmental effects for resemblance between siblings), were only modest in size and cannot be taken as a substantial source of bias in the present study. Secondly, although parents and offspring did differ significantly for some traits (body mass, sprint running speed, swimming endurance, BMR, $\dot{V}O_2$max, but not for any of the suborganismal traits studied), the differences were always quite small in magnitude. For no trait did the difference in mean values, adjusted for effects of covariates, exceed 5% ([9,10], Dohm et al. unpublished). Therefore, according to figures published in Kennedy et al. [29], and our estimated heritabilities for these traits, differences between parents and offspring for the traits in question do not warrant substantial concern as a source of bias in our statistical tests for gene effects.

Our last statistical point addresses degrees of freedom. We statistically removed affects of sex, age, body mass, and measurement block prior to both quantitative genetic analyses [9,10] and tests for effects of single genes (this study). As a consequence, our statistical tests for single-gene effects do not correct for the loss of degrees of freedom (df) attributable to these additional sources of variation. This approach was necessary because maximum likelihood programs that can account for the breeding design used by us and both fixed effects and covariates are not yet available. However, we note that our findings of statistical significance for eight of 56 single-locus tests, and one of 14 multi-locus tests, would not change even if we subtracted the 15–20 degrees of freedom related to these statistical corrections.

3. Results

3.1. Allozyme variation

Seven enzyme stains were used in this study. Three (Ldh, Pgm, Me) revealed two loci (i.e. isozymes) each, whereas the other four (Gpi, Mdh, 6Pgd, Got) showed banding patterns indicative of a single locus; these results are similar to those in other strains of laboratory *Mus domesticus* [19,44]. Both Ldh loci and both Me loci were monomorphic, as were Pgm-1 and Got-1. Mdh-1, 6Pgd and Pgm-2 were polymorphic with two alleles each; for each of these loci, the fast-running allele was identified as 'a' and the slow-running allele as 'b' (following Ref. [19]). Gpi-1 was also polymorphic, with two alleles; in this case, the fast-running allele was identified as 'b' and the slow-running allele as 'a' (following Ref. [19]). Thus, of the 10 loci scored, four showed polymorphic banding patterns with two alleles each: Gpi-1, Pgm-2, 6Pgd and Mdh-1. Table 1 presents frequency information and statistics for these polymorphic loci in both generations of mice. All loci in the parental generation were in Hardy–Weinberg equilibrium, using the χ^2 statistic to compare observed and expected genotypic frequencies [47,51].

3.2. Relationships between allozyme genotypes and physiological phenotypes

Because of the similarity of genotype frequencies between generations, because generation was a statistical blocking variable used to produce trait residuals, and because generations were pooled for other analyses [9,10], data from the two generations were pooled for analysis by genotype. Multi-locus heterozygosity was calculated by summing the number of heterozygous loci for each individual (range = 0 to 4). The coefficients and P values from Pearson product–moment correlations between multi-locus heterozygosity and each trait are in the final column of Table 2. In contrast to results from most previous studies, BMR was positively correlated with multi-locus heterozygosity ($P < 0.001$), with heterozygote class 4 being approximately 10.7% higher than heterozygote class 0 (Fig. 1). However, $\dot{V}O_2$max was not significantly correlated with multi-locus heterozygosity ($r = -0.059$; $P = 0.3$; Table 2; Fig. 2), nor did $\dot{V}O_2$max differ among multi-locus genotype classes (oneway ANOVA, $F = 0.7026$, $P = 0.59$; $df = 3, 302$). No other traits showed statistically significant correlations with multi-locus heterozygosity.

Table 2 also presents P values from ANOVA of trait residuals by genotype for each locus. Eight of 56 analy-

ses (14%) demonstrated significant ($P < 0.05$, unadjusted for multiple tests) differences among genotypes; four of these were for oxygen consumption traits (BMR and $\dot{V}O_2$max). 6Pgd, which catalyzes a step in the pentose shunt, never showed differences among genotypes for any trait; Mdh-1, which is involved in the Krebs cycle, showed differences among genotypes for two traits; and Gpi-1 and Pgm-2, which are at the head of the glycolytic pathway, showed differences among genotypes for three traits each.

4. Discussion

We found that levels of allozyme variation in outbred Hsd:ICR mice are similar to those reported 18 and 25 years ago [24,44], and to those measured in wild populations of house mice [44,55]. Of the 10 allozyme loci scored in the 325 mice studied here, four (40%) were polymorphic, and the average heterozygosity across all 10 loci was 11.2 and 12.8% for the parent and offspring generations, respectively (computations and terminology following Ref. [23]). The lack of deviations from Hardy–Weinberg expectations demonstrates that the inbreeding coefficient (F) for the parental mice is not significantly different from 0 (Table 1: cf. Refs. [23,28]). Thus, this sample of the outbred Hsd:ICR strain, which consisted of mice purchased from Harlan Sprague Dawley, did not show evidence of inbreeding. Low levels of inbreeding are expected for a sample drawn from a large, outbred population of laboratory mice [44,50]. The size of the commercial population from which these mice were drawn has been maintained at several hundred to thousands of individuals since 1983; presently, 1000 females are bred each generation, and inbreeding is estimated at one-tenth of one percent each generation (D. Renner personal communication 10 July 1997; L. White personal communication, 7 October 1994; see Ref. [9] for further details).

Allozyme variation in wild *Mus* populations is quite variable (see Refs. [44,55] for reviews). The allozyme variation we measured is similar to that reported by Selander and Yang [49] for *Mus domesticus* in populations from North America and Denmark. Within various wild populations, they found that 41% of loci were polymorphic and that average heterozygosity was 8.5%, whereas we found that 40% of loci were polymorphic and that average heterozygosity was 11–12%. Although wild populations undoubtedly have more allozyme variants than do outbred laboratory strains [19,49], the behavior and population dynamics of wild mice result in small, highly structured sub-populations, in which founder effects and drift can have large effects on local genetic diversity [49]. In contrast, large random bred or outbred laboratory populations are more likely to maintain genetic variation [50]. In any case, levels of genetic variation in Hsd:ICR mice reported herein and 18 and 25 years ago [24,44] are similar to those reported for wild populations of house mice. In addition, physiological traits that have been studied show, at most, only small quantitative differences between Hsd:ICR mice and wild mice from a Wisconsin population [11,16,45]. Therefore, outbred Hsd:ICR mice represent an appropriate model for studies of the genetics and evolution of physiological aspects of the phenotype.

The data set presented herein on relationships between allozyme genotype and physiological phenotype is one of the largest and most thorough for mammals, both in terms of sample size and number of traits measured (see also Ref. [7]). Eight of 56 analyses of trait differences among single-locus genotypes were statistically significant. Because α for the statistical tests was chosen a priori to be 0.05, three of these significant results were expected by chance alone. We had no expectations for phenotypic differences among genotypes at specific loci in these mice, as, for example, was the case for Gpi in *Colias* butterflies [54] and Ldh in the teleost fish *Fundulus* [42]. Such significant relationships

Table 1
Summary of genetic measures for parents and offspring for the four variable loci[a]

Locus		p		H_o	F	X^2 for H-W	n
Mdh-1	Parents	0.928	a	0.144	−0.078	0.63	104
	Offspring	0.864	a	0.262	−0.115		221
6Pgd	Parents	0.981	a	0.038	−.019	0.04	104
	Offspring	0.952	a	0.096	−0.050		219
Gpi-1	Parents	0.577	b	0.442	0.094	0.92	104
	Offspring	0.552	b	0.507	−0.025		221
Pgm-2	Parents	0.639	a	0.505	−0.094	0.89	101
	Offspring	0.638	a	0.419	0.093		217

[a] Note: frequency and identity (the a or b allele) of most common allele (p); heterozygote frequency (H_o); inbreeding coefficient (F); X^2 value from test of Hardy–Weinberg equilibrium in parent generation, degrees of freedom = 1 and critical value = 3.84 for each test, and none are significant at $P < 0.05$ (the value for X^2 is the statistic itself, not the P value); and sample sizes (n), for each generation and each locus.

Table 2
Significance of tests relating genotypes to phenotypic traits[a]

Trait	Mdh-1	6Pgd	Gpi-1	Pgm-2	Multi-locus heterozygosity
Body mass at 35 days	0.674	0.269	0.076	**0.002 bb**	0.572 $r = -0.035$
Maximal sprint running speed	**0.004 ab**	0.402	0.801	0.802	0.161 $r = 0.081$
Swimming endurance	0.296	0.912	0.234	0.078	0.238 $r = 0.073$
Maximal oxygen consumption	0.914	0.167	**0.032 bb**	0.233	0.309 $r = -0.059$
Basal metabolic rate	0.045 ab	0.430	**0.042 ab**	**0.007 ab**	0.001 $r = 0.209$
Triceps surae muscle mass	0.125	0.239	0.269	**0.014 aa**	0.825 $r = -0.012$
Heart mass	0.268	0.455	0.693	0.997	0.598 $r = 0.029$
Liver Mass	0.606	0.630	0.446	0.981	0.636 $r = -0.026$
Tail length	0.876	0.620	**0.003 ab**	0.364	0.183 $r = 0.075$
Hemoglobin	0.872	0.687	0.606	0.476	0.453 $r = -0.042$
Hematocrit	0.923	0.204	0.258	0.493	0.350 $r = -0.052$
Cholesterol	0.220	0.114	0.846	0.400	0.692 $r = 0.022$
Thyroxin	0.539	0.174	0.814	0.852	0.490 $r = 0.040$
Triiodothyronine	0.593	0.985	0.569	0.664	0.573 $r = -0.033$

[a] Note: significance levels are from ANOVA for differences among single-locus genotypes and from Pearson correlation analysis for the multi-locus genotype. For significant ANOVA results, the genotype (e.g. aa, ab, or bb) with the highest value of the trait is listed next to the P value; for the correlation analysis, the value of r is listed next to the P value. All significant results are in bold type ($P<0.05$, not adjusted for multiple tests). All phenotypic traits are residuals from multiple regressions on covariates and blocking variables (see Section 2).

between single-locus genotype and a physiological trait may be caused by variation at the locus itself, or may reflect linkage disequilibrium between that locus and another which actually affects the trait [37]. To identify which mechanism accounts for the significant single-locus results presented here, additional experiments examining functional differences among allozymes at each locus are required [12].

We also found that multi-locus heterozygosity was positively correlated with mass-corrected BMR, but was not significantly correlated with $\dot{V}O_2max$ nor with any of the other 12 morphological or physiological traits measured. Although BMR in endotherms is not identical to standard metabolic rates measured in ectotherms, it is analogous. The positive correlation between BMR and multi-locus heterozygosity is striking in that it is opposite in sign to nearly all previously reported results for resting or standard metabolic rates in ectotherms (e.g. snails [17], clams [18], oysters [32,46], trout [8], and tiger salamanders [39]).

How multi-locus heterozygosity might affect BMR in these mice is unclear. One possibility is that the genetic variation has no physiological effect at all, but that the studied loci are in linkage disequilibrium with polymorphic loci which do have such effects. Although this cannot be excluded, it seems unlikely because all the polymorphic loci we studied are involved in central metabolism and are the very loci predicted to affect such traits as BMR [31]. A second possible explanation, related to the first, is that the allozyme variation has no physiological effect, but is simply an index of inbreeding [37]. If either of these explanations are true, then correlations between BMR and any polymorphic molecular marker (e.g. microsatellite DNA loci) should reveal the same results [37]; this hypothesis can be tested in future studies.

On the other hand, correlations between multi-locus heterozygosity and BMR may have a physiological basis. Hawkins and Day [25] have argued that 20–40% of standard metabolic rate is spent catabolizing and synthesizing proteins, and have demonstrated that overall protein turnover rate in mussels decreases with enzyme heterozygosity. However, precisely why het-

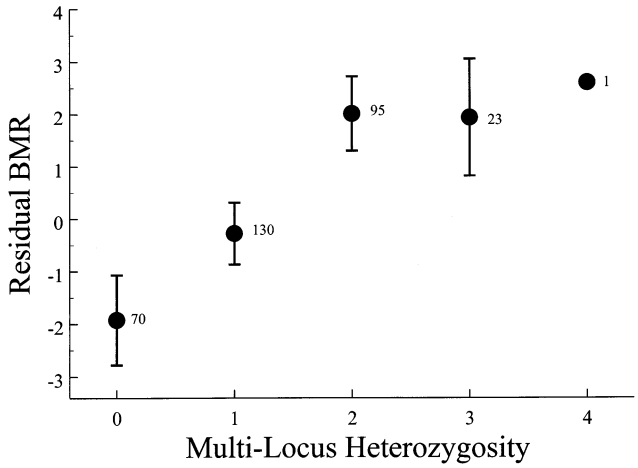

Fig. 1. Means and standard errors of residual basal metabolic rate (BMR) vs. multi-locus heterozygosity. Pearson product–moment correlation coefficient = 0.209, two-tailed $P < 0.001$. Sample sizes of each heterozygosity class appear next to the symbol on the graph. The mean BMR for all mice was 39.84 ml O_2 h^{-1}; the mean residuals for heterozygote class 0, 1, 2, 3 and 4 are -1.92, -0.28, 2.01, 1.94, and 2.61 ml O_2 h^{-1}, respectively. Because residuals are in the units of original measurement, percentage differences among heterozygote classes can be calculated. For example, heterozygote class 0 is $(39.84 - 1.92)/(39.84 + 2.61)$ or 89.3% of heterozygote class 4.

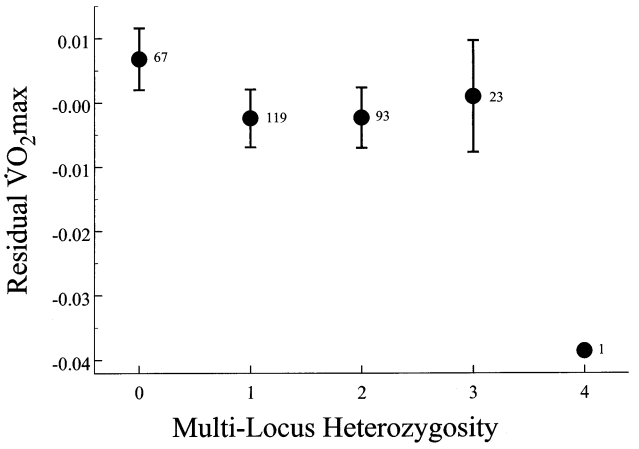

Fig. 2. Means and standard errors of $\log_{10}$ transformed residual maximal oxygen consumption (VO_2max) during forced exercise vs. multi-locus heterozygosity. Pearson product–moment correlation coefficient = −0.059, two-tailed $P = 0.309$. If heterozygote class 4 is excluded from the analysis, the Pearson product–moment correlation coefficient = −0.052 and the two-tailed $P = 0.367$. Sample sizes of each heterozygosity class appear next to the symbol on the graph.

erozygous allozymes are catabolized more slowly has yet to be elucidated. In a related vein, Clark and Koehn [6] suggested that a more heterozygous allozyme pool might provide a higher flux through metabolic pathways at a lower cost of protein maintenance; however, this idea has yet to be tested empirically. If either or both of these explanations are true for ectotherms, then our data oppose the generality of these explanations with regard to endotherms. This highlights an excellent opportunity to study protein turnover in relation to metabolic rate and multi-locus heterozygosity in endotherms, for comparison with existing information on ectotherms. Such comparative data will help resolve questions of how allozyme heterozygosity affects metabolism, and may elucidate different evolutionary trajectories followed by ectotherms and endotherms with regard to genetic variation and metabolism.

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