

Quantitative genetics of maximal oxygen consumption in a garter snake

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GARLAND, THEODORE, JR., AND ALBERT F. BENNETT. *Quantitative genetics of maximal oxygen consumption in a garter snake*. Am. J. Physiol. 259 (Regulatory Integrative Comp. Physiol. 28): R986–R992.—Broad-sense heritabilities and genetic correlations of maximal oxygen consumption ($\dot{V}O_{2\max}$), blood hemoglobin levels, and ventricle mass were estimated in a natural population of snakes. Traits were measured for six or fewer presumed full-sibling offspring from each of 45 wild-caught gravid garter snakes (*Thamnophis sirtalis*). $\dot{V}O_{2\max}$ was highly reproducible between replicate trial days ($r = 0.88$). In an attempt to reduce maternal effects, correlations of each character with body mass, snout-vent length, age at testing, litter size, dam mass, and dam snout-vent length were removed by computing residuals from multiple-regression equations. These residuals were used in subsequent genetic analyses. Approximate coefficients of variation of residuals were 16% for $\dot{V}O_{2\max}$, 19% for hemoglobin level, and 13% for ventricle mass. Broad-sense heritabilities were highly significant for all characters [$P < 0.0001$; $\dot{V}O_{2\max}$ heritability (h^2) = 0.88; hemoglobin level $h^2 = 0.63$; ventricle mass $h^2 = 0.41$], suggesting that they could respond genetically to selection. Phenotypic correlations (r_P) among residual characters were significant only between $\dot{V}O_{2\max}$ and ventricle mass ($r_P = +0.27$). $\dot{V}O_{2\max}$ and ventricle mass exhibited a significant (broad-sense) genetic correlation of +0.64; this might facilitate the correlated evolution of these two traits in response to natural or artificial selection. Ventricle mass and hemoglobin level showed a significant environmental correlation of +0.43. Treadmill endurance crawling time (Evolution 42: 335–350, 1988) showed a weak but significantly positive r_P with $\dot{V}O_{2\max}$ ($r_P = +0.17$).

aerobic capacity; evolution; exercise; hemoglobin; heritability; *Thamnophis sirtalis*

MAXIMAL AEROBIC POWER is one of the most important factors influencing an individual's physical endurance capacity. As measured by the maximal rate of organismal oxygen consumption ($\dot{V}O_{2\max}$), it sets an upper limit to the intensity of physical activity (work load) that can be supported aerobically, without requiring supplemental anaerobic metabolism (which is linked ultimately to fatigue). $\dot{V}O_{2\max}$ is generally considered to be "the best single measure of aerobic capacity" (3). From a more comparative and ecological perspective, $\dot{V}O_{2\max}$ is important, because it may be a significant determinant of the levels of activity that free-living animals can sustain. High sustainable activity levels are presumed in some cases to facilitate foraging, searching for mates, escaping from predators, and other behaviors that may have important influences on Darwinian fitness (4, 5, 18, 23, 27, 33).

$\dot{V}O_{2\max}$ shows considerable interspecific variation within mammals (34), reptiles (4, 5, 18), and amphibians (27, 33). Substantial variation in $\dot{V}O_{2\max}$ exists among individuals within species as well (6, 8, 16, 19, 27, 36). Whether these individual differences are genetically based is poorly understood.

On first consideration, one might doubt that individual differences in $\dot{V}O_{2\max}$ would be heritable, simply because numerous studies show that $\dot{V}O_{2\max}$ is a rather plastic physiological trait. For example, exercise conditioning and/or general level of physical activity affects $\dot{V}O_{2\max}$ in humans and other mammals (9, 10) and in captive lizards (7, 20). In fact, an increase in $\dot{V}O_{2\max}$ is considered to be "the single most objective indication of a training effect" (29). Natural seasonal variation in $\dot{V}O_{2\max}$ has been demonstrated in lizards (19, 23). Both seasonal variation and laboratory acclimation of $\dot{V}O_{2\max}$ to both temperature and altitude occur in deer mice (see references in 13, 22). However, the plasticity of a trait between environments or in response to changes in environmental factors bears no necessary relationship to the heritability of that trait within a single environment. Quantitative genetic parameters are specific to particular populations and the environments within which they exist and within which they are measured. If all individuals within a population were to respond similarly to a given environmental factor (e.g., a change in temperature), then heritability (defined below) would not necessarily be affected. If, on the other hand, environmental factors (broadly defined) affected individuals in different ways (e.g., some showed increases and others decreases), then measured differences among individuals would be inflated and heritability would be lowered. This is a real possibility for $\dot{V}O_{2\max}$, because, for example, individual differences in the response to exercise training have been documented in humans (9) and possibly in lizards (see Fig. 1 in Ref. 20).

$\dot{V}O_{2\max}$ shows continuous variation within populations and almost certainly is affected by alleles segregating at multiple loci (see references in 8, 9, 13, 22). For such "polygenic" traits, the techniques of quantitative genetics allow estimation of the relative influence of genetic and environmental factors in determining the phenotype. Quantitative genetics is used routinely in animal and plant breeding and has achieved many obvious successes. However, for both historical and practical reasons, the application of quantitative genetics to vertebrate physiology has been limited (for exceptions see Refs. 9, 17, 21, 24, 25, 31, 32). One reason for the infrequent application

of quantitative genetic analyses to physiological variation is that appropriate breeding designs often are impractical with the relatively large animals studied by physiologists or with natural populations of vertebrates. A second reason is that large sample sizes are required to obtain accurate estimates of quantitative genetic parameters, and physiological traits are more time consuming and technically difficult to measure than are traits typically examined by quantitative geneticists (such as body size or bristle number in *Drosophila*). Consequently, little information exists concerning heritabilities or genetic correlations of physiological characters (2, 26).

Quantitative genetic analyses are complementary to analyses of genes with major effects. When present, effects of "major genes" act in conjunction with the smaller, often additive effects of allelic variation at all other loci affecting the phenotype (31). Two quantitative genetic parameters are of primary interest from the standpoint of basic physiological knowledge. The first, "narrow-sense" heritability (h_N^2), is defined as the proportion of phenotypic variance due to the additive effects of genes [$h_N^2 = \text{additive genetic variance} / (\text{additive genetic variance} + \text{dominance variance} + \text{epistatic variance} + \text{environmental variance})$; 15]. Narrow-sense heritability indicates the potential for evolutionary change via individual selection, that is, whether a trait could respond genetically to natural or artificial selection. In the absence of correlated environmental effects, narrow-sense heritability also determines the extent of parent-offspring resemblance. "Broad-sense" heritability [$h_B^2 = \text{additive genetic variance} + \text{dominance variance} + \text{epistatic variance} / (\text{additive genetic variance} + \text{dominance variance} + \text{epistatic variance} + \text{environmental variance})$], on the other hand, is somewhat less informative for evolutionary analyses. Broad-sense heritability estimates the ratio of total genetic variance to phenotypic variance, whereas only the additive portion of genetic variance determines the resemblance of offspring to their parents and the potential for response to selection. Nevertheless, estimation of broad-sense heritability often is much easier than estimation of narrow-sense heritability, and the former is a useful and practical first step for many natural populations (1, 17), including humans (8, 9).

The response to selection depends on a second factor, genetic correlation, in addition to heritability. Genetic correlations are based on the additive genetic component of the phenotypic correlation between traits. They reflect the fact that many genes affect more than one trait. Such "pleiotropic" gene action appears to be pervasive, and virtually all traits probably show significant genetic correlations with at least some others (37). (Genetic correlations also may reflect linkage of genes, but this usually will be of less importance than is pleiotropy.) Genetic correlations are important because they indicate the extent to which selection for one trait will affect traits that are genetically correlated with it (2). Genetic and phenotypic correlations often differ substantially in magnitude and even in sign (15, 35), and the former can only be estimated from data on relatives.

The purpose of this paper is to provide basic information on the quantitative genetics of maximal aerobic

power and some possible mechanistic correlates in a natural population of vertebrates. The inheritance of $\dot{V}O_{2\max}$ has not been studied previously in a natural (nonhuman) population of vertebrates. Garter snakes are convenient models for such studies, because large numbers of gravid females can be captured in the wild, thus providing ready-made sets of siblings for analysis (1). Previously, we reported significant heritabilities for both maximal sprint crawling speed and treadmill endurance and a positive genetic correlation between them (17). A subsequent paper will consider potential biochemical correlates of locomotor performance and $\dot{V}O_{2\max}$ (maximal in vitro catalytic rates of citrate synthase and pyruvate kinase in ventricle, liver, and skeletal muscle).

MATERIALS AND METHODS

Animal collection and maintenance. Gravid garter snakes (*Thamnophis sirtalis*) were captured 9 June-4 July, 1984, in the vicinity of Eagle Lake, Lassen, CA (State of California Department of Fish and Game Scientific Collecting Permit no. 0983 to S. J. Arnold). After transportation to the University of California, Irvine, dams were housed individually in plastic shoeboxes, maintained in an environmental chamber on a 12:12 h photophase (30°C days, 20°C nights). Water was always available. Dams were offered cooked fish mixed with chopped earthworms and a multivitamin-mineral supplement weekly. All remained in apparent good health before parturition.

Births occurred 2 August-6 September, 1984 (see Ref. 17 for further details). Offspring were weighed and checked for obvious deformities at birth. Six (or all individuals if litter size was <6) nondeformed newborns were chosen randomly from each litter and placed in individual transparent plastic containers (volume = 0.5 l) with ground corn cob for substrate and water dishes. All newborns were removed from the environmental chamber and housed at room temperature (~20°C) until the day before performance testing. Food was withheld from all newborn snakes, but water was available ad libitum (17). Logistical constraints dictated that not all individuals could be measured at the same age. We withheld food, because two previous studies (see references in Ref. 17) have demonstrated that recent feeding (that is, a full stomach) may decrease locomotor performance of snakes. Moreover, previous studies (for example see Ref. 1) and our own observations demonstrate that individual newborn snakes vary greatly in their willingness to eat in captivity. Fasting all individuals was done in order to eliminate these possible sources of variation. Previous experience indicated that newborn snakes held at 20°C can fast for at least 2 mo with no apparent harm. Length of fasting may, of course, affect physiological performance, so length of fast (equivalent to age at testing) was controlled for statistically, as explained below. The average age at time of measurement of $\dot{V}O_{2\max}$ was 31.6 days (median = 28, SD = 15.34, range = 11-58). Comparison of masses measured during $\dot{V}O_{2\max}$ trials with those measured at birth indicated that snakes lost an average of 12% of their body mass (median = 11, SD = 4.7, range = 4-29).

Testing schedule. A total of 245 neonatal snakes was

tested. For logistical reasons, families were divided into blocks of six (36 individuals) for testing, based on order of birth date; that is, the first six families born were tested first. An alternative would have been to test some individuals from each family in each set. We did not choose this alternative because it would have greatly increased the range of variation in age at time of testing. The testing schedule was as follows: *days 1* and *2*, measurement of maximal sprint crawling speed; *days 3* and *4*, measurement of treadmill endurance (17); *days 5* and *6*, measurement of $\dot{V}O_{2\max}$. All measures were conducted during the normal photophase at 30°C, which approximates the mean body temperature of *T. sirtalis* when active in the field (C. R. Peterson, personal communication; references in Ref. 17).

$\dot{V}O_{2\max}$. $\dot{V}O_{2\max}$ was measured as snakes crawled on a motorized treadmill while wearing lightweight transparent masks. Masks were fashioned from the tips of nitrocellulose ultracentrifuge tubes or disposable plastic test tubes, held on the neck with loose-fitting rubber diaphragms fashioned from balloons. Air entered around the snake's neck and through small holes around the circumference of the mask. A downstream diaphragm pump drew air into the mask, through a column of Drierite-Ascarite-Drierite, through a calibrated Brooks rotameter, and finally into the sensor of an Applied Electrochemistry model S-3A oxygen analyzer. Flow rates averaged 213 ml/min STPD; this relatively high flow rate ensured that all expired gases were captured (otherwise oxygen consumption would be underestimated) and allowed a rapid response time for the system. A continuous record of percent oxygen in excurrent air was made on a 10-in. flatbed chart recorder (Varian) with span being 21.0–20.9% O_2 . These measurement conditions pushed the limits of accuracy of the oxygen analyzer. They were, however, adequate for these experiments because 1) baselines were recorded both before and after each individual was tested (<10 min) and 2) the reproducibility of our values for replicate trial days was almost 0.9 (see RESULTS), similar to that reported previously for (larger) lizards (19). This is important because repeatability sets an upper limit to heritability (15); if a character cannot be measured reproducibly, then it cannot be heritable. All measurements were conducted in an environmental chamber at 30°C.

The standard procedure for measuring $\dot{V}O_{2\max}$ is to put animals through a stepped series of increasing speeds (e.g., 3, 16, 18, 19). The small size of the newborn snakes in relation to the size and mass of the mask made it difficult to employ such a step test in the present study. We therefore tested all newborns at a single speed, chosen to yield a workload slightly above that which could be maintained (compare Refs. 13, 22 and references therein). Without a mask, newborns crawled an average of 6 min before exhaustion at 0.4 km/h (17). Consequently, to compensate for the work of wearing a mask, we tested all snakes at a treadmill speed of 0.3 km/h. When wearing a mask, newborns could maintain this workload for ~4–10 min (but no longer), which was presumed to provide sufficient time for individuals to exhibit their $\dot{V}O_{2\max}$ before exhaustion. Snakes were removed from their individual plastic containers, fitted

with the mask, placed on the moving treadmill, and induced to crawl by gentle tapping about the tail and posterior body with a foam rubber-tipped pointer.

Individuals were tested on 2 consecutive days, and for each trial the highest 2-min period of oxygen consumption was recorded. Values from both days were analyzed for repeatability. However, following previous studies of lizards (16, 18, 19), the single higher value was taken as an individual's $\dot{V}O_{2\max}$ in subsequent analyses. (This is analogous to human exercise physiologists' attempts to reduce motivational effects by using personal best performances.) In practice this made little difference because, due to the high repeatability of our measures, the average for the two trial days was very highly correlated with the single higher value ($r = 0.987$).

Hemoglobin. Snakes were killed by decapitation, and blood samples were taken from the severed neck with heparinized microhematocrit tubes. Duplicate subsamples of this blood were drawn into 20- μ l Drummond microcaps, rinsed into test tubes containing 5.0 ml Drabkin's solution (11), and vortexed. Approximately 15 min at room temperature were allowed for color development, then samples were refrigerated for several hours. Absorbance at 540 nm was recorded at room temperature on a Beckman model 25 spectrophotometer and compared with cyanmethemoglobin standards (Sigma stock no. 525-18, technical bulletin no. 525). Means of duplicate assays were analyzed.

Ventricle mass. Body cavities were opened with a mid-ventral incision. Forceps were used to lift the heart by the apical ligament, thus allowing the ventricle to be dissected free from the atria. Mass was recorded to the nearest 0.1 mg.

Statistical analyses. For a comparative measure of variability, we log transformed each character, regressed them on the appropriate significant covariates (see below), then compared standard deviations of residuals (multiplied by 2.3026 because variables were log rather than ln transformed). This quantity is approximately equivalent to a coefficient of variation, but with the effects of body size eliminated (16).

Snakes used in the present analyses are presumed to represent sets of full siblings (see discussion in Ref. 17). Full-sibling analyses may overestimate narrow-sense heritabilities, because dominance, epistasis, genetic and nongenetic maternal effects, and common family environment before birth contribute to among-family variation, in addition to the additive effects of genes (1, 15). Maternal effects in reptiles may be mediated largely through maternal size and condition, which affect the size and condition of offspring. We therefore used multiple regression analyses in an attempt to control statistically for the possible effects of offspring body size and condition (indexed by mass and snout-vent length), maternal body mass and snout-vent length, and litter size (17). We conducted stepwise multiple regressions of $\dot{V}O_{2\max}$, ventricle mass, and hemoglobin concentration on offspring mass, snout-vent length, age, age², and mass loss before testing, as well as litter size and dam mass and snout-vent length. After determining which of these were statistically significant as independent variables, we used residuals from multiple regression equations for

further analyses (17). Use of such procedures is routine in quantitative genetic analyses of humans, including studies estimating heritabilities of $\dot{V}O_{2\max}$ (8) and hemoglobin levels (21).

Broad-sense heritabilities for each character were estimated as twice the among-family component of variance derived from a one-way analysis of variance among families (1, 15). Bulmer's (12, p. 84) algorithm was used to calculate 95% confidence intervals for these estimates. We also used restricted maximum likelihood to estimate heritabilities of single-character data sets (17, 30).

Phenotypic correlations (Pearson product-moment) between residual characters were calculated and tested for significance in the usual way. Genetic correlations were estimated in the following three ways: first, by restricted maximum likelihood (30); second, by multivariate analysis of variance partitioning covariance among vs. within families; third, as correlations between weighted litter means (1, 17). Note that, because data are for sets of full siblings only, these estimates of genetic correlations may overestimate the magnitude of correlation due strictly to additive genetic effects. Environmental correlations were estimated both by restricted maximum likelihood and by least squares (*Eq. 19.1* in Ref. 15; *Eq. 2* in Ref. 1). Significance tests of genetic and environmental correlations were attempted only for the maximum likelihood estimates, and involved comparing the likelihood of a constrained model (one or more parameters constrained to zero) with that of the unconstrained model (30). Twice the difference in likelihoods is distributed approximately as a χ^2 with *df* = the number of parameters constrained to zero (30). Point estimates of genetic and environmental variances and covariances were based on parameter estimates for the complete (3- or 4-character) data set, with no parameters constrained to zero. Shaw's (30) program was used for restricted maximum likelihood estimation; SPSS/PC+ was used for other procedures. Statistical significance was judged at $P < 0.05$ in all cases.

RESULTS

Repeatability. Daily measures of $\dot{V}O_{2\max}$ were highly repeatable. For whole animal $\dot{V}O_{2\max}$ (ml O_2 /h), the Pearson product-moment correlation between replicate trials was 0.882 ($n = 242$), whereas the intraclass correlation (proportion of variance among individuals as opposed to between trial days) was 0.864. These repeatabilities are higher than those reported previously for maximal crawling speed, treadmill endurance, and anti-predator behavior (17). Considering $\dot{V}O_{2\max}$ expressed per gram body mass, the Pearson correlation between replicate trials was 0.796. Because $\dot{V}O_{2\max}$ scaled approximately in direct proportion to body mass (see below), this is approximately equivalent to the repeatability of a mass-corrected residual (see Ref. 19). A paired *t* test indicated that $\dot{V}O_{2\max}$ showed a slight (~3%) but significant ($P = 0.002$) decrease from *day 1* (mean = 3.04 ml O_2 /h) to *day 2* (mean = 2.95 ml O_2 /h). A similar decrease was noted in a previous study of lizards (19).

Descriptive statistics and variability. Distributions of $\dot{V}O_{2\max}$, hemoglobin concentration, and ventricle mass were approximately normally distributed and so were not

transformed before further analyses (coefficients of skewness were -0.116, -0.201, and 0.053, respectively; coefficients of kurtosis were 0.306, 0.174, and -0.133, respectively). Approximate coefficients of variation for residuals of the three traits (controlling for variation in body mass, age, etc.) ranged from 13 to 19% (Table 1). These values are considerably lower than for residual treadmill endurance in the same individuals (48% from Ref. 17).

Attempts to reduce maternal effects. Stepwise multiple regression indicated that log offspring body mass (partial $r^2 = 47.2\%$, sign of partial regression coefficient is positive) and offspring age (23.9%, negative sign) both were significant predictors of $\dot{V}O_{2\max}$ (multiple $r^2 = 71.1\%$). Thus larger individuals consumed more oxygen, and those tested at older ages (which is equivalent to days of fasting since birth) showed somewhat lower values. (Note that amount of mass lost between birth and time of testing was not a significant predictor of $\dot{V}O_{2\max}$, after accounting for correlations with mass and age at testing.) When the foregoing multiple regression equation is used, $\dot{V}O_{2\max}$ at birth is predicted to have been 4.03 ml O_2 /h, at which time body mass of these 245 individuals averaged 2.53 g.

For hemoglobin concentration, a total of 49.9% of the variance was explainable by offspring age (partial $r^2 = 36.0\%$, negative sign), offspring body mass (8.3%, positive sign), litter size (2.7%, positive sign), offspring age² (1.9%, positive sign), and the amount of mass lost from birth to time of sacrifice (1.1%, negative sign). Predicted mean hemoglobin level at birth was 6.82 g/100 ml. For ventricle mass, 60.6% of the variance was explainable by offspring body mass (partial $r^2 = 46.9\%$, positive sign) and offspring age (12.9%, negative sign) and age² (0.8%, positive sign). Predicted ventricle mass at birth was 4.81 mg. For all three characters, residual values computed from the foregoing multiple regressions were approximately normally distributed (compare Fig. 1 in Ref. 17).

Allometry. Coefficients of "static allometry," representing scaling relationships within an age class (that is, considering only newborn snakes), were computed as the partial regressions of log $\dot{V}O_{2\max}$, hemoglobin concentration or ventricle mass on log body mass, holding constant age, and other significant independent variables (previous paragraph; 17). These partial regression coefficients indicated that $\dot{V}O_{2\max}$ scaled as $\text{mass}^{1.110 \pm 0.129}$ ($\pm 95\%$ confidence interval), hemoglobin concentration scaled as $\text{mass}^{0.784 \pm 0.171}$, and ventricle mass scaled as $\text{mass}^{1.023 \pm 0.113}$.

Variation among families and heritabilities. Analysis of variance indicated highly significant components of variance among families for residuals of all three characters (Table 2). Corresponding broad-sense heritabilities ranged from 0.41 for relative ventricle mass to 0.88 for $\dot{V}O_{2\max}$. Heritabilities estimated by restricted maximum likelihood were highly significant for all three characters and were very similar to those estimated by least squares (Table 2), which is not surprising, because the data set was nearly balanced (mostly 6 individuals/family).

Evidence for major genes. The available data allow a rough test for the presence of single genetic factors with relatively large effect. Briefly, and as discussed previ-

TABLE 1. Descriptive statistics for characters measured in *Thamnophis sirtalis*

Character, units	n_f	n_i	Means \pm SD	Range	CV, %	SD of residuals
Body mass, g	45	245	2.22 \pm 0.350	1.22–3.34	15.7	
Snout-vent length, mm	45	245	187.3 \pm 9.18	160–207	4.9	
$\dot{V}O_{2\max}$, ml O_2 /h	45	245	3.15 \pm 0.845	0.82–5.50	26.8	16.2
Hemoglobin, g/100 ml	45	244	5.51 \pm 1.322	1.91–9.29	24.0	18.6
Ventricle mass, mg	45	244	4.18 \pm 0.861	1.80–6.80	20.6	13.1

n_f , no. of families; n_i , no. of individuals. CV, coefficient of variation.

TABLE 2. Analyses of variance among vs. within families and associated broad-sense heritabilities

Residual Character	MS Between Families, MS Within Families, Between-Family Variance Component	F	df	P	Bulmer's h^2 and 95% CI, Least Squares h^2 , Maximum Likelihood
$\dot{V}O_{2\max}$	0.6146 0.1166 0.0916	5.27	44,200	<0.0001	0.62 < 0.89 < 1.19 0.88 0.88
Hemoglobin	2.1127 0.6015 0.2790	3.51	44,199	<0.0001	0.38 < 0.63 < 0.94 0.63 0.64
Ventricle	0.5705 0.2303 0.0628	2.48	44,199	<0.0001	0.19 < 0.41 < 0.70 0.43 0.43

MS, mean squares. CI, confidence intervals.

ously (17, p. 343), if a major gene is present (e.g., a single locus with large phenotypic effects), then the most extreme families (averaging either high or low on the phenotypic scale) will be composed of individuals homozygous for either “high” or “low” alleles at the locus of large effect; the variance within such families will be relatively low. Intermediate families, on the other hand, will tend to be composed of some individuals that are homozygous and some that are heterozygous for the locus in question; the variance within such families will be relatively high.

Levene's tests [a one-way analysis of variance (ANOVA) using absolute deviations of each individual's value from its family mean; 17] indicated that families differed significantly in variance for both residual $\dot{V}O_{2\max}$ ($F = 2.85$, $df = 44, 200$, $P < 0.0001$) and residual hemoglobin concentration ($F = 1.52$, $df = 44, 199$, $P = 0.0286$). However, quadratic regressions (17) indicated that for both traits intermediate families tended to be less variable. These analyses do not therefore offer any evidence favoring the presence of single genetic factors with large effects on $\dot{V}O_{2\max}$, hemoglobin concentration, or ventricle mass.

Phenotypic, genetic, and environmental correlations. Phenotypic correlations were significant only between residual $\dot{V}O_{2\max}$ and ventricle mass ($r_p = +0.27$, $n = 242$, $P < 0.001$; Table 3). Multiple regression analysis indicated that residual hemoglobin concentration did not add significantly to prediction of $\dot{V}O_{2\max}$ ($F = 0.003$, $P = 0.956$).

The positive phenotypic correlation between $\dot{V}O_{2\max}$ and ventricle mass was due to a positive genetic correlation ($r_G = +0.64$) acting in combination with a somewhat weaker negative environmental correlation ($r_E = -0.47$; Table 3). Maximum likelihood tests indicated that the genetic correlation was highly significant ($\chi^2 = 7.95$, $df = 1$, $P < 0.005$). The environmental correlation be-

tween hemoglobin level and ventricle mass ($r_E = +0.43$) was also significant ($\chi^2 = 6.50$, $df = 1$, $P < 0.02$).

Treadmill endurance (from Ref. 17) was weakly positively correlated with $\dot{V}O_{2\max}$ ($r^2 = 2.8\%$, $F = 6.529$, $n = 245$, $P = 0.009$), but multiple-regression analysis indicated that neither hemoglobin concentration (partial $F = 0.278$, $P = 0.599$) nor ventricle mass (partial $F = 0.724$, $P = 0.396$) significantly improved predictive power. Treadmill endurance was not significantly genetically correlated with $\dot{V}O_{2\max}$ ($r_G = +0.032$), hemoglobin concentration (-0.393), or relative ventricle mass (-0.163). Environmental correlations with endurance were all positive: endurance \times $\dot{V}O_{2\max}$ $r_E = +0.640$ ($\chi^2 = 2.63$, $df = 1$, $P > 0.1$); endurance \times hemoglobin concentration $r_E = +0.623$ ($\chi^2 = 6.25$, $df = 1$, $P < 0.02$); endurance \times ventricle mass $r_E = +0.153$.

DISCUSSION

Heritabilities. Arnold (2) notes, “Most geneticists would expect physiological traits to show heritable variation in a natural population, but this is merely an educated guess.” He points out that there are very few estimates of heritabilities or of genetic correlations among physiological characters. Our data suggest that maximal oxygen consumption, blood hemoglobin concentration, and relative ventricle mass show heritable variation in garter snakes. The most recent studies of humans indicate a low and perhaps nonsignificant narrow-sense heritability for maximal oxygen consumption but a possibly significant maternal effect (8, 25). [Studies of monozygotic twins indicate that the trainability of $\dot{V}O_{2\max}$, how much an individual's $\dot{V}O_{2\max}$ increases in response to physical conditioning, is genotype dependent, but heritabilities per se have not been estimated (9).] The heritability of $\dot{V}O_{2\max}$ apparently has not been studied in any other vertebrate, but oxygen consumption during tethered flight is heritable in *Drosophila* (14).

TABLE 3. Correlations among $\dot{V}O_{2\max}$, hemoglobin concentration, and relative ventricle mass, based on residuals from multiple regression equations

Variable	Correlation	Variable	
		Hemoglobin	Ventricle
$\dot{V}O_{2\max}$	Phenotypic	0.005 ($n = 242$)	0.268* ($n = 242$)
	Genetic	-0.059 (0.011, -0.025)	0.639* (0.652, 0.472)
	Environmental	0.217 (-0.015)	-0.477 (-0.506)
Hemoglobin	Phenotypic		0.008 ($n = 243$)
	Genetic		-0.342 (-0.357, -0.170)
	Environmental		0.430* (0.424)

Phenotypic correlations are standard Pearson product-moment. Genetic correlations are restricted maximum likelihood estimates (for 3 character data set), followed (in parentheses) by 1) least-squares estimates, obtained by partitioning covariance among vs. within families, and 2) correlations among weighted litter means. Environmental correlations are restricted maximum likelihood estimates, followed (in parentheses) by least-squares estimates. * Significant correlations ($P < 0.05$). Significance tests for least-squares estimates of genetic and environmental correlations were not attempted.

Because our estimates are based on sets of (presumed) full siblings, they may overestimate true narrow-sense heritabilities, because dominance, epistasis, and both genetic and nongenetic maternal effects (should any of these exist) are confounded with additive genetic variance (common family environments were disrupted at birth)(1, 15, 17). Possible magnitudes of nonadditive genetic effects for these traits in garter snakes are unknown. Studies of domestic (15) and of wild vertebrates (references in Refs. 17, 26) show variable differences between broad- and narrow-sense heritabilities, and no simple correction factor is possible. Maternal effects on $\dot{V}O_{2\max}$ are possible, as has been suggested for humans (8, 25). However, our use of residuals presumably reduced the influence of maternal effects (see MATERIALS AND METHODS and Ref. 17) and also may have removed some genetic effects, to the extent that the traits of interest were genetically correlated with body size (35).

Left ventricular volume shows a heritability of 0.3–0.7 in humans (8). Heritability of hematocrit or of hemoglobin content of the blood has been shown for humans (0.60 and 0.77, respectively; 21) and chickens (24) and has been suggested for deer mice (32). Thus our heritability estimates for hemoglobin concentration and for ventricle mass are consistent with those reported previously.

Correlations among characters. Both hemoglobin concentration (an indicator of blood oxygen carrying capacity) and ventricle mass (a possible index of stroke volume, which is a partial determinant of cardiac output) might be expected to correlate positively with $\dot{V}O_{2\max}$ (10). Phenotypically, residual $\dot{V}O_{2\max}$ was positively correlated with relative ventricle mass ($r = 0.268$; Table 3) but not with hemoglobin levels ($r = 0.005$). $\dot{V}O_{2\max}$ has been reported to correlate positively with hematocrit, but not with hemoglobin concentration or total heart mass, in two species of lizards (16, 19). In two additional species of lizards (*Callisaurus draconoides* and *Cnemidophorus tigris*), $\dot{V}O_{2\max}$ did not correlate with any of these three traits (Garland, unpublished observations). However, Schall et al. (28) reported a positive correlation between $\dot{V}O_{2\max}$ and hemoglobin levels in a fifth species. (In the present study, we were unable to measure hematocrit in addition to hemoglobin levels due to the small volume of blood available from newborn garter snakes.) Walsberg

et al. (36) reported that both hemoglobin concentration and ventricle mass explained significant fractions of the variation in exercise oxygen consumption among individual bullfrogs but that variation in maximum heart rate did not. However, their data for both $\dot{V}O_{2\max}$ and ventricle mass were expressed and analyzed on a per gram body mass basis rather than computing residuals from regression equations (3, 16, 17, 19, present study), so it is not clear that possible confounding effects of body mass were effectively eliminated.

The positive phenotypic correlation between $\dot{V}O_{2\max}$ and ventricle mass is attributable to a relatively strong and positive genetic correlation ($r_G = +0.64$ by maximum likelihood) acting in combination with a weaker (and not significant) negative environmental correlation ($r_E = -0.48$; Table 3). The genetic correlation suggests that some genes influence both $\dot{V}O_{2\max}$ and ventricle mass in similar directions. This genetic coupling might reflect the presumed mechanistic effect of ventricle size on stroke volume, cardiac output, and hence oxygen delivery to the tissues, or perhaps more subtle interactions. Note that our estimates of genetic correlations are also broad sense, because they include nonadditive genetic effects, if present. Genetic correlations may also be inflated by maternal effects and by common family environmental effects, although the latter was disrupted at birth.

In simple linear regressions, only residual maximal oxygen consumption was a statistically significant predictor of residual treadmill endurance times ($r^2 = 2.8\%$, $P = 0.009$). Thus individuals with a relatively high $\dot{V}O_{2\max}$ tended to have relatively high endurance capacities, after removing the confounding effects of body size, age at time of testing, etc. Neither residual hemoglobin concentration nor residual ventricle mass added significantly to this predictive ability in a multiple regression. The correlation between treadmill endurance and $\dot{V}O_{2\max}$ is lower than has been found in three species of lizards (*Callisaurus draconoides*, Garland, unpublished observations; *Ctenosaura similis*, 16; *Dipsosaurus dorsalis*, 23), but no significant correlation exists in two other species of lizards (*Cnemidophorus tigris*, Garland, unpublished observations; *Ctenophorus nuchalis*, 19). How well variation in suborganismal characters predicts individual variation in endurance depends in a complicated fashion on how well they indicate metabolic capacities that are

important for the particular type of endurance test (see discussions in Refs. 18, 19). In the present study, the low predictive power of $\dot{V}O_{2\max}$ and of two indicators of oxygen transport and delivery (hemoglobin levels and ventricle mass) may reflect the fact that average endurance times of 6 min (17) were short enough that anaerobic metabolism was of considerable if not primary importance in supplying ATP for muscular contraction (4, 5).

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