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UNIVERSITY OF CALIFORNIA,
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Predictors of Maximal Oxygen Consumption During Exercise, and Ecological and Behavioral Correlates in Lizards and Mammals

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Ralph Lacerda de Albuquerque

September 2019

Dissertation Committee:
Dr. Theodore Garland, Jr., Chairperson
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*“It’s a long way to the top
if you wanna rock and roll.”*

- Angus Young, Malcolm Young, and Bon Scott.

“For those about to rock, we salute you.”

- Angus Young, Malcolm Young, and Brian Johnson.

“[...] o único sentido oculto das cousas é elas não terem sentido oculto nenhum”

“[...] the only hidden meaning of things is that they have no hidden meaning”

- Fernando Pessoa.

ABSTRACT OF THE DISSERTATION

Predictors of Maximal Oxygen Consumption During Exercise, and Ecological and Behavioral Correlates in Lizards and Mammals.

by

Ralph Lacerda de Albuquerque

Doctor of Philosophy,
Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, September 2019
Dr. Theodore Garland, Jr., Chairperson

The current paradigm in animal organismal biology links morphology, performance, behavior, and Darwinian fitness in a sequence of proximate causal relations. First, the combination of lower-level organismal traits (e.g., leg length, heart mass, muscle enzyme activities) generate performance levels (e.g., maximal sprint speed, maximal oxygen consumption during exercise [$\dot{V}O_{2max}$]); in turn, maximal performance constrain behavioral options, and the consequences of behavioral choices influence fitness. Because behavior and performance are the last steps leading to fitness, selection acts more directly on them than on lower-level (subordinate) traits. Performance and behavior are, therefore, pivotal points in understanding evolutionary adaptation. A primary determinant of an animal's aerobic performance capacity is $\dot{V}O_{2max}$ which sets the limit to the intensity of physical effort that can be sustained over prolonged periods. Only during relatively rare occasions (e.g., during predator-prey

interactions) do animals perform at levels above $\dot{V}O_2\text{max}$; therefore, most activities will be constrained by their capacity to consume oxygen. Many key events involve mostly aerobic levels of activity (e.g., foraging, patrolling territories), making $\dot{V}O_2\text{max}$ a likely target for natural and sexual selection.

This dissertation investigated $\dot{V}O_2\text{max}$ in two contexts. In chapters one and two I put $\dot{V}O_2\text{max}$ into an historical evolutionary context, evaluating the coadaptation of $\dot{V}O_2\text{max}$ and ecological traits among lizard and mammal species. These studies revealed that $\dot{V}O_2\text{max}$ is weakly positively correlated with home range area among species of mammals, that viviparous lizards have lower $\dot{V}O_2\text{max}$ than non-viviparous species, and that varanids, helodermatids, and skinks have higher $\dot{V}O_2\text{max}$ than other groups of lizards.

In the third chapter, I use a mechanistic approach to elucidate the proximate causes and consequences of $\dot{V}O_2\text{max}$ at the level of among-individual variation. I found that $\dot{V}O_2\text{max}$ in adult male *Sceloporus occidentalis* lizards from Hampton Butte, Oregon during the breeding season is positively related to hematocrit (or hemoglobin) levels in the blood and to territorial behaviors (number of push-ups using two or four legs per bout of push-ups).

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GENERAL INTRODUCTION

The maximal amount of oxygen an animal can consume during exercise ($\dot{V}O_2\text{max}$) is a key aspect of exercise physiology and performance. $\dot{V}O_2\text{max}$ is not a measure of locomotor performance *per se* (Careau & Garland, 2012), but it sets the upper limit to any kind of effort that needs to be sustained over relatively long periods (more than a minute or so; Seeherman *et al.*, 1981; Jones & Lindstedt, 1993; Levine, 2008; Spurway *et al.*, 2012). It is tightly linked to maximum sustainable speed and endurance capacity, and hence may potentially limit activity levels during ecologically relevant tasks (e.g., patrolling a territory, fighting, courting, foraging). From an evolutionary perspective (ultimate causation), $\dot{V}O_2\text{max}$ varies among phylogenetic lineages in relation to their ecology (e.g., Clemente *et al.*, 2009, p. 200; Killen *et al.*, 2016). $\dot{V}O_2\text{max}$ also varies among individuals within populations, and this variation can be exploited to elucidate the proximate causes of variation in $\dot{V}O_2\text{max}$ (e.g., Garland, 1984; Garland & Else, 1987).

Ultimate causes of $\dot{V}O_2\text{max}$ (ecological correlates among species)

In principle, individual variation in $\dot{V}O_2\text{max}$ should be one of numerous factors that contribute to variation in reproductive success. Because $\dot{V}O_2\text{max}$ is a heritable trait (Garland & Bennett, 1990; Garland *et al.*, 1990b; Dohm *et al.*, 2001; Wone *et al.*, 2015), natural and/or sexual selection across generations can lead to differentiation among populations, species, and eventually major

phylogenetic lineages. For example, higher capacity to consume oxygen during exercise can be advantageous because animals would be able to maintain higher activity levels aerobically (Chappell & Snyder, 1984; Hayes, 1989) leading to the simultaneous evolution (coadaptation) of $\dot{V}O_2\text{max}$ and activity levels. This relation was empirically tested in laboratory house mice selectively bred for voluntary aerobic exercise (Swallow *et al.*, 1998). In that experiment, the four selected lines of mice showed significantly higher $\dot{V}O_2\text{max}$ than the four control (random-bred) lines after 10 generations, providing evidence for the potential adaptive significance of $\dot{V}O_2\text{max}$ to high activity levels.

Some lineages within lizards and mammals have relatively high $\dot{V}O_2\text{max}$, possibly associated with specific ecological conditions, and higher activity levels. Among mammals, $\dot{V}O_2\text{max}$ is significantly higher in canids and in *Equus caballus*, *Antilocapra americana*, and *Phyllostomus hastatus* (Dlugosz *et al.*, 2013), whereas among lizards, helodermatids and varanids have higher $\dot{V}O_2\text{max}$ than other lineages (Beck *et al.*, 1995; Clemente *et al.*, 2009). Even though mammals have much higher $\dot{V}O_2\text{max}$ than lizards (approximately six-fold difference on average), some species of varanids show $\dot{V}O_2\text{max}$ values almost as high as those of some mammals (Garland & Albuquerque, 2017).

Several studies have investigated ecological correlates of $\dot{V}O_2\text{max}$ among species or higher taxa in an attempt to describe evolutionary patterns for that trait. For example, among lizards, researchers found higher $\dot{V}O_2\text{max}$ in more active species in nature (e.g., active foragers) than closely related counterparts

that are more sedentary (Bennett *et al.*, 1984; Clemente *et al.*, 2009). Among species of geckos, nocturnal species have lower $\dot{V}O_{2\max}$, but that difference seems to be caused mostly by the lower temperatures at which they operate in nature (chapter 2; Autumn *et al.*, 1999). Among varanids, $\dot{V}O_{2\max}$ is positively correlated with maximal endurance and stamina, which are higher in active foraging species and in species from xeric environments (Clemente *et al.*, 2009). Among mammals, $\dot{V}O_{2\max}$ is weakly, but positively, correlated with home range area (chapter 1, also published as Albuquerque *et al.*, 2015b), a trait that should be associated with higher activity levels. And finally, among teleost fish, $\dot{V}O_{2\max}$ is higher for pelagic species and for species from higher trophic levels (Killen *et al.*, 2016).

Proximate causes and consequences of $\dot{V}O_{2\max}$ among individual lizards

As for any other whole-animal performance capacity, $\dot{V}O_{2\max}$ arises from lower-level suborganismal traits, and variation in $\dot{V}O_{2\max}$ may in turn have consequences for behavior and Darwinian fitness (Garland & Kelly, 2006). This flow of causal relations among levels of biological organization above and below whole-animal performances can be identified today as the dominant paradigm in organismal biology, termed the 'ecomorphology paradigm'. This paradigm, originally proposed by Arnold (1983) and subsequently expanded by other authors (e.g., Garland & Losos, 1994; Aerts *et al.*, 2000; Lailvaux & Husak, 2014; Storz *et al.*, 2015; Orr & Garland, 2017), structures ecomorphological relations in

causal steps from morphology to performance, from performance to behavior, and finally from behavior to Darwinian fitness. The directionality of these causal links implies that an animal's reproductive success should be proximally influenced (and possibly limited) by their capacities at simpler tasks, such as sprinting or sustaining effort. These capacities are limited by lower-level traits ranging from biochemical to morphological, that in combination determine whole-animal performance (Garland & Kelly, 2006). Moreover, behavior may serve as an important "filter" between selection and performance (Garland *et al.*, 1990b; Garland, 1994b; Garland & Carter, 1994; Garland & Losos, 1994).

Morphology here is a shorthand term that refers to any lower-level, subordinate trait, so the pathway can be expanded and detailed as much as it suits the study system. For example, one can include under "morphology" measures of tissue-specific enzyme activities, organ masses, or external linear measurements (e.g., Garland, 1984; Garland & Else, 1987; Kohlsdorf & Navas, 2012). Performance refers to the animal's capacity to accomplish a task that requires the use of the whole body when maximally motivated (Careau & Garland, 2012). To enhance ecological relevance (Arnold, 1983; Irschick & Garland, 2001), these performances should be directly related to key aspects of the animal's ecology or day-to-day activities, such as escaping predators, defending territory, foraging, or searching for mates. If they are related to these types of natural behaviors, then they should affect overall reproductive success (Darwinian fitness).

Very few studies have investigated the proximate causes of intraspecific variation in lizard $\dot{V}O_2\text{max}$. In adult male *Dipsosaurus dorsalis*, mass-specific $\dot{V}O_2\text{max}$ was positively correlated with mass-specific gastrocnemius muscle citrate synthase activity (John-Alder, 1983). However, some of this positive correlation may have been related to effects of body mass, as residuals from regressions on body mass were not analyzed. Two other studies analyzed residuals from regressions on body mass. These studies included juveniles, both sexes, and/or lizards collected throughout the year and, consequently, encompassed a much wider variation in $\dot{V}O_2\text{max}$ and its subordinate traits (Garland, 1984; Garland & Else, 1987). In the first study, $\dot{V}O_2\text{max}$ in *Ctenosaura similis* was positively related to citrate synthase activity in the liver and thigh muscle, lactate dehydrogenase activity in the heart, and hematocrit levels (Garland, 1984). In the second study, $\dot{V}O_2\text{max}$ in *Amphibolurus nuchalis* was negatively related to thigh pyruvate kinase, liver mass, and positively related to hematocrit levels (Garland & Else, 1987).

Garland and Losos (1994) expanded Arnold's paradigm and included behavior as an additional step between performance and fitness (see also Garland *et al.*, 1990b; Garland, 1994b; Garland & Carter, 1994). Because performance limits constrain what an animal can do, they may constrain behavior in the wild. For example, when their body temperature is too cold to attain high sprint speeds, some lizard species switch from running to attacking when facing a predator (Hertz *et al.*, 1982; Crowley & Pietruszka, 1983; Herrel *et al.*, 2007;

Barros *et al.*, 2010). Because performance and behavior are the last steps leading to fitness, natural and sexual selection act directly on them (Garland & Kelly, 2006), and evolutionary changes in lower-level traits (e.g., morphology, physiology, biochemistry) happen as a consequence. Therefore, understanding performance and behavior variation and the (causal) relations between them is crucial to understand animal morphological and physiological adaptations.

Behaviors related to sexual selection (e.g., male-male disputes or courtship) can be costly (Daly, 1978; Höglundi *et al.*, 1992; Mowles & Jepson, 2015) and should be strongly affected by performance capacities. In territorial iguanid lizards, activity levels increase significantly during the breeding season (Ruby, 1978; John-Alder, 1984b; Baird *et al.*, 2001). During that period, in addition to the everyday foraging and thermoregulatory activities, individuals must patrol territories, engage in disputes and chase intruders away, and invest in courtship and mating activities (Fitch, 1940; Stamps, 1978; Sheldahl & Martins, 2000). These additional activities should increase the frequency and intensity of movements (e.g., number of moves and distance traversed) and social interactions (e.g., push-up displays).

Finally, very few studies have tried to connect lower-level traits, performance, and behavior simultaneously, as mentioned by Sinervo (1995): "Whereas several of the chapters build on the conceptual aspects of Arnold's paradigm (Garland and Losos add analysis of behavior, Chapter 10), it is clear that there are very few studies in which the complete paradigm has received

empirical treatment." That observation is still true today. Connecting multiple levels of biological organization in a single study is difficult. Each type of variable requires different technical resources and trained researchers to use them. Observing lizard behavior requires many hours of observation to obtain reliable data. Measuring performance in the laboratory requires specialized and expensive equipment (e.g., racetrack, oxygen analyzer). And measurement of several lower-level traits requires time-sensitive procedures during dissection (e.g., enzyme activities, hemoglobin concentration), so multiple trained investigators are needed.

In this dissertation, I investigate the ecological correlates of $\dot{V}O_{2\max}$ among lizard and mammal species (chapters 1 and 2), casting some light on the possible evolutionary meaning of that trait, and I execute the first study to connect multiple levels of biological organization centered on variation in $\dot{V}O_{2\max}$ among individual lizards (chapter 3).

CHAPTER 1. - Relationship between maximal oxygen consumption ($\dot{V}O_2\text{max}$) and home range area in mammals.

Abstract

Home range is defined as the area traversed during normal daily activities, such as foraging, avoiding predators, and social or antagonistic behaviors. All else being equal, larger home ranges should be associated with longer daily movement distances and/or higher average movement speeds. The maximal rate of oxygen consumption ($\dot{V}O_2\text{max}$) generally sets an upper limit to the intensity of work (e.g., speed of locomotion) that an animal can sustain without fatigue. Therefore, home range area and $\dot{V}O_2\text{max}$ are predicted to evolve in concert (coadapt).

We gathered literature data on home range and $\dot{V}O_2\text{max}$ for 55 species of mammals. We computed residuals from log-log (allometric) regressions on body mass with two different regression models: ordinary least squares (OLS) and phylogenetic generalized least squares (PGLS). Residuals were weakly positively related for both the OLS ($r = 0.278$, one-tailed $P < 0.05$) and PGLS ($r = 0.210$, $P < 0.05$) regressions. For $\dot{V}O_2\text{max}$, the PGLS regression model had a slightly higher likelihood than the OLS model, but the situation was reversed for home range area. In addition, for both home range area and $\dot{V}O_2\text{max}$, models that fit better than either OLS or PGLS were obtained by modeling residual variation with the Ornstein-Uhlenbeck process to mimic stabilizing selection

(RegOU), indicating that phylogenetic signal is present in both size-adjusted traits, consistent with findings of previous studies. (However, residuals from the RegOU models cannot be tested for correlation due to mathematical complexities.) We conclude that the best estimate of the residual correlation is probably somewhere between these two values reported above. Possible reasons for the low correlation between residual home range area and $\dot{V}O_2\text{max}$ are discussed.

Introduction

The idea that physiological performance abilities should evolve in a correlated fashion (coadapt) with aspects of an organism's behavioral ecology is a central tenant of ecological and evolutionary physiology (Garland & Carter, 1994; Garland & Losos, 1994; Angilletta *et al.*, 2006). For example, among species of *Anolis* lizards, maximal sprint speed measured in the laboratory correlates positively with both escape speed and feeding speed measured in the field (Irschick & Losos, 1998). Also, among lizard species, treadmill endurance capacities are positively correlated with daily movement distance, the percentage of time spent moving, and the number of moves per minute (Garland, 1999). Other than those two studies, we are not aware of any that have demonstrated a cross-species correlation between quantitative measures of locomotor behavior in the field and aspects of exercise abilities through phylogenetically based statistical analyses. Therefore, the purpose of the present study was to take advantage of a recent comparative analysis of mammalian maximal oxygen consumption (Dlugosz *et al.*, 2013) to test for a positive association with home range size, a widely reported measure of field locomotor behavior in mammals (Kelt & Van Vuren, 2001).

As defined by Burt (1943), home range is the area traversed by an animal in its normal activities of food gathering, mating, and caring for young. All else being equal, a larger home range area would imply an increase in the distance moved per unit time (e.g., per day), the amount of time spent moving, or both.

For terrestrial locomotion, it is less costly to cover a greater distance by increasing speed rather than increasing the amount of time spent moving at a given speed (e.g., Rezende *et al.*, 2009 and references therein). Moving faster to cover a greater distance would also minimize the amount of time spent exposed to predators or other environmental hazards, such as high temperatures. The speed of locomotion (intensity of activity) that can be sustained by an animal for a prolonged period of time is limited by its aerobic capacity, which, in terrestrial vertebrates, is typically measured as the maximal rate of oxygen consumption during forced treadmill exercise (Seeherman *et al.*, 1981). In general, $\dot{V}O_2\text{max}$ defines the upper limit to the rate of work that can be sustained for more than a brief period. If a larger home range area implies a higher average rate of movement, then a positive correlation between home range area and $\dot{V}O_2\text{max}$ would be expected.

To test this prediction in an evolutionary framework, we gathered literature data for 55 species of mammals. We computed residuals from log-log regressions on body mass and then tested the residuals for correlation by use of both conventional and phylogenetically informed statistical methods (Rezende & Diniz-Filho, 2012).

Methods

All of the $\dot{V}O_2\text{max}$ data were obtained from Dlugosz *et al.* (2013). We used various internet search tools (especially Google, Google Scholar, and Web of

Science) to obtain home range data for as many species as possible that were also included in the study by Dlugosz et al. (2013).

We found usable home range data for 55 species. Home range area can be affected by several methodological factors. For example, precision of location data and whether it is constrained to occur on a pre-defined grid will vary among techniques, e.g., traps versus radio telemetry. Also, the different ways to calculate the home range area from location data can result in different area values, even if calculated from the same data set. For example, whereas inclusive boundary strip adds half the distance between traps as part of the home range, the minimum convex polygon method would only consider the area inside the polygon created from connecting the outermost points. Therefore, we also noted the method used to locate animals (direct observation, trapping, and telemetry [we considered radio, satellite, and radioactive telemetry as one category), the area calculation method (Minimum Convex Polygon, Kernell, inclusive/exclusive boundary strips, and 95% probability ellipses), the season in which the animals were captured (fall, spring, summer, winter, and all year [we considered the study all year if it included all seasons or both winter and summer), the sex (males, females, pooled), and duration of the study in months. These were used as cofactors when computing residual home range area. When body mass was not reported in the original home range study, it was taken from another paper with an average for the species used, or the mass from Dlugosz et

al. (2013) was used. For more details on how the data were treated, refer to the Supplementary Material.

In Mesquite version 2.75, we trimmed the phylogenetic tree of Dlugosz et al. (2013) to 55 species (tips), to match the species with home range data, including the Arctic Fox (*Alopex lagopus*), which was inadvertently omitted from the analyses presented in that paper. Following Dlugosz et al. (2013), branch lengths were set by the arbitrary method of Pagel (1992) (Fig. 1.1). The phylogeny was exported from Mesquite as a PDI file, then imported to the DOS PDDIST program to create the phylogenetic variance-covariance matrix (Garland & Ives, 2000).

In Matlab version 6.1, we used the Regressionv2.m program of Lavin et al. (2008) to regress $\log_{10} \dot{V}O_{2\max}$ and \log_{10} home range area on \log_{10} body mass using Ordinary Least Squares (OLS), Phylogenetic Generalized Least Squares (PGLS), and a phylogenetic regression model that allows the branches to vary in length according to an Ornstein-Uhlenbeck process to mimic stabilizing selection on the residuals (RegOU). These models are discussed in detail in Lavin et al. (2008; see also Rezende & Diniz-Filho, 2012). Briefly, the OLS method analyzes the data assuming a star phylogeny with contemporaneous tips (usually called conventional or nonphylogenetic analysis), PGLS uses the phylogenetic tree as input, and RegOU allows the nodes of the tree to move between the root and tips of the tree and hence the tree varies continuously between a star and the original tree, or even a more hierarchical tree. In the RegOU model, a parameter named

" d " is estimated by restricted maximum likelihood, simultaneously with estimation of the regression parameters, to determine the optimal amount of "stretching" or "compressing" of the tree as the nodes move between the root and tips (Garland & Ives, 2000; Blomberg *et al.*, 2003). A d value higher than 1 means that the nodes of the tree were pushed closer to the tips (i.e., making the tree more hierarchical), while a value smaller than 1, indicates the nodes were pulled towards the root, making it somewhat closer to a star phylogeny. In the limit, d can be estimated as zero, which indicates that a star phylogeny best fits the regression residuals. Any estimated d value greater than zero indicates the presence of phylogenetic signal in the residuals.

The residuals obtained from the $\dot{V}O_{2\max}$ and home range regressions for OLS and PGLS models were then correlated (one for each regression model) in SPSS Version 22.0. We did not use residuals from the RegOU models because they are not strictly comparable when the estimated d values differ. For home range area, we computed two sets of regressions, one including body mass plus all the co-factors listed above and another set including only body mass so that it would be directly comparable to the $\dot{V}O_{2\max}$ regressions. When correlating 55 residual data points, the nominal degrees of freedom would be 53, with a corresponding critical value of 0.224 for a 1-tailed test. However, it is appropriate to reduce d.f. to account for the parameters estimated when computing the regression models used to calculate residuals. For $\dot{V}O_{2\max}$, only body mass was used as an independent variable, but for the full model used with home range

area an additional 10 dummy variables were included to account for variation related to calculation method, season, etc. The exact number of d.f. that should be subtracted is not entirely obvious, so we also consider significance levels as if we had 43 d.f., for which the 1-tailed critical value would be 0.248.

In principle, a better approach to test for a relation between two variables could be to include one (e.g., $\dot{V}O_2\text{max}$) as an additional independent variable in a multiple regression model predicting the other (e.g., home range size)(e.g., see Freckleton, 2002). That would solve certain statistical issues, including the calculation of d.f., and also allow use of the more flexible RegOU model. Unfortunately, multiple regressions can be unreliable when the correlation between independent variables is strong (collinearity or multicollinearity). In the present case, log body mass (for home range area) and log $\dot{V}O_2\text{max}$ have a correlation of 0.972. Adding log $\dot{V}O_2\text{max}$ to the multiple regression model for log home range area results in a very high Variance Inflation Factor (VIF) of 21.360 for log body mass and 21.798 for log $\dot{V}O_2\text{max}$. Most statistical sources warn strongly about VIF values above 10, and some warn about values of even 4 or 5. Therefore, we not comfortable with the estimated partial correlations or P values for log $\dot{V}O_2\text{max}$ in the present analysis. In addition, we have separate estimates of body mass for the two traits of interest, and the multiple regression approach would only accommodate one, perhaps an average, but that would induce additional error into the estimate of body mass.

Results

Figure 1.2A shows a scatterplot of home range area with body mass. As shown in Table 1.1, the ln maximum likelihood for the RegOU multiple regression (-60.1488) was high enough to be considered significantly better ($P = 0.0159$ and $P = 0.0016$, respectively) than the OLS (-63.0550) or PGLS (-65.1521) models, based on a ln likelihood ratio test with one degree of freedom to account for the additional parameter estimated in the RegOU model (Lavin *et al.*, 2008; e.g., see Gartner *et al.*, 2010). Hence, the tree used in the best-fitting model was intermediate between a star and the original tree (estimated OU parameter, $d = 0.337$), and residual home range area contains statistically significant phylogenetic signal. The foregoing comparisons are based on "full" models predicting home range, which included additional cofactors and covariates related to methodology. For the models that used only body mass as an independent variable, the estimated d value in the RegOU model was zero, indicating that a star phylogeny better fit the data than did the hierarchical tree shown in Figure 1.1. We then compared the full model fitted by RegOU (ln maximum likelihood = -60.1488) with the minimal OLS model (only log body mass as an independent variable, ln maximum likelihood = -70.9329), and found that twice the difference in ln likelihoods (21.5682) would be considered statistically significant with 12 d.f. ($P = 0.0427$). Therefore, we conclude that the full RegOU model is the best-fitting for home range area.

Figure 1.2B shows that, for a given body mass, $\dot{V}O_{2\max}$ is much less variable than home range area (Fig. 1.2A). For the $\dot{V}O_{2\max}$ regressions (Table 1.1), the ln maximum likelihood for the RegOU regression (9.9644) was high enough to be considered significantly better ($P = 0.0029$ and $P = 0.0016$, respectively) than the OLS (5.5224) or PGLS (6.8137) models, based on a ln likelihood ratio test with one degree of freedom. So, as for home range area, residual $\dot{V}O_{2\max}$ contains significant phylogenetic signal, but not as much as would be implied by the tree shown in Figure 1.1, similar to the findings of Dlugosz et al. (2013) for the larger data set.

The correlation of OLS residuals (Fig. 1.3A) was positive ($r = 0.278$) and statistically significant (1-tailed $P < 0.05$ with either 53 or 43 d.f.). The correlation of residuals from PGLS models was lower and statistically non-significant ($r = 0.210$, 1-tailed $P > 0.05$). Note that for home range area, the OLS model fits the data somewhat better than the PGLS model, based on a comparison of the likelihoods (Table 1.1), whereas for $\dot{V}O_{2\max}$ the converse is true.

Discussion

Contrary to our expectation, we found only weak evidence for a positive correlation between residual home range area and $\dot{V}O_{2\max}$. Several explanations are possible, and they are not mutually exclusive.

One possibility is that the data include too much "measurement error" (in the broad sense of Ives *et al.*, 2007), which obscures any correlation. Methods

for calculating home range area are much more variable than for measurement of $\dot{V}O_2\text{max}$, and this difference could partly account for the much greater variability of home range area at any given body mass (Fig. 1.2). Indeed, the phylogenetic RegOU model that included additional independent variables to partially account for measurement-related variation fit the home range data significantly better. Many studies have reported variation in home range size, adjusting for variation in body mass, among populations of the same species of mammal (McNab, 1963; Grant *et al.*, 1992; Herfindal *et al.*, 2005). As the home range and $\dot{V}O_2\text{max}$ data analyzed here did not come from the same populations of each species, population differences likely contributed to the "measurement error" in our data, as this also refers to some aspects of real biological variation in the trait being studied (Ives *et al.*, 2007). Similarly, variation related to age, sex or season could have diluted the true correlation between home range area and $\dot{V}O_2\text{max}$.

The d values observed in RegOU models indicate how the tree was altered to best fit the data in the statistical models. For both home range area and $\dot{V}O_2\text{max}$, the estimated d was significantly greater than zero, indicating that the variance-covariance matrix of the residuals was not the identity matrix or, equivalently, that the best-fitting phylogenetic tree was not a star. At the same time, the d values were less than one, indicating that the altered tree is somewhat less hierarchical than the one shown in Figure 1.1 (i.e., the nodes of the tree were pulled towards the root, making the tree less hierarchical). Thus, for

this set of species, phylogenetic signal is not as strong for home range area as it is for $\dot{V}O_2\text{max}$, a result consistent with the overall pattern documented previously (Blomberg *et al.*, 2003). Whether this result means that home range is more biologically variable or more evolutionarily labile than $\dot{V}O_2\text{max}$ cannot be addressed without a detailed consideration of measurement error in the broad sense (Blomberg *et al.*, 2003; Ives *et al.*, 2007; Revell *et al.*, 2008), which is not possible with the data presently available.

Although we have emphasized that measurement error of various types may have obscured a true correlation between home range size and $\dot{V}O_2\text{max}$, it is important to note that other studies have successfully identified interspecific correlates of home range size and $\dot{V}O_2\text{max}$, or related measures, with phylogenetically informed analyses. For example, $\dot{V}O_2\text{max}$ has been shown to correlate positively with brain size among species of mammals (Raichlen & Gordon, 2011). Kelly *et al.* (2006) reported positive correlations between residual home range area and both residual hindlimb length and residual metatarsal/femur ratio in both Carnivora and ungulates (although the home range - leg length correlation failed to reach statistical significance in the ungulate sample). Among species of lizards, treadmill endurance capacity is positively related to daily movement distance, the percentage of time spent moving, and the number of moves per minute (Garland 1999). Bowman *et al.* (2002) found that dispersal distance and distance moved after translocation in mammals also correlate with home range area, after controlling for body size, although this

study used only conventional regression and correlation analyses. Harris and Steudel (1997) observed that residual hind-limb length in Carnivora is not correlated with home range or daily movement distance (contra Kelly et al. 2006), but is related to prey capture mode. Hence, we do not believe that the lack of correlation we observed reflects only high measurement error in the data for home range area and/or $\dot{V}O_2\text{max}$.

Finally, we note that the somewhat different correlations observed with the residuals obtained from conventional and phylogenetic analyses provide another example of how ignoring phylogenetic relationships can affect results (Garland *et al.*, 1993, 2005; Rezende & Diniz-Filho, 2012; White & Kearney, 2014; references therein). As an example closely related to the present study, Harris and Steudel (1997) reported a positive correlation ($r = 0.263$, 2-tailed $P = 0.0597$) between home range area and residual hind-limb length that became non-significant with use of phylogenetically independent contrasts. Examination of the present data (Fig. 1.3A) indicate that the more positive correlation in the non-phylogenetic analysis may be largely attributable to Carnivora tending to have both large home ranges and high $\dot{V}O_2\text{max}$ for their body size.

Future Directions

Home range area is not necessarily the same as an animal's territory, which is a smaller area, inside the home range, that is actively defended against conspecifics and/or interspecific intruders (Burt 1943). Possibly, a tighter relationship exists between $\dot{V}O_2\text{max}$ and territory size, given that the movements

and attention dedicated to the territory may generally be more intense than for the home range as a whole. Unfortunately, information on territory size is much less available.

Alternatively, daily movement distance (Garland 1983) or possibly average movement speed or some other metric (e.g., see Garland 1999; Perry 1999) might have a tighter relationship with $\dot{V}O_{2\max}$ than the area of the home range. Some animals might have a small home range area but be more active and move more frequently inside that area, possibly close to the maximum aerobic capacity (e.g., squirrels), thus requiring a higher $\dot{V}O_{2\max}$, all else being equal. Movement distances and speeds, like home range area, will also be affected by such ecological factors as substrate availability, habitat type, productivity, density of conspecifics and other species, etc., which were not considered here. It will also be of interest to test for a relationship between $\dot{V}O_{2\max}$ and dispersal distance in mammals (Bowman et al. 2002).

Finally, as more data become available, it will be important to test possible clade differences in relationships, as apparently is the case with Carnivora (see Fig. 1.3A).

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Figure Legends

Figure 1.1. Phylogeny used to compute phylogenetic regressions (PGLS and RegOU). This tree was trimmed from the original provided by Dlugosz et al. (2013), and we added *Alopex lagopus*, which was unintentionally omitted in their paper. Branch lengths were then set using the arbitrary method of Pagel (1992), following Dlugosz et al. (2013). The 2-character codes at the start of the scientific names correspond to codes in the file of raw data provided in the online Supplemental Material.

Figure 1.2. Scatterplots of (A) \log_{10} home range area and (B) $\log_{10} \dot{V}O_2\text{max}$ versus \log_{10} body mass for 55 species of mammals.

Figure 1.3. Scatterplot of residual \log_{10} home range area versus residual $\log_{10} \dot{V}O_2\text{max}$ based on (A) conventional OLS regressions and (B) phylogenetic generalized least squares (PGLS) models. For (A), the correlation using all data points ($r = 0.278$) is statistically significant, but for (B) it is not ($r = 0.212$).

Figure 1.1.

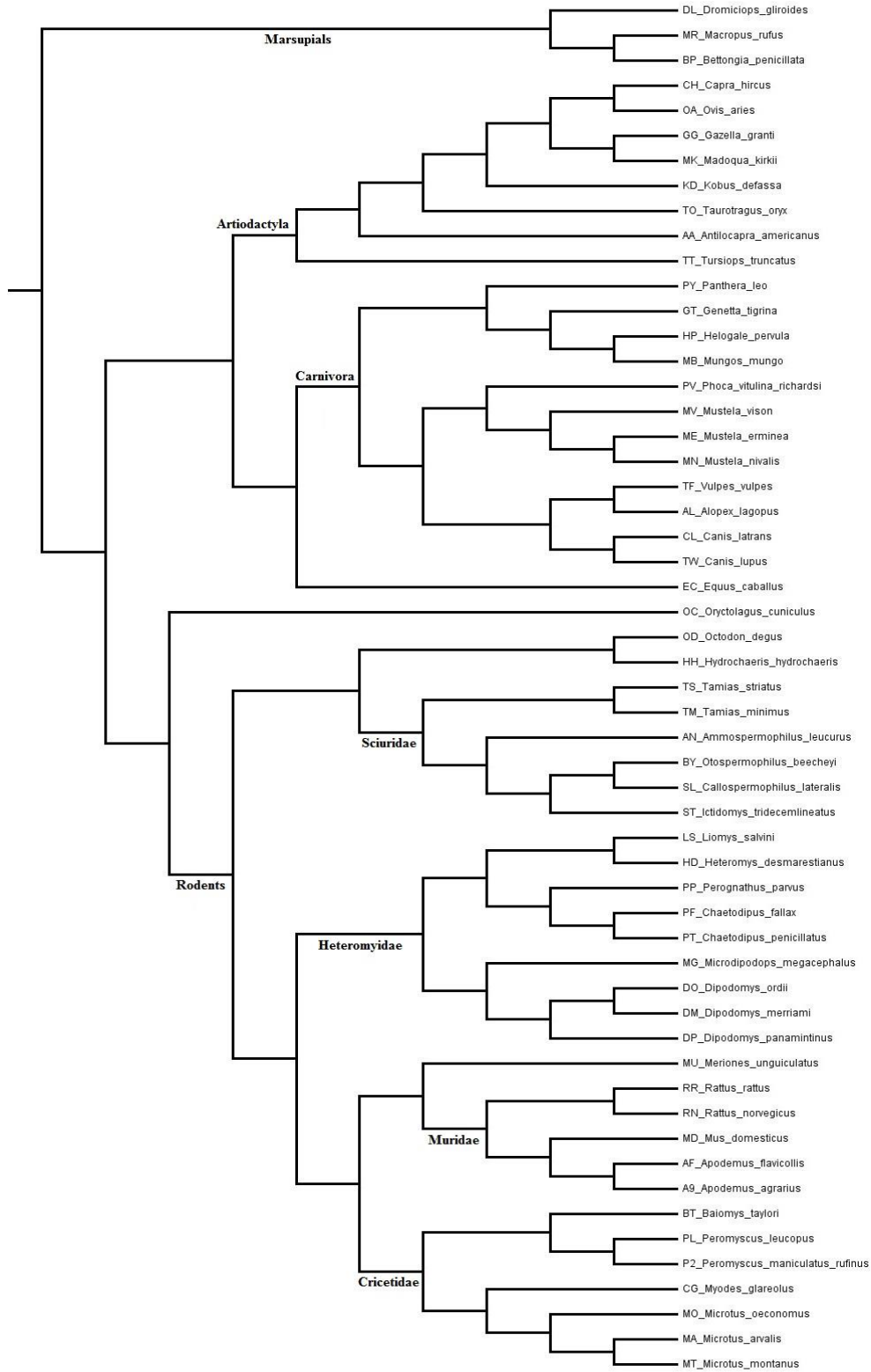
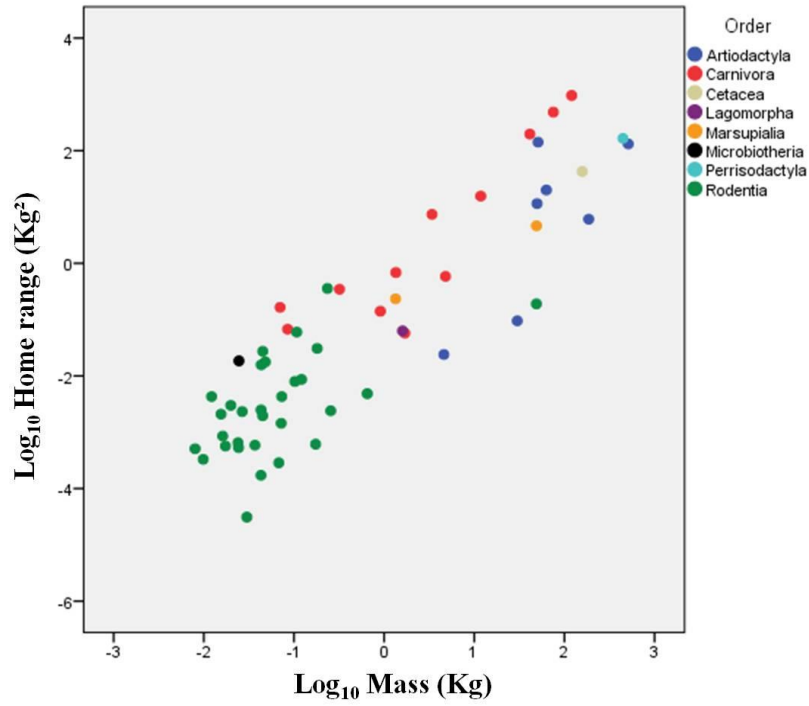


Figure 1.2.

(A)



(B)

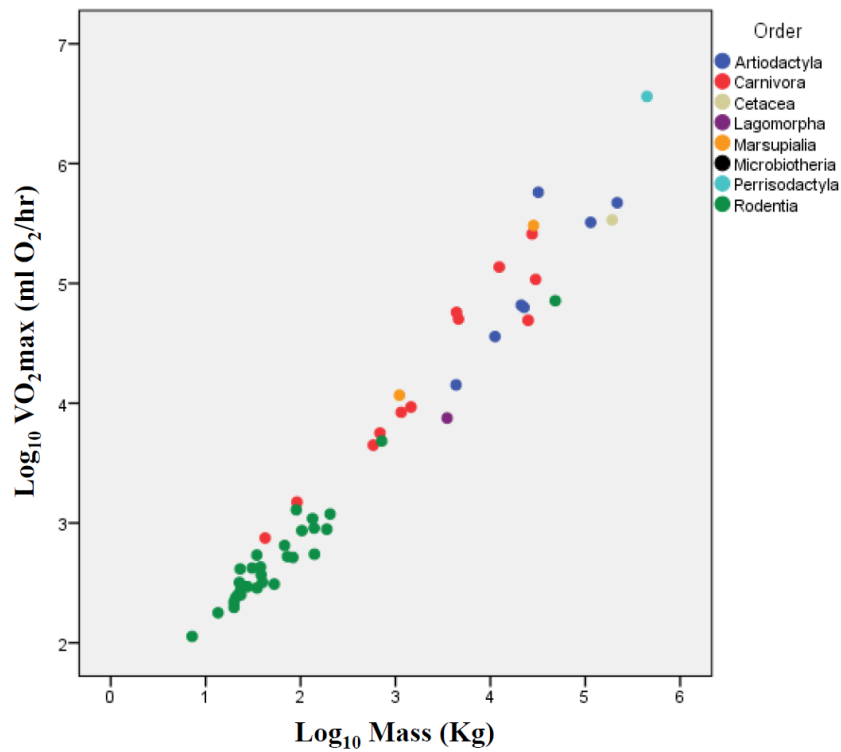
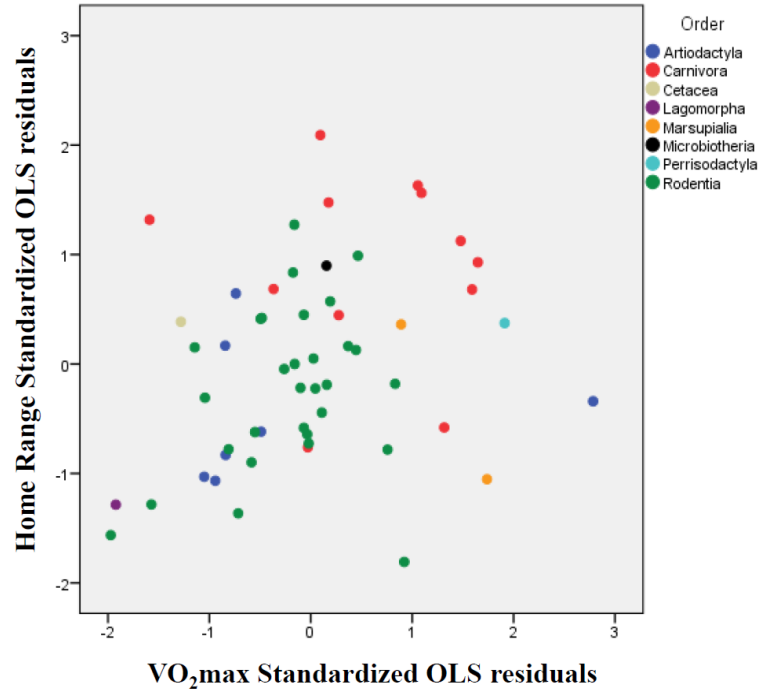


Figure 1.3.

(A)



(B)

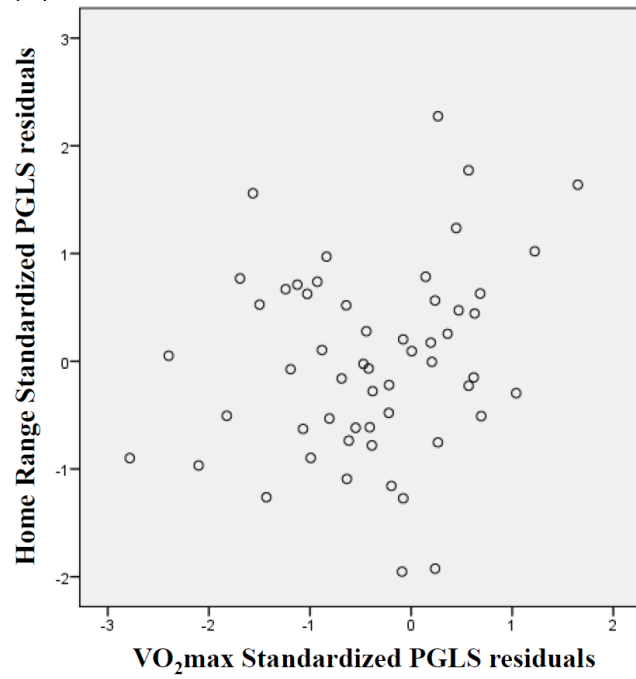


Table 1.1. Parameter estimates for regressions of $\log_{10} \dot{V}O_{2\max}$ (ml O₂/hr) and \log_{10} home range area (square kilometers) on \log_{10} body mass (kilograms). For the home range regressions, we also included covariates and cofactors related to methodological variation (see Methods: those additional parameter estimates are not shown here). The OLS and PGLS regression models were used to compute residuals, which were then tested for correlation (see Results and Figure 1.3). For both dependent variables, the best-fitting models, based on ln maximum likelihood ratio tests, were the RegOU (see Results).

Dependent Variable	Model	<i>d</i>	Y intercept	SE	Slope	SE	ln max. likelihood	Mean Squared Error	SE of Estimate	R ² &
Home Range	OLS		-5.3312	1.2661	1.3204	0.1303	-63.0550	0.7593	0.8714	0.8390
Home Range	PGLS		-4.7801	1.5022	1.1421	0.1360	-65.1521	0.8195	0.9053	0.7684
Home Range	RegOU	0.3369	-5.0512	1.2501	1.2625	0.1267	-60.1488	0.6832	0.8266	0.7996
$\dot{V}O_{2\max}$	OLS		1.2264	0.0673	0.8686	0.0224	5.5224	0.0497	0.2229	0.9660
$\dot{V}O_{2\max}$	PGLS		1.2900	0.1807	0.8392	0.0285	6.8137	0.0474	0.2178	0.9425
$\dot{V}O_{2\max}$	RegOU	0.4768	1.2597	0.0992	0.8489	0.0271	9.9644	0.0425	0.2061	0.9488

& Values are not comparable between OLS and the phylogenetic models. “*d*” is the OU transformation parameter that estimates the best-fitting amount of stretching or compressing of branch lengths (see Methods). For both traits, these values are significantly greater than zero, based on likelihood ratio tests comparing RegOU with PGLS models, thus indicating the presence of phylogenetic signal in the residuals (see text).

CHAPTER 2. - Phylogenetic analysis of maximal oxygen consumption during exercise ($\dot{V}O_2\text{max}$) and ecological correlates among lizard species

Abstract

Maximal aerobic capacity ($\dot{V}O_2\text{max}$), measured as the maximum amount of oxygen consumed during forced exercise, sets the upper limit to the rate of work that can be sustained over relatively long periods and, therefore can limit activity levels during ecologically relevant tasks. Among ectotherms, $\dot{V}O_2\text{max}$ is primarily affected by body size and body temperature, but it should also coadapt with behavior, ecology, and aspects of the life history. We compiled published data for 58 species of lizards plus 7 populations (total 65 data points) from 11 different families and tested whether $\dot{V}O_2\text{max}$ was related to diet (herbivore, insectivore, insectivore/carnivore, carnivore, and omnivore), climate category (tropical, temperate, and arid), nocturnality, viviparity, or phylogenetic lineage (monophyletic taxonomic family or infraorder), with body mass and body temperature as covariates. Preliminary analysis revealed that most of the effect of phylogenetic lineage was caused by Helodermatidae, Varanidae, and Scincidae, all with relatively high $\dot{V}O_2\text{max}$, in agreement with previous studies. We therefore analyzed models that included a set of three dummy variables coding for helodermatids, varanids, and skinks as compared with all other lizards. We fitted 48 models that included body mass, measurement temperature, and all possible combinations of other independent variables using both ordinary least-

squares (OLS) and phylogenetic regressions that assume an Ornstein-Uhlenbeck model of residual trait evolution (RegOU) (N = 96 total models). The sum of Akaike weights for each independent variable revealed viviparity ($\sum w_i = 0.996$) and the combined set of dummy variables coding for helodermatids, varanids and skinks ($\sum w_i = 0.996$) as the most important predictors. Viviparity had a negative effect on $\dot{V}O_{2\max}$, whereas helodermatids, varanids, and skinks all had relatively high $\dot{V}O_{2\max}$. The average allometric slope of $\dot{V}O_{2\max}$ from the top eight models (which accounted for 99% of the cumulative evidence) was 0.803 (95% confidence interval = 0.747-0.859), which is similar to that reported previously for lizards and mammals in general but not for varanids or nocturnal geckos.

Keywords: aerobic capacity, allometry, comparative methods, habitat, phylogeny, temperature, viviparity

Introduction

Maximal aerobic capacity, measured as the maximum rate of oxygen consumption during forced exercise (Seeherman *et al.*, 1981), sets the upper limit to the rate of work that can be sustained over relatively long periods of time (more than a few minutes) (Seeherman *et al.*, 1981; Jones & Lindstedt, 1993; Levine, 2008; Spurway *et al.*, 2012). It directly affects maximal sustainable speed and endurance and hence may potentially limit activity levels during ecologically relevant tasks (e.g., patrolling a territory, fighting; courting; foraging). Relatively few studies have tested for coadaptation between $\dot{V}O_2\text{max}$ and aspects of behavior, ecology or life history that intuitively might depend on high sustained metabolic rates (Bennett *et al.*, 1984; Autumn *et al.*, 1999; Clemente *et al.*, 2009; Albuquerque *et al.*, 2015b; Killen *et al.*, 2016).

In two lizard species, Bennett *et al.* (1984) found that the widely foraging *Eremias lugubris* has higher $\dot{V}O_2\text{max}$ and activity levels in the wild, as compared with the closely related *E. lineocellata*, but two-species comparisons cannot provide strong evidence regarding adaptation or coadaptation (Garland & Adolph, 1994). Autumn *et al.* (1999) compared eight species of nocturnal geckos with 24 species of diurnal lizards. Nocturnal geckos are active at lower body temperatures, and when measured at their field-relevant body temperatures, they had a lower $\dot{V}O_2\text{max}$ than diurnal lizard species. However, assuming a Q_{10} of 2.5 (from previous studies of lizards in general; Bennett, 1982, 1983), correcting to the same body temperature yielded similar values for nocturnal and diurnal

species. Finally, in a study of 18 varanid species, Clemente et al. (2009) showed that $\dot{V}O_2\text{max}$ was positively correlated with endurance, measured as maximum distance and time running when chased around a circular racetrack until exhaustion. Endurance was higher in widely foraging species and in species from xeric as compared with tropical areas, but these comparisons were not reported for $\dot{V}O_2\text{max}$.

Variation in $\dot{V}O_2\text{max}$ has also been studied in other taxa. Among mammals, Albuquerque et al. (2015b) found that $\dot{V}O_2\text{max}$ was marginally positively correlated with home range area. All else being equal, larger home ranges should be associated with higher activity levels. However, the correlation between home range and $\dot{V}O_2\text{max}$ was weak ($r = 0.278$) and statistically non-significant in phylogenetically based analyses. The authors argue that measurement error (Ives *et al.*, 2007), including a mismatch between the populations for which home range and $\dot{V}O_2\text{max}$ were measured, and the fact that activity levels should be higher inside the defended portion of the home range (territory), might account for the weak correlation between home range and $\dot{V}O_2\text{max}$. Killen et al. (2016) showed that size-corrected $\dot{V}O_2\text{max}$ is higher for pelagic teleost fishes and for species from higher trophic levels.

In the present study, we tested whether $\dot{V}O_2\text{max}$ in lizards is associated with diet (herbivore, insectivore, insectivore/carnivore, carnivore, and omnivore), which presumably relates to foraging mode, climate (tropical, temperate, and arid), nocturnality, and viviparity, while controlling for the effect of body mass and

temperature using conventional ordinary least squares and phylogenetic regressions to account for species relatedness. We included viviparity because retaining embryos for longer gestation periods should have important impacts on activity levels and perhaps the intensity of effort during daily tasks. Pregnancy can reduce locomotor capacities in lizards not only through the effects of added body mass, but also through unidentified changes in physiology (Garland & Else, 1987; Olsson *et al.*, 2000; Zani *et al.*, 2008). Therefore, all else being equal (e.g., in the absence of compensatory behavior: Bauwens & Thoen, 1981; Brodie, 1989), a higher $\dot{V}O_{2\max}$ may be required to maintain similar activity levels in viviparous versus oviparous species.

Methods

Data collection

We obtained $\dot{V}O_{2\max}$ data from scientific publications. We started with the dataset provided by Garland and Albuquerque (2017) and added data for species outside of the 35-40°C range used by them. We searched for “lizard $\dot{V}O_{2\max}$ ”, “lizard aerobic capacity”, and “lizard maximal metabolic rate” in Google Scholar and Web of Science. We considered only studies that measured oxygen consumption while gradually increasing speed on a treadmill. When the studies reported mass-specific $\dot{V}O_{2\max}$ we multiplied the reported mass-specific $\dot{V}O_{2\max}$ by the average body mass.

Additionally, we measured $\dot{V}O_2\text{max}$ for 14 adult male *Sceloporus occidentalis* from Riverside, CA, USA (California Department of Fish and Wildlife permit SC - 013534). We obtained each lizard's $\dot{V}O_2\text{max}$ while running on a speed-controlled treadmill (e.g., Garland, 1984; Garland & Else, 1987). Prior to each trial, animals were maintained for at least 2 hours in an environmental chamber at 35° C. We placed each lizard at the rear of the treadmill belt between adjustable Plexiglas walls. Lizards wore a light-weight translucent plastic mask through which room air was pulled and conducted along tubes to an S-3A Applied Electrochemistry oxygen analyzer. Flow rate through the mask (500 SCCM, controlled by a mass-flow controller) was sufficient to capture all exhaled gas. A set of heat lamps above the treadmill was adjusted such that body temperature remained near 35° C during the trial. We stimulated lizards to run by gentle taps on their tails and hindlimbs as we slowly increased the treadmill speed while monitoring oxygen concentration from the exhaled air. When we observed no further decline in oxygen concentration despite increases in speed for at least 60 seconds, we stopped the trial. $\dot{V}O_2\text{max}$ was taken as the highest one minute of O_2 consumption during the trial (e.g., Garland, 1984; Garland & Else, 1987). We ran each lizard twice, once in two consecutive days, and used the highest trial as $\dot{V}O_2\text{max}$.

A small tube with calcium sulfate, soda lime, and calcium sulfate in series removed carbon dioxide and water vapor from air before it entered the oxygen analyzer. A data Acquisition Module (ADAM-4019) converted the analog signal

from the oxygen analyzer into a digital signal sent to a Macintosh computer. We used LabHelper software (WarthogSystems, www.warthog.ucr.edu) to record and visualize the data in real time. We used Warthog LabAnalyst to calculate oxygen consumption from records of oxygen concentration using the Mask Mode 1 conversion.

We gathered literature data on climate, diet, nocturnality, and viviparity for each species for which we had $\dot{V}O_2$ max data. We used a Google Earth layer based on the updated Köppen-Geiger climate classification provided by Peel et al. (2007) to determine the climate category for the locality where each species was collected. If a species was collected in multiple localities and separate measures of $\dot{V}O_2$ max were not reported, we used the climate category that encompassed the majority of collection sites for the individuals collected. When captive species were used or when collection sites were not described in the original papers, we used the climate category that encompassed most of the distribution of the species reported in the IUCN website (IUCN, 2019).

Categorizing an animal's diet can be complicated (Peters, 1977). We classified species' diet as herbivore, insectivore, insectivore/carnivore, carnivore, or omnivore following the 90% rule (Peters, 1977; Harestad & Bunnell, 1979; Perry & Garland, 2002) when studies that reported stomach contents in detail were available. For those studies, if less than 10% of the volume of stomach contents reported was composed of plant material, then we considered the lizard to be primarily insectivorous. If more than 10% of the stomach contents volume

was composed of plant material, the species was considered omnivorous. Stomach contents of exclusively carnivorous and herbivorous species might not be as well preserved as the contents of species that eat mostly arthropods, so we also considered descriptions in general ecology papers or field guides, which usually involve direct observations in the wild. Finally, for one species, *Teratoscincus przewalskii*, none of the sources of information described above were available, so we classified the species as insectivorous based on descriptions of what caretakers fed the animals in captivity, as taken from descriptions on pet websites or forums (e.g., Good Life Herps, 2018). We classified species as viviparous or oviparous and as diurnal or nocturnal according to the information provided in the Reptile Database (Uetz, 2019), Animal Diversity (Myers *et al.*, 2019), or from data available in Mesquita *et al.* (2015).

Phylogenetic tree construction

We used the time calibrated phylogeny provided as online supplemental material with Tonini *et al.* (2016) and removed species not present in our study. We then added seven new branches to represent the two populations that we had for *Sceloporus occidentalis*, *Tiliqua rugosa*, *Varanus gilleni*, *Varanus gouldii*, *Varanus panoptes*, *Varanus rosenbergi*, and *Varanus tristis* for which we found $\dot{V}O_2\text{max}$ data. We arbitrarily set the branch length between the two populations of these seven species as equal to half the shortest branch length between any

two species in our tree. The shortest bifurcation in the phylogeny for our species was between *Varanus panoptes* and *Varanus gouldii* (5.47 million years), so we set the seven bifurcations to 2.74 million years (Fig. 2.1).

Statistical analysis

We computed multiple regressions in two ways (reviewed in Garland *et al.*, 2005; Lavin *et al.*, 2008) using the Matlab REGRESSIONv2.m program (Lavin *et al.*, 2008). First, we used conventional, nonphylogenetic, ordinary least squares (OLS) and then regressions in which the residuals are modeled as having evolved via an Ornstein–Uhlenbeck process (RegOU), which is intended to mimic stabilizing selection on the specified phylogenetic tree. The OLS models assume a “star phylogeny” with no hierarchical structure, whereas the RegOU models alter the branch lengths of the tree to increase or decrease its hierarchy (respectively, pulling nodes towards the tips of the tree or closer to the root). As compared with an OLS regression, the RegOU regression model contains one additional parameter, d , that estimates the transformation of the phylogenetic tree (Blomberg *et al.*, 2003; Lavin *et al.*, 2008) to better fit the residuals obtained with a given set of independent variables. A d -value greater than 1 means that the analysis altered the tree to be more hierarchical; conversely, values less than 1 mean that the nodes were pulled closer to the root, as compared to the original tree. The minimum value for this parameter is zero, which would indicate that the tree was altered to a star phylogeny.

Our dataset included $\dot{V}O_2\text{max}$ for 58 species distributed among 11 currently recognized monophyletic taxa (all families except for Gekkota, which is an infraorder) and the number of species per taxa ranged from 1 (Crotaphytidae) to 24 (Varanidae). These 11 taxa (henceforth referred to as families) correspond to major branches in our phylogenetic tree (Fig. 2.1). Several previous studies have suggested that some of these families have relatively high $\dot{V}O_2\text{max}$ and/or endurance capacity (e.g., see Garland, 1994a; Beck *et al.*, 1995; Clemente *et al.*, 2009). Therefore, we initially tested for differences among the 11 families (e.g., see Gartner *et al.*, 2010; Foster *et al.*, 2018). Preliminary analysis revealed that most of the effect of family was caused by Helodermatidae, Varanidae, and Scincidae, all with relatively high $\dot{V}O_2\text{max}$. In addition, models with the 11-category family variable had relatively high AICc (see below), suggesting that this variable was too fine-grained. We therefore decided to instead use a set of three dummy variables coding for helodermatids, varanids, and skinks as compared with all other lizards. Only models that included (or excluded) all three of these dummy variables were considered. We made the *a priori* decision to include body mass and body temperature during $\dot{V}O_2\text{max}$ trials in all models, because these are known to be highly influential predictors of $\dot{V}O_2\text{max}$ (see Introduction, and also Garland 1994 regarding endurance capacity).

We fitted a total of 48 multiple regression models, including all possible combinations of the independent variables climate (3 categories), diet (5 categories), nocturnality, viviparity, and either family (11 categories) or the set of

three dummy variables for putatively aerobic taxa (helodermatids, varanids, and skinks). Note that models never included both family and the 3-dummy set. All 48 models were fitted by both OLS and RegOU procedures, yielding a total of 96 regression models.

For each model, we report the partial regression coefficient and p-value for each independent variable, the d parameter (see above), the \ln maximum likelihood (LnML) for the model, and the Akaike information criterion corrected for sample size $\{AICc = (-2 * LnML) + [2 * p * 2/(n - p - 1)]\}$, where p is the number of parameters and n is the sample size (lower AICc values indicate better model fit to the data) (Burnham & Anderson, 2002).

To assess the importance of each independent variable, we used the following approach: first, we calculated the Akaike weight (w_i) for each model, which is the probability that model i would be the best fitting model, if the data were collected again under identical circumstances. The sum of w_i for all 96 models equals 1. Then, for each independent variable, we calculated the sum of Akaike weights ($\sum w_i$). If an independent variable is mostly present in the best-fitting models (the ones with lowest AICc), it would then have a high w_i and would therefore be a relatively important variable for model fit. We calculated a weighted average of the partial regression slope for body mass and the standard error (SE) of the coefficient using the relative importance (measured as the Akaike weight) of each regression model as the weighting factor (Burnham & Anderson, 2002). This weighted averaging procedure allowed us to obtain the

average allometric scaling exponent of $\dot{V}O_2\text{max}$ across all the models within 10 AICc from the best (these top eight models accounted for 99% of the cumulative w_i) while accounting for the proportional contribution of each model based on their AICc values. We used the average standard error of the body mass partial regression coefficient to calculate the confidence intervals of the allometric slope of $\dot{V}O_2\text{max}$. The average SE was calculated from models that ranged in degrees of freedom (df) from 55 to 58 (some models included more variables). Therefore, we considered 55 df to calculate the boundaries of 95% confidence interval of the partial regression coefficient for body mass.

Results

We obtained eight models within 10 AICc from the best (Table 2.1). The difference in AICc scores from the 8th to 9th model was 5.14, which can be considered a large difference in model fit (Burnham & Anderson, 2002). Additionally, the eight best models accounted for 99% of the cumulative evidence (cumulative w_i), so we are confident that these models encompass the most accurate explanation of the (co)variation in our dataset.

Considering these eight models, the average allometric slope for $\dot{V}O_2\text{max}$ was 0.803 (95% confidence interval = 0.747-0.859). All these models included the three dummy variables coding for helodermatids, varanids, and skinks. The best model (based on AICc values) that included “family” (with its 11 categories) had an AICc of -26.94 and was placed at the 23rd position. The best model that

did not include “family” or the three dummy variables had an AICc of -31.80 and was placed at the 10th position. The allometric slope including only body mass and temperature was 0.861 (95% CI 0.795 to 0.927) from OLS regression and 0.857 (95% CI 0.782 to 0.931) from RegOU (Table 2.2).

The most influential independent variables were viviparity ($\sum w_i = 0.996$), the combined set of dummy variables coding for helodermatids, varanids and skinks ($\sum w_i = 0.996$), and climate ($\sum w_i = 0.734$). Nocturnality was the next most influential variable, but its cumulative evidence was only 0.291. Diet and “family” with 11 categories had very low cumulative evidence ($\sum w_i < 0.01$ each). The variables coding for viviparity, Varanidae, and Helodermatidae were always statistically significant ($P < 0.05$) in the top regression models (Table 2.1). Viviparity had a negative effect on $\dot{V}O_2\text{max}$, whereas being part of helodermatids, varanids, or skinks positively affected $\dot{V}O_2\text{max}$, including in the phylogenetic regressions (RegOU).

The best eight models included a mix of OLS and RegOU (Table 2.1). The d-values of the RegOU models were all below 0.013. This value is much closer to 0 than it is to 1, meaning that the nodes of the tree were considerably moved towards the root. Consequently, the tree that best explains the residual variation in this dataset is much more similar to a star phylogeny than to its original configuration. For completeness, we also present full models including all independent variables (mass, temperature, family, diet, climate, nocturnality, and viviparity) in Table 2.2.

Discussion

$\dot{V}O_2$ max allometry

In the present study, we estimated that $\dot{V}O_2$ max scales as $\text{mass}^{0.803}$ in lizards, based on a sample of 58 species/populations. This allometric slope is the average of 8 models that included measurement temperature (range: 20 to 40° C) as a covariate and also different combinations of viviparity, climate, and nocturnality as independent variables (Table 2.1). The average 95% confidence interval of the allometric slope of $\dot{V}O_2$ max from these top eight models (0.747-0.859) includes the 0.779 allometric exponent reported in our previous study restricted to lizard species measured within the 35-40° C range and estimated without regard to phylogeny (Garland & Albuquerque, 2017). It also includes the value of 0.77 reported for 24 species of diurnal lizards (Autumn *et al.*, 1999), but not the value of 0.96 for eight nocturnal species (Autumn *et al.*, 1999) or the 0.74 slope reported for varanids (Clemente *et al.*, 2009). Additionally, the allometric slope reported here is very similar to the value of 0.839 previously reported for a large size range of mammals analyzed in a phylogenetic context (Dlugosz *et al.*, 2013).

The best 8 models included four OLS and four RegOU regressions with identical combinations of independent variables. These variables represented, in order of importance: viviparity, varanids, helodermatids, skinks, climate, and nocturnality and are discussed below.

Differences among phylogenetic groups

Partial regression coefficients showed that helodermatids and varanids had greater $\dot{V}O_2\text{max}$ than other lizards. These lineages, in general, are composed by active predators (King & Green, 1993; Beck *et al.*, 1995; Gienger *et al.*, 2014)] and their high $\dot{V}O_2\text{max}$ has been noted previously (Beck *et al.*, 1995; Clemente *et al.*, 2009). Most varanid species use mobile foraging strategies that routinely cover long distances (Clemente *et al.*, 2009) and probably benefit from relatively high aerobic capacity to sustain effort. Also, Clemente *et al.* (2009) showed that the coadaptation of high activity levels, endurance, and $\dot{V}O_2\text{max}$ is probably plesiomorphic for Australian varanids (Clemente *et al.*, 2009), so even though some of the species included here are sit-and-wait foragers, their high $\dot{V}O_2\text{max}$ levels may be explained by evolutionary history.

The two living species of helodermatids (*Heloderma horridum* and *H. suspectum*, both included in this study) are sporadic, binge-feeders that prey on ephemeral and sparsely distributed food resources (Stahnke, 1950; Beck & Lowe, 1991; Beck, 2005). Gienger *et al.* (2014) showed that *H. suspectum* have a high proportional cost of activity relative to total energy expenditure during the active season, although they are largely inactive for most of the year. Previous studies also showed that helodermatids have relatively high endurance when accounting for body mass and temperature simultaneously (see Figure 11.7 in Garland, 1994a). Perhaps the high $\dot{V}O_2\text{max}$ observed in helodermatids is related

to relatively higher activity levels during the short period when they are active above ground in search of food or mates (John-Alder *et al.*, 1983; Jones, 1983). However, Beck *et al.* (1995) also suggested that the high $\dot{V}O_2\text{max}$ (and aerobic scope) of helodermatids can be attributed to strenuous male-male disputes, rather than foraging or activity levels. Future studies could answer test that hypothesis testing for correlations between $\dot{V}O_2\text{max}$ and foraging success or the outcome of male-male disputes at the level of individual variation in these species.

We also found that Scincidae tend to have higher $\dot{V}O_2\text{max}$ than other lizard groups (even though that difference was not statistically significant in some models – Table 2.1). The vast majority of skinks are widely foraging species (Huey & Pianka, 1981; Cooper, 1994a; b). Cooper *et al.* (2000) showed that omnivorous and insectivorous skinks quickly respond to chemical cues from active prey, and that this behavioral trait seems to be plesiomorphic for the family. The four skink species included in the present study (*Tiliqua rugosa*, *Oligosoma macgregori*, *O. nigriplantare*, *Plestiodon skiltonianus*) are either omnivores or insectivores that would probably show the same behavior. However data on movement patterns or foraging strategy is not available for these species (but see Kerr & Bull, 2006), so we cannot make direct comparisons of activity levels and $\dot{V}O_2\text{max}$.

Diet, climate, nocturnality, and viviparity correlates

Viviparity is strongly related to $\dot{V}O_2\text{max}$ among species of lizards, with an overall importance of 0.996 and present in all of the top eight models (Table 2.2). However, contrary to our prediction, it had a negative effect on $\dot{V}O_2\text{max}$. Viviparity is estimated to have evolved independently ~115 times in squamates (three of these included in our tree: Figure 2.1) and it seems to originate most commonly in cold climates and in lizards that typically have low body temperatures (Shine, 2005; Meiri *et al.*, 2013; Pyron & Burbrink, 2014; Mesquita *et al.*, 2016). However, the low $\dot{V}O_2\text{max}$ that we observed in viviparous lizards is not related to low body temperature per se, as all models included measurement temperature as a covariate. Additional ecological or behavioral factors (e.g., activity levels) may explain this negative correlation, and it would also be of interest to study the mechanistic underpinnings of the low $\dot{V}O_2\text{max}$ of skinks.

Climate was significantly related to $\dot{V}O_2\text{max}$ in four of the eight top models, but its importance was 0.734, which is below the 0.8 threshold suggested to be considered an influential variable in model averaging (Calcagno & Mazancourt, 2010). Climate was significantly related to endurance (and possibly $\dot{V}O_2\text{max}$) among 18 species of varanids (Clemente *et al.*, 2009). Inspection of the partial regression coefficient of each climate category showed that “temperate” had a significant negative effect on $\dot{V}O_2\text{max}$. It is possible that some of the correlation between climate and $\dot{V}O_2\text{max}$ in lizards is caused by different temperatures under which each species evolved. The structural

complexity of the main habitats in each climate category (e.g., forests and deserts) might be related to the remaining correlation. Arid habitats should, on average, be more open and, perhaps, scarcer in food resources, which could lead to higher activity levels associated with foraging. Tropical habitats, such as ombrophilous forests, can also impose relative physical constraints on movements, which might increase the effort required to travel a given distance. Forests are also more shaded and offer fewer basking sites, creating the need for the lizard to move around more during thermoregulatory behavior.

None of the top eight models included diet (importance = 0.003), and the best model that did had an AICc of -31.4303, which is considerably higher than any of the top eight models. These results indicate that diet, at least in the categories used here, is not associated with $\dot{V}O_2\text{max}$ among species of lizards. Carnivorous and insectivorous lizards are higher in trophic chains and, all else being equal, animals that have to hunt for prey should have to move around more and possibly have larger home ranges (Peters, 1977; Garland *et al.*, 1993; Perry & Garland, 2002). These broad diet categories should also be related to ecological and behavioral traits, such as home range (Harestad & Bunnell, 1979; Perry & Garland, 2002), and activity levels (all else being equal, herbivores need to move less to obtain food). However, expecting a simple correlation between diet and $\dot{V}O_2\text{max}$ may be unrealistic because, among insectivorous and carnivorous lizards, a wide range of foraging strategies exist, including extreme sit-and-wait predators (e.g., Andrews, 1979; and to a smaller extent, *Gambelia*

wislizenii in Rose, 2004) that might move even less than herbivores and have a reduced need for aerobic capacities. Perhaps, activity levels are more closely related to how active the prey is (Huey & Pianka, 1981). Diet is also a considerably variable trait throughout seasons and among populations within species (Kolodiuk *et al.*, 2009; e.g., Albuquerque *et al.*, 2018), so a mismatch between the populations used for $\dot{V}O_2\text{max}$ and diet data would reduce the correlation.

Even though nocturnality occurred in four of the top eight models, its overall importance was only 0.291 and it was not a significant predictor of $\dot{V}O_2\text{max}$ in any of them. Because we accounted for measurement temperature in all models, this result corroborates the previous finding that the lower $\dot{V}O_2\text{max}$ in nocturnal lizards is mostly explained by their lower body temperatures (Autumn *et al.*, 1999).

Limitations of the Present Study and Future Directions

Several factors unmeasured in the present study can affect $\dot{V}O_2\text{max}$ and add noise to the potential correlations with traits evaluated. For example, sex differences may have added noise to the data, e.g., see Beck *et al.* 1995. Additionally, seasonality, especially in species with seasonal reproduction and territorial species, can have a significant effect on $\dot{V}O_2\text{max}$ (John-Alder, 1984b; John-Alder *et al.*, 2009). The $\dot{V}O_2\text{max}$ data used here came from different studies conducted during different seasons and using individuals from different

sexes (some studies report values for males and females separately, but others pool them together or do not report the sex). In future studies we plan to include sex and season as cofactors as well as other aspects, such as home range size (Perry & Garland, 2002), activity levels (e.g., daily movement distances: Garland & Albuquerque, 2017), foraging mode, territoriality, and long-term captives vs. wild-caught individuals, while concomitantly measuring maximal oxygen consumption in new species spanning a wider range of ecological traits and behavioral traits. We would also like to include more species from clades such as Phrynosoma, which show particularly low $\dot{V}O_{2max}$ for their temperature to test for a general pattern in the group. Finally, additional viviparous species and measures of their activity in nature would help us understand why this reproductive mode is associated with relatively low $\dot{V}O_{2max}$.

Acknowledgments

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Figure Legends

Figure 2.1 – Phylogenetic tree including 58 species of lizards of 11 different families for which $\dot{V}O_2$ max data is available in the literature. For seven of those species, we obtained $\dot{V}O_2$ max data for populations in two different localities (represented as a “2” after the species name), so the tree has 65 tips. Time calibrated branch lengths were calculated using all available DNA data for squamates and included 7 mitochondrial and 10 nuclear genes from a fully-sampled Squamata tree (Tonini *et al.*, 2016) before we removed the species absent in our study. Viviparous species are indicated by “(V)”, branch colors represent families, x-axis in million years.

Figure 2.2. Scatterplots of $\log_{10} \dot{V}O_2$ max on \log_{10} body mass for 58 lizard species plus 7 populations (total 65 data points) and color coded by (A) temperature measured, (B) monophyletic taxonomic groups or “family” (but note that Gekkota is an infraorder), (C) viviparity, (D) climate.

Figures

Figure 2.1

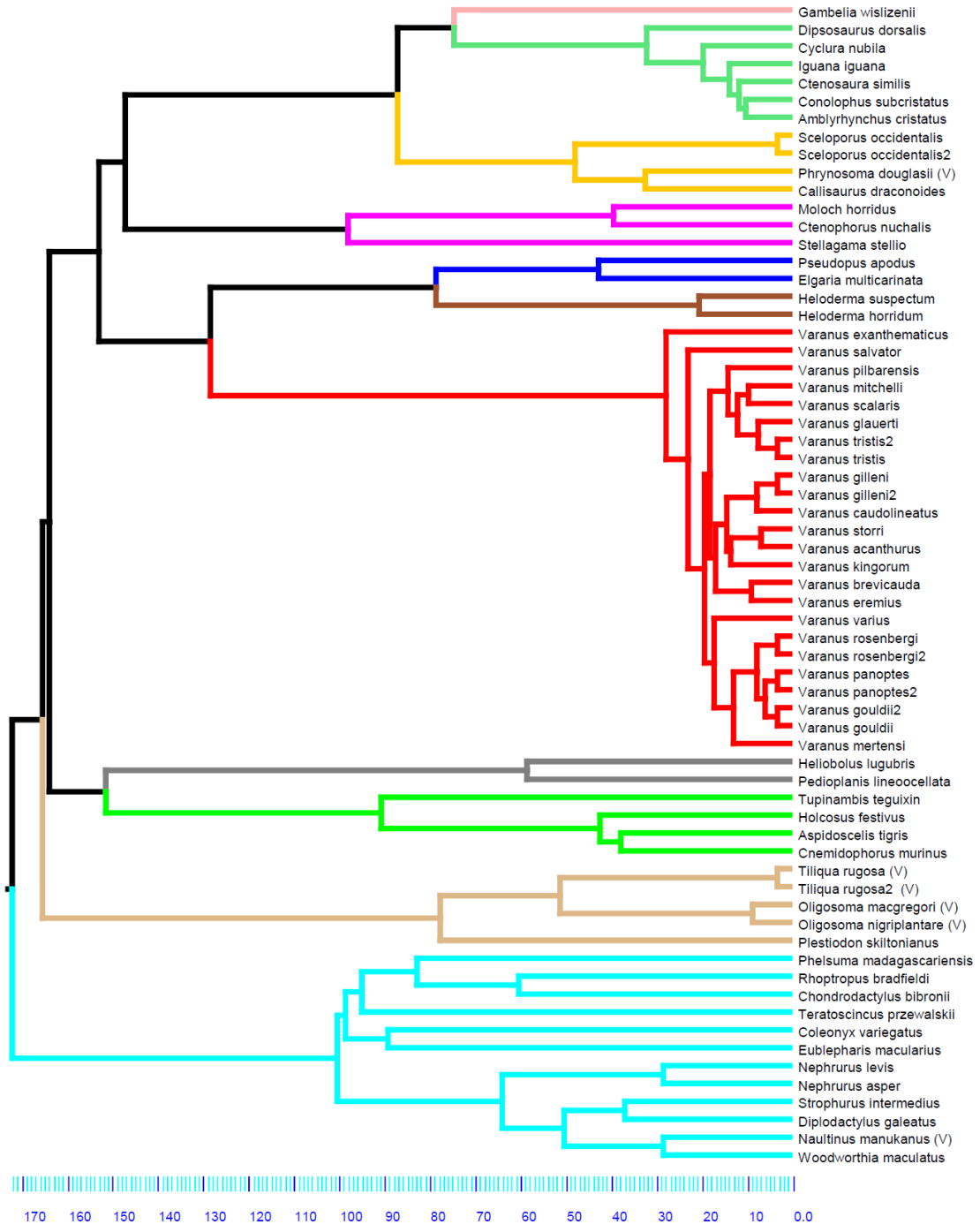
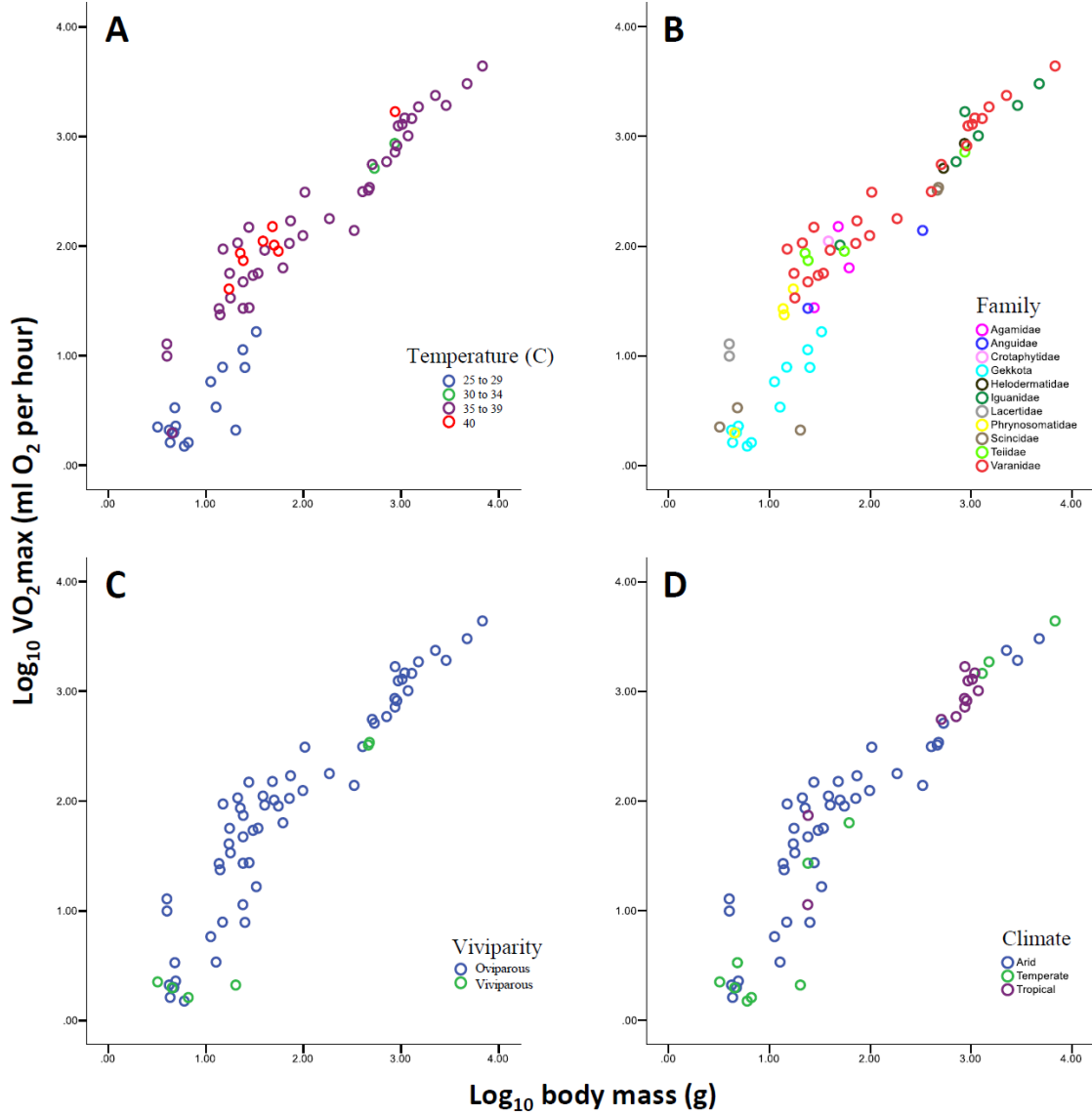


Figure 2.2



Tables

Table 2.1 - R², likelihood, and AICc of top 8 regressions models (according to AICc scores) from models including all possible combinations of family, nocturnality, diet, climate, and viviparity as predictors of log₁₀ $\dot{V}O_2$ max among 58 lizard species plus 7 populations (total 65 data points). The slopes (partial regression coefficients) for log₁₀ body mass and for temperature always had P values < 0.001 (not shown).

Method	I.V. included	R ²	ML	AICc	Df	MSE	S.E.E.	d
OLS	3DumFam, viviparity, climate	0.978	33.425	-42.776	56	0.0243	0.1559	NA
RegOU	3DumFam, viviparity, climate	0.975	34.154	-41.327	56	0.0238	0.1543	0.0034
OLS	3DumFam, viviparity, climate, nocturnality	0.978	34.033	-41.085	55	0.0243	0.1558	NA
OLS	3DumFam, viviparity	0.975	29.654	-40.736	58	0.0263	0.1623	NA
RegOU	3DumFam, viviparity	0.971	30.471	-39.669	58	0.0258	0.1606	0.0079
RegOU	3DumFam, viviparity, climate, nocturnality	0.975	34.828	-39.656	55	0.0238	0.1542	0.0013
OLS	3DumFam, viviparity, nocturnality	0.976	29.916	-38.559	57	0.0266	0.1631	NA
RegOU	3DumFam, viviparity, nocturnality	0.971	30.856	-37.638	57	0.0260	0.1611	0.0122

Table 2.1 (continued)

Method	I.V. included	Mass Slope	Mass SE	Temp. Slope	Temp. SE	Diet	Family
OLS	3DumFam, viviparity, climate	0.801	0.0278	0.052	0.0040	NA	NA
RegOU	3DumFam, viviparity, climate	0.802	0.0292	0.052	0.0041	NA	NA
OLS	3DumFam, viviparity, climate, nocturnality	0.803	0.0279	0.047	0.0063	NA	NA
OLS	3DumFam, viviparity	0.803	0.0263	0.054	0.0041	NA	NA
RegOU	3DumFam, viviparity	0.810	0.0286	0.053	0.0042	NA	NA
RegOU	3DumFam, viviparity, climate, nocturnality	0.804	0.0294	0.046	0.0063	NA	NA
OLS	3DumFam, viviparity, nocturnality	0.803	0.0264	0.050	0.0064	NA	NA
RegOU	3DumFam, viviparity, nocturnality	0.810	0.0291	0.048	0.0064	NA	NA

Method	I.V. included	Helo. Slope	Helo. P	Vara. Slope	Vara. P	Scin. Slope	Scin. P
OLS	3DumFam, viviparity, climate	0.312	0.0111	0.233	0.0000	0.239	0.0255
RegOU	3DumFam, viviparity, climate	0.306	0.0137	0.223	0.0000	0.233	0.0335
OLS	3DumFam, viviparity, climate, nocturnality	0.279	0.0277	0.220	0.0000	0.226	0.0361
OLS	3DumFam, viviparity	0.334	0.0090	0.227	0.0000	0.228	0.0394
RegOU	3DumFam, viviparity	0.326	0.0121	0.217	0.0002	0.209	0.0665
RegOU	3DumFam, viviparity, climate, nocturnality	0.269	0.0357	0.207	0.0002	0.218	0.0493
OLS	3DumFam, viviparity, nocturnality	0.312	0.0184	0.218	0.0000	0.219	0.0498
RegOU	3DumFam, viviparity, nocturnality	0.296	0.0283	0.204	0.0008	0.195	0.0924

Table 2.1 (continued)

Method	I.V. included	Clim. P	Vivi. Slope	Vivi. P	Noct. Slope	Noct. P
OLS	3DumFam, viviparity, climate	0.0388	-0.355	0.0007	NA	NA
RegOU	3DumFam, viviparity, climate	0.0446	-0.355	0.0009	NA	NA
OLS	3DumFam, viviparity, climate, nocturnality	0.0307	-0.365	0.0006	-0.096	0.3127
OLS	3DumFam, viviparity	NA	-0.428	0.0001	NA	NA
RegOU	3DumFam, viviparity	NA	-0.432	0.0001	NA	NA
RegOU	3DumFam, viviparity, climate, nocturnality	0.0374	-0.366	0.0007	-0.105	0.2694
OLS	3DumFam, viviparity, nocturnality	NA	-0.437	0.0001	-0.065	0.4994
RegOU	3DumFam, viviparity, nocturnality	NA	-0.445	0.0001	-0.088	0.3644

Table 2.2 - R², likelihood, and AICc of regressions models including all independent variables considered in this study (top four rows) and regression models including only body mass and temperature (bottom 2 rows) as predictors of log₁₀ $\dot{V}O_2$ max among 58 lizard species plus 7 populations (total 65 data points). Family includes 11 categorical variables refereeing to monophyletic taxonomic groups present in our tree (see methods and Fig. 2.1), 3DumFam refer to three dummy variables coding for helodermatids, varanids, and skinks, diet includes 5 categories (herbivore, carnivore, insectivore, carnivore/insectivore, and omnivore), climate includes three categories (tropical, temperate, and arid), viviparity and nocturnality are dummy variables coding for nocturnal and viviparous species. The slopes (partial regression coefficients) for log₁₀ body mass and for temperature always had P values < 0.001 (not shown).

Method	I.V. included	R ²	ML	AICc	Df	MSE	S.E.E.	d
OLS	Family, climate, viviparity, nocturnality, diet	0.981	38.877	-9.659	44	0.026	0.162	NA
RegOU	Family, climate, viviparity, nocturnality, diet	0.978	39.086	-5.246	44	0.026	0.162	0.007
OLS	3DumFam, climate, viviparity, nocturnality, diet	0.979	34.422	-29.048	51	0.0259	0.161	NA
RegOU	3DumFam, climate, viviparity, nocturnality, diet	0.975	35.050	-26.767	51	0.0255	0.160	0.006
OLS	Only body mass and temperature	0.952	7.7653	-6.864	62	0.0483	0.2199	NA
RegOU	Only body mass and temperature	0.933	15.046	-19.076	62	0.0387	0.1966	0.179

Method	I.V. included	Mass Slope	Mass SE	Temp Slope	Temp SE	Diet P	Fam P
OLS	Family, climate, viviparity, nocturnality, diet	0.809	0.0378	0.056	0.0136	0.5104	0.0289
RegOU	Family, climate, viviparity, nocturnality, diet	0.813	0.0419	0.055	0.0139	0.6530	0.0822
OLS	3DumFam, climate, viviparity, nocturnality, diet	0.803	0.0353	0.047	0.0067	0.8581	NA
RegOU	3DumFam, climate, viviparity, nocturnality, diet	0.807	0.0384	0.046	0.0067	0.9890	NA
OLS	Only body mass and temperature	0.861	0.0331	0.056	0.0054	NA	NA
RegOU	Only body mass and temperature	0.857	0.0373	0.052	0.0055	NA	NA

Table 2.2 (continued)

Method	I.V. included	Helo Slope	Helo P	Vara Slope	Vara P	Sci Slope	Scin P
OLS	Family, climate, viviparity, nocturnality, diet	NA	NA	NA	NA	NA	NA
RegOU	Family, climate, viviparity, nocturnality, diet	NA	NA	NA	NA	NA	NA
OLS	3DumFam, climate, viviparity, nocturnality, diet	0.301	0.0404	0.220	0.0008	0.235	0.0439
RegOU	3DumFam, climate, viviparity, nocturnality, diet	0.284	0.0564	0.206	0.0032	0.228	0.0565
OLS	Only body mass and temperature	NA	NA	NA	NA	NA	NA
RegOU	Only body mass and temperature	NA	NA	NA	NA	NA	NA

Method	I.V. included	Clima P	Vivi Slope	Vivi P	Noct Slope	Noct P
OLS	Family, climate, viviparity, nocturnality, diet	0.1348	0.002	0.0018	0.247	0.2469
RegOU	Family, climate, viviparity, nocturnality, diet	0.2237	0.003	0.0027	0.235	0.2355
OLS	3DumFam, climate, viviparity, nocturnality, diet	0.0365	-0.350	0.0019	-0.089	0.3749
RegOU	3DumFam, climate, viviparity, nocturnality, diet	0.0531	-0.356	0.0020	-0.098	0.3344
OLS	Only body mass and temperature	NA	NA	NA	NA	NA
RegOU	Only body mass and temperature	NA	NA	NA	NA	NA

CHAPTER 3 - Lower-level predictors and behavioral correlates of maximal aerobic capacity ($\dot{V}O_2\text{max}$) and sprint speed among adult male *Sceloporus occidentalis* during the breeding season.

Abstract

The standard paradigm of organismal biology recognizes that lower-level, subordinate traits (e.g., aspects of morphology and physiology) affect organismal performance abilities (e.g., maximal sprint speed), which in turn constrain behavior. However, few studies have simultaneously examined lower-level traits, performance, and behavior. We measured key morphological and physiological traits (blood [hemoglobin] & hematocrit; organ masses; citrate-synthase and lactate dehydrogenase activity), two organismal performance traits (maximal sprint speed and maximal oxygen consumption [$\dot{V}O_2\text{max}$]), and several aspects of field behavior in adult male *Sceloporus occidentalis* lizards during the breeding season. We used three, 20-min focal observations per individual to record movement behaviors and push-up displays in the field, then captured animals and returned them to the lab for measures of performance, physiology, and morphology. In the lab, we also scored ecto- and endoparasites as potential predictors of sprint speed and $\dot{V}O_2\text{max}$. We found significant individual repeatability of field behaviors, based on analysis of variance, but intra-class correlation coefficients were relatively low (range = 0.05 to 0.31). Sprint speed and $\dot{V}O_2\text{max}$ showed significant repeatability using both raw data (speed:

Pearson's $r = 0.70$, $P < 0.0001$; $\dot{V}O_2\text{max}$: $r = 0.94$, $P < 0.0001$) and residuals from regressions on body mass (speed: $r = 0.70$, $P < 0.0001$; $\dot{V}O_2\text{max}$: $r = 0.88$, $P < 0.0001$). We used multiple regressions to test for relations of lower-level traits and parasites to performance, and of performance to behavior. We used residuals from regressions on body mass for all (sub)organismal traits. Both calf (standardized partial regression [path] coefficient $B = 0.53$) and thigh ($B = -0.37$) muscle masses were significant predictors of sprint speed; hemoglobin concentration ($B = 0.42$) was a significant predictor of $\dot{V}O_2\text{max}$. In turn, $\dot{V}O_2\text{max}$ predicted the maximum number of 4-legged push-ups per bout ($B = 0.39$). In path analysis, log likelihood ratio tests indicated that the best-fitting model did not include any direct path from lower-level traits to behavior. Overall, our results show that aspects of individual variation in field behaviors can be related to whole-organismal performance abilities, which in turn reflect differences in morphology and physiology, although not parasite load, for this population of lizards. Moreover, given the low repeatability of behavior in the field, some of the relationships between behavior and performance may be stronger than suggested by our results.

Introduction

A prevailing view in organismal biology is that lower-level subordinate traits (e.g., morphology, physiology) affect organismal performance abilities (e.g., maximal sprint speed), which in turn constrain behavior (Arnold, 1983; Garland & Losos, 1994; Storz *et al.*, 2015). Many whole-organism performance traits, such as maximal sprint speed and endurance capacity, are potentially relevant for many natural behaviors, including foraging, escaping from predators, territorial defense, courtship, and mating. Two key measures of whole-animal performance are maximal sprint speed and maximal oxygen consumption ($\dot{V}O_2\text{max}$) (Garland & Losos, 1994; Albuquerque *et al.*, 2015a; b). These measures are 'fueled' via different metabolic pathways (crudely, anaerobic versus aerobic, respectively) and should be important for the ability to engage in different types of behavior that – intuitively, at least – seem likely to be ecologically relevant.

On the anaerobic side, maximal sprint speed should be important for short and intense 'burst' activities, such as escaping from predators, chasing conspecific intruders or capturing insect prey. In male *Sceloporus occidentalis* lizards, maximal sprint speed is positively correlated with dominance during staged territorial disputes in the laboratory (Garland *et al.*, 1990a). If that pattern holds for other territorial iguanids, then it could explain why faster *Crotaphytus collaris* individuals are better at defending their territories and also sire more offspring (Husak *et al.*, 2006, 2008).

On the aerobic end, $\dot{V}O_2\text{max}$ sets the upper limit for the intensity of work that can be sustained over longer periods (minutes or more), so it can be used as a predictor of maximal aerobic speed or stamina in various activities (Garland & Losos, 1994; Autumn, 1999; Clemente *et al.*, 2009). In male lizards of two species (*Dipsosaurus dorsalis* and *Sceloporus undulatus*), $\dot{V}O_2\text{max}$ increases during the breeding season, when activity levels are also at their peak (John-Alder, 1984b; John-Alder *et al.*, 2009). During the breeding season, males in these and other species of lizards (Ruby, 1978; Baird *et al.*, 2001) increase activity levels, home range size, the frequency of interactions with females, and the intensity and frequency of male-male agonism. Comparisons among species of lacertid lizards also indicate a positive relation between $\dot{V}O_2\text{max}$ and activity levels (e.g., Bennett *et al.*, 1984), and varanids, many of whom are highly active, have relatively high $\dot{V}O_2\text{max}$ (Clemente *et al.*, 2009 and references therein).

In some organisms, behaviors related to sexual selection (e.g., male-male disputes or courtship) can be energetically costly and/or physically demanding (Daly, 1978; Höglundi *et al.*, 1992; Mowles & Jepson, 2015). For example, territorial disputes among male *S. occidentalis* can be long and apparently strenuous, with durations of up to 45 minutes (Fitch, 1940). During these disputes, lizards perform push-up displays and head bobs. Push-up displays are a conspicuous, sexually selected signal used during courtship, male-male disputes, or broadcasting to an unknown audience in *S. occidentalis* and other iguanian (Pyron *et al.*, 2013) lizards (Carpenter & Ferguson, 1978; Stamps,

1978). The number of push-ups performed in sequence (hereafter called a bout of push-ups), the number of legs flexed (zero, two, or four legs), and the addition of body postures to the display (arched back, lateral flattening, raised tail and gular extension) are indicators of the intensity of the interaction (Carpenter & Ferguson, 1978; Martins, 1993). In the case of male-male disputes, lizards will move closer, perform more push-ups, and use more legs as the dispute intensifies. If a winner is not established by then, lizards will engage in fighting, and finally chasing (Carpenter, 1962; Martins, 1994; Baird *et al.*, 2003), but that seems to occur very rarely (Sheldahl & Martins, 2000). Thus, maximal sprinting abilities or stamina might be related to the success of an individual lizard in male-male agonistic interactions. In support of this expectation, Baird *et al.* (2003) found a positive correlation between display frequency and copulation rate in *Crotaphytus collaris* lizards. In further studies, Baird and colleagues also observed that display frequency, intensity, and duration are correlated with reproductive success in males (Baird *et al.*, 2007; Baird, 2013).

The first goal of the present study was to test whether movement rates or aspects of the push-up display, measured in the field, reflect individual variation in maximal sprint speed and/or $\dot{V}O_2\text{max}$ in adult males of the lizard *S. occidentalis*, for which a wealth of background information exists. The second goal was to explore the mechanistic basis of variation in organismal performance by examining relevant suborganismal traits (e.g., blood hematocrit and hemoglobin levels; heart and calf muscle masses; citrate synthase and lactate

dehydrogenase activity in thigh muscle, heart, and liver). Third, we tested whether organismal performance might be associated with parasite counts (number of ticks, intracellular parasites in red blood cells, free living nematodes in blood and digestive tract). Finally, we used path analytic models to test for the presence of unmeasured aspects of performance that might mediate relations between lower-level traits and field behavior, as well as the presence of direct paths from lower-level traits to behavior. Few studies have been able to measure the relationship among traits at all three of these hierarchical levels for the same set of individuals.

Materials and methods

Field site and behavior observations

We conducted field work in a sagebrush – juniper woodland in the municipality of Hampton Buttes, Oregon. The landscape was dominated by small and medium sized bushes (*Artemisia tridentata*, *Ribes sanguineum*, *Ericameria nauseosa*, *Bromus tectorum*, and *Achnatherum hymenoides*). The field site included three rock cliffs of approximately 20 meters height and spanning over a total of approximately 1,500 meters in length. A population of *Sceloporus occidentalis* inhabits rock outcrops in that area, especially near cliffs or the cliff walls. The density of juniper trees and rock outcrops increased with proximity to the cliffs providing abundant shelter and thermoregulation sites for the lizards.

We performed focal observations between 8 am and 3 pm Pacific Time from 26 May to 29 June 2017. The first observation each day started at least an hour after we found the first lizard, to avoid observing non-active lizards. We gave each lizard an identification number represented by a unique combination of toe clips (Tinkle, 1967) and wrote that number on the animal's back with Painters® acrylic paint markers to allow identification from a distance. We started observations a week after toe-clipping. We observed each lizard for 20 minutes from at least 5 meters with binoculars. If the lizard moved out of sight for less than five minutes, then we extended the observation period to obtain a total of 20 minutes. If the lizard was out of sight for more than 5 five minutes, then we ended the focal. We used a digital recorder to register the number of moves, distance moved, number of push-ups using no legs, and the number of 2- and 4-legged push-ups during each bout of push-ups, as described in several ethogram studies for the genus (e.g., Carpenter & Ferguson, 1978; Martins, 1994). Events were transcribed from the recordings, and we calculated the number of moves per minute, distance moved per minute, number of push-ups per minute, number of push-ups per bout, and average, maximum, and standard deviation of the distance per move and number of push-ups per bout.

We considered as a bout of push-ups any continuous sequence of push-ups (even if they included only one push-up). If the lizard stopped for more than 3 seconds after the last push-up or if it altered its position (e.g., performing 180 degrees turn or moving a few centimeters), then we considered that as the end of

the bout. Because push-ups on different number of legs should represent a different amount of effort, we analyzed the data for each separately. Regressions of each behavioral trait on total observation time revealed a significant positive relation only for the number of bouts of 4-legged push-ups per minute. As 4-legged push-ups were relatively rare, and some individuals were observed for as few as 20 min (see below), this likely reflects inadequate sampling for the individuals with low total observation time. When we used only the lizards with 60 or more minutes of observation, the relationship was not statistically significant, so we excluded lizards with less than three focals (60 minutes of observation) from analyses that involved this behavior. During some bouts, lizards started doing push-ups using all four legs but switched to only two legs halfway through the bout (as if getting tired). Therefore, we excluded those bouts when calculating the number of push-ups and bouts of push-ups per minute to assure statistical independence.

We performed an average of 3.26 (1 - 5) focal observations on 57 lizards. From those, 44 were captured by noose, over the last 2 days of the field work, and kept in cloth bags protected from high temperatures until transported to the University of California, Riverside. There, we housed lizards individually in 10-gallon terraria and gave them a 2-day acclimation period with ad lib access to water, plus misting on the first day, but no food. We performed sprint speed trials on days 3 and 4 and the $\dot{V}O_2$ max trials on days 7 to 10 (lizards were transferred to the terraria on day 1). On day 5, lizards were given ad lib access to crickets,

but were fasted during day 6, allowing for >24 hours until the first $\dot{V}O_2$ max trial (most lizards did not eat during the fifth day, and no feces were found in cloth bags during $\dot{V}O_2$ max trial days).

Sprint speed and $\dot{V}O_2$ max trials

We measured sprint speed (e.g., Garland, 1985; Albuquerque *et al.*, 2015a) four times per individual lizard (twice per day with at least 4 hours between runs) on a 12-meter racetrack with photocell sensors spaced every 0.5 meters. We used the fastest consecutive 1 meter as the maximal sprint speed of that trial and the fastest of all four trials as the maximal sprint speed of each individual. Prior to all trials, animals were maintained for at least 2 hours in an environmental chamber at 35 C.

We measured $\dot{V}O_2$ max twice (once per day, with one rest day between trials) per individual lizard on a speed-controlled treadmill (e.g., Garland, 1984; Garland & Else, 1987). Prior to each trial, animals were maintained for at least 2 hours in an environmental chamber at 35 C. We placed the lizard on the rear of the treadmill belt between adjustable Plexiglas walls, while they wore a light-weight translucent plastic mask over their heads through which room air was pulled and conducted along tubes to an S-3A Applied Electrochemistry oxygen analyzer. A set of heat lamps above the treadmill warmed the moving belt and lizard. Soda lime and calcium sulfate removed the carbon dioxide and water vapor, respectively, from the air before it entered the oxygen analyzer. An

ADAM-4019 data Acquisition Module converted the analog signal from the oxygen analyzer into a digital signal sent to a personal computer. We used the LabHelper software (WarthogSystems, www.warthog.ucr.edu) to record and visualize the data in real time. We used Warthog LabAnalyst to calculate oxygen consumption from records of oxygen concentration using Mask Mode 1. We stimulated lizards to run by gentle taps on their tails and hindlimbs as we slowly increased the treadmill speed and monitored the oxygen consumption. When we observed no increase in oxygen consumption despite increases in speed for at least 60 seconds, we stopped the trial. Because $\dot{V}O_2$ max trials take longer than sprint speed trials, we ran half (22) of the lizards on the 7th and 9th day and the other half on the 8th and 10th day (while the first half rested).

We measured cloacal temperature with an Amprobe TMD-52 thermocouple thermometer immediately after sprint speed trials and both before and after $\dot{V}O_2$ max trials. We used the average of the body temperature before and after trials to evaluate the effect of temperature over $\dot{V}O_2$ max. All body temperatures fell within 33.7 to 35.5 C for sprint speed trials (only one trial lower than 34 C) and within 34.5 to 36.9 C for $\dot{V}O_2$ max trials (only 4 trials higher than 35.9). Regressions of each performance measure on body mass and body temperature revealed no significant effect of temperature on either performance, so we excluded body temperature from further analysis.

External morphology and dissections

After the last $\dot{V}O_2$ max trial, we measured hindlimb span, forelimb span, snout-vent length, and tail length (Garland, 1985) with a ruler and placed lizards back in terraria with ad lib water and food. We also misted lizards and terraria with water to avoid dehydration. The day after the last $\dot{V}O_2$ max trial (day 11), we sacrificed lizards by decapitation and obtained blood samples directly from the neck into a weight boat containing sodium heparin powder. From the weight boat, we collected two 75 μ l microcapillary tubes for hematocrit measures and pipetted two 20 μ l samples into 5 ml of Drabkin's reagent (SIGMA product number D5941-6VL) for hemoglobin measures. The microcapillary tubes were centrifuged at 11,700 RPM for 5 minutes and hematocrit data consisted of the proportion of red blood cells (red area at the bottom of tubes) to total volume of blood, measured with a ruler. We placed the mixture of blood plus Drabkin's reagent in a refrigerator (approximately 5 °C) protected from light after 15 minutes at room temperature and measured their absorbance 540 nm wavelength after we finished dissections. The absorbance value was used to calculate hemoglobin levels based on a regression using known hemoglobin concentrations (Fisher Scientific catalog number AAJ6383814) in milligrams per milliliter. Dissections followed immediately after decapitation, and we weighed heart (ventricle mass, after blotting to remove blood, and free of atria), liver, lung, calf, and thigh muscles on a precision scale (0.0001g) and then immediately froze tissues in liquid nitrogen. Heart, liver, and thigh were dissected and frozen

within 10 minutes to minimize the chance of enzyme degradation. We then stored all tissues in a -80°C freezer for enzyme analysis.

Parasite inspection

Because malaria parasites (*Plasmodium mexicanum*) affect behavior, blood hemoglobin level, and potentially maximal aerobic capacity in a *S. occidentalis* population from northern California (Schall *et al.*, 1982; Schall & Dearing, 1987; Schall & Sarni, 1987), we tested for the presence of *P. mexicanum* in our lizards. We fixed 2-4 blood smears per lizard with methanol and stained with Giemsa to look for *P. mexicanum* and other parasites. We found no *P. mexicanum* in any of the blood smears. We looked for intracellular parasites in red blood cells on 10 evenly spaced areas containing similar density of cells on each blood smear at 1000x magnification and counted all free-living nematodes by scanning the entire blood smear at 10x magnification. We counted the number of nematodes inside stomach and intestines, and the number of ticks attached to the skin.

Enzyme assays

Before enzyme assays, we removed tissues from the -80 freezer and kept them in liquid nitrogen until homogenization started. We homogenized tissue samples using a Biospec Tissue Tearor model 985370-395 in a small glass vial placed on ice and containing 1 part tissue in 19 parts homogenization buffer. We

used the lowest speed (5090 RPM) for no more than 10 seconds continuously to avoid buildup of heat or frothing. Each 10-second cycle was repeated until no solid particles were visible. The homogenization buffer consisted of 100 mM potassium phosphate and 5 mM EDTA buffer containing 0.1% Triton-x, final pH 7.4 (Suarez *et al.*, 1990). We split the homogenates into two 1.5 ml microcentrifuge tubes (one to be used for citrate synthase and the other for lactate dehydrogenase assays), then we refroze them in liquid nitrogen. We thawed the citrate synthase homogenates on ice and refroze them in liquid nitrogen three times. After the third freeze-thaw cycle, we sonicated homogenates for 10 seconds three times, with a 10 second interval in between, using a Fisher Scientific Sonic Dismembrator model 100, also keeping samples on ice. Finally, we vortexed homogenates, aliquoted to final assay dilution, centrifuged the aliquot at 15000 RPM for 5 minutes at 2°C and used a sample of the supernatant for assays (Garland, 1984; Suarez *et al.*, 1986, 1990; Garland & Else, 1987).

We determined citrate synthase (CS) and lactate dehydrogenase (LDH) activities in liver, heart, and mixed thigh muscle tissues spectrophotometrically, following the procedures described by Srere (1969) and by Somero and Childress (1980), respectively. Assays were performed at 35 °C with nonlimiting concentrations of substrates and co-factors. CS assay starting solutions contained 50 mM Tris-HCL buffer pH8.0, 0.3 mM acetyl-coA (Sigma product number A2181), 0.1 mM DTNB, and the tissue sample. We measured the

increase in absorption at 412 nm wavelength for 3 minutes and used the slope of the last minute as a baseline to account for endogenous levels of thiol or deacetylase activity. We then started the reaction by adding 0.5 mM oxaloacetate (Sigma product number O4126) and followed the increase in absorption for 7 minutes.

We measured LDH activity in the pyruvate reductase direction (production of lactate) by following the decrease in absorption at 340 nm wavelength for 7 minutes. The assay mixture contained 50 mM Tris-HCl buffer pH 7.5, 100 mM KCl, 150 mM NADH, and 2 mM pyruvate, and we started reactions by adding homogenate samples. For both CS and LDH, we used the steepest slope (either positive or negative) of the change in absorption during 60 consecutive seconds after we started the reaction to calculate maximum activity. Enzyme activities are expressed as micromoles of product per minute per gram of wet tissue at 35°C. We ran duplicates for all assays. If the absorption graphs varied substantially between duplicates (e.g. no reaction or much slower change in absorption in one of the graphs), then we repeated the assay for that sample.

Statistical analysis

We used SPSS v24 for most analyses. The data file for field observations consisted of one row for each lizard for each event (either a move or a bout of push-ups). From these data, we calculated individual behavioral values for each lizard based on all minutes of observation (i.e., pooling data from all focal

periods). Specifically, we computed the mean, standard deviation, and maximum (longest move and bout with the greatest number of push-ups). We also computed rates for each behavior (e.g., number moves divided by total minutes of observation) for each lizard.

For field behavior traits, we used the intra-class correlation (ICC) as a measure of repeatability. We calculated ICCs as the proportion of the total variance represented by the variation among individuals from one-way ANOVAs (Lessells & Boag, 1987), using individual lizard as the factor and each move or bout of push-ups as replicates, following the procedure described on Box 9.1 in Sokal and Rohlf (1994). Thus, we did not examine repeatability at the level of among-focal observation periods.

We calculated Pearson correlation coefficients among the mean values for all behavioral traits. Several of the observed or calculated behavioral traits should be correlated simply because of mathematical interdependence (e.g., mean and maximum values will be positively correlated in samples drawn from a normal distribution). In these cases, the usual null hypothesis of zero correlation is not appropriate. One way to determine whether traits are correlated for biological (as opposed to simply mathematical) reasons is to calculate correlation coefficients from simulated data and compare them to the coefficients obtained from the observed data.

To check if the correlation coefficients among the behavioral traits were purely mathematical or caused by biological factors, we sampled behavior events

(movement distances or bouts of push-ups) from normal distributions with the same mean and standard deviation as for our real data. For example, we observed 1,555 movement events, with distances that ranged from 0.01 to 15 m per move. As these data were highly right-skewed, they were transformed as log base 10. On the \log_{10} scale, movements ranged from -2 to 1.18, with a mean of -0.4548 and a S.D. of 0.67442 (Table 3.1). We thus sampled 1,555 values from a normal distribution with mean and S.D. as indicated from the observed real data on the \log_{10} scale, and then truncated any that exceeded the range of values observed for the real data. We then sampled from this simulated data set, without replacement, to obtain data sets for each of 57 lizards, matching the number of moves per lizard as observed for the real data set. In other words, some of the simulated lizard data sets had only two values, whereas one simulated lizard data set had 97 values, with an overall mean of 27.3 (see Table 3.1 and 3.2). For these 57 values, we then calculated the correlation between, for example, mean \log_{10} distance per move by maximum \log_{10} distance per move. We then repeated this procedure 20 times and calculated the mean correlation (Table 3.3, right column). We repeated the process for push-ups using no legs, 2-legged push-ups, and 4-legged push-ups (Table 3.3). We applied the same transformations to the simulated dataset as used on the observed data (log and rank when necessary for some traits).

We used the mean correlation coefficients from the simulated data as our null hypothesis. We calculated 95% confidence intervals of the correlation

coefficients from our observed dataset using the “cor.test” function in R version 3.4.3 and compared them to the correlation coefficients obtained from the simulated data. If the 95% confidence interval of the correlations from the observed lizards did not include the correlation coefficient from the simulated data, then we considered the observed correlations to be biologically significant.

For laboratory measures of performance (sprint speed, $\dot{V}O_2\text{max}$), and associated body masses, we calculated repeatability as the Pearson correlation between the first and second measures, and we tested for significant differences between days using a paired t-test. For sprint speed, we used the highest value of days 1 and 2 as the two replicates.

We used multiple regressions (both forward entry and backward elimination) to explore the predictive ability of lower-level traits (enzyme activities, organ masses, and external morphology) and parasite scores for whole-animal performances (sprint speed, $\dot{V}O_2\text{max}$). The multiple regression predicting $\dot{V}O_2\text{max}$ included the five parasite scores (intracellular parasites in red blood cells, free living nematodes in blood, stomach and intestines, and ticks) along with heart, liver, lung, thigh and calf muscle masses, upper and forearm dry muscle masses, hemoglobin concentration in blood, and citrate synthase activity in the heart, liver and thigh muscle. We used canonical correlation to test for relations between parasites and behavioral traits.

Analyses of sprint speed require further explanation. One lizard was uncooperative during sprint speed trials and so was excluded. Two lizards had

lost their longest hind limb digits, which might reduce performance, so they also were excluded from analysis of sprint speed. Finally, of the 41 lizards with reliable sprint speed data, 8 had broken but fully regenerated tails, 5 had partially regenerated tails, and 8 lost a small portion of their tail while in cloth bags before sprint speed trials. Because lizards can use their tails during locomotion, or the lost mass can affect sprint speed (Daniels, 1983; McElroy & Bergmann, 2013; Jagnandan *et al.*, 2014), we used dummy variables coding for each tail condition listed above. The final data set used in the multiple regressions predicting sprint speed included the parasite scores as well as calf and thigh muscle masses, upper and forearm dry muscle masses, hind limb span, lactate-dehydrogenase activity in the thigh muscle and heart, citrate synthase activity in the thigh (all residuals from regressions with body mass), and 3 dummy variables coding for damaged tail condition.

We calculated coefficients of variation (CV) of traits that did not scale with body mass (behavior traits) by dividing the standard deviation of each trait by their mean. For traits that scaled with body mass, we calculated residuals from regressions of each \log_{10} -transformed trait on \log_{10} transformed body mass, then multiplied the standard deviation of the residuals by 2.3026 (See details in Garland, 1984, 1985; Garland & Else, 1987).

We used multiple regressions to measure the predictive ability of sprint speed and $\dot{V}O_2\text{max}$ for the behavior variables (number of moves, distance moved, number of push-ups, number of bouts of push-ups per minute, and

number of push-ups per bout). Again, push-ups performed using no legs, two legs, or four legs were analyzed separately.

We used the results from the regressions described above to build an initial path model that included all of the statistically significant ($P < 0.05$) relationships identified from lower-level traits to the two measures of organismal performance, and from performance to behavior (Fig. 3.2). We used this model (herein called model 1.00) as a starting point from which to add or remove paths. We set the starting values of all path coefficients to 0.5 and used maximum likelihood to estimate the final path coefficients in Onyx (Oertzen *et al.*, 2014). Then, we tested if removing or adding single paths significantly changed the model by log likelihood ratio tests with 1 d.f., and by examining standard errors of the path coefficients. We tried to simplify the model by removing correlations or causal paths between variables one at a time. However, we never removed the correlation paths between sprint speed and $\dot{V}O_{2\max}$ or between hemoglobin and calf muscle mass (all residuals). We also made the model more complicated by adding paths from each lower-level trait directly to behavior (see discussion in Garland & Losos, 1994).

Results

Behavior in the field

We observed a total of 57 lizards for a grand total of 59 hours and 58 minutes. Pooling all observations and considering each move or bout of push-

ups as one independent event yields 2,424 total events (= rows in the data set). The total observation time per lizard averaged 63.1 min (range = 20 – 100 min). During these observations, we recorded 1,555 moves and 869 bouts of push-ups. The majority of push-up bouts were performed using two legs (53.4%), followed by push-ups using no legs (or head bobs - 29.1%) and four legs (14.3%). In 28 bouts (3.2%), lizards started on four legs and switched to two legs about halfway through the bout.

During field work, we observed a 24-minute long intense combat between two males that were already fighting when we first saw them. We performed 20-minute focals on each, then moved on to find other lizards before the combat was over, so we do not know the total length of their interaction. During the fight, the lizards would align their bodies side-to-side with snouts pointing in opposite directions and try to bite each other on their tails, hindlimbs or trunk. When a successful bite was connected to the hindlimb or lateral portion of the trunk, the aggressor would try to flip the other lizard on its back or throw it into the air (sometimes successfully). If the bite was connected to the tail, the aggressor would hold the tail and make abrupt movements sideways, apparently trying to break the opponent's tail. Lizards moved frequently during this interaction while chasing each other. Most moves were short (under 30 cm) but during a few moves the lizards traversed distances of up to 2 m. Very few resting periods in between movements and attacks were longer than 60 seconds and most were shorter than 30 seconds. Both focal observations were included in the analyses

described below, and values for these two observations included the most extreme number of moves per minute (2.68) and the 4th most extreme value (1.50).

Variability of movements

Lizards averaged 0.43 moves per minute (1,555 moves/59 hours and 58 minutes). Movement lengths ranged from 0.01 to 15 m (Table 3.1), with a mean of 0.99 ± 1.65 (\pm S.D.) meters, but lizards used short moves much more frequently, resulting in a highly right-skewed distribution (Fig. 3.1) (median = 0.30, mode = 0.1 m). A \log_{10} -transformation of movement distances yielded an approximately normal distribution, but with a peak corresponding to \log_{10} of 0.1 m (Fig. 3.1). Examination of the frequency of movement distances for individual lizards also indicated right-skewed distributions for most animals (only 3 lizards were not skewed, but each of these had <7 moves). For most individuals (50 of 57), a \log_{10} -transform again yielded distributions closer to normal. Therefore, we used the mean and standard deviation of \log_{10} -transformed values as two indicators of individual movement behavior. For each individual, we also obtained its maximum movement distance, distance moved per minute (total distance/total observation time), and number of moves per minute (total distance/total observation time), and number of moves per minute (total moves/total observation time). Histograms of these values (maximum distance, moves per minute, and distance per minute) were also right skewed and so we

again analyzed \log_{10} -transformed values. The maximum movement distance for each lizard was positively correlated with body mass ($r = 0.38$, $P = 0.031818$). Table 3.2 provides descriptive statistics of movement traits among individuals. The movement trait with lowest coefficient of variation was the standard deviation of distance per move ($CV = 0.26$) and the highest was the distance moved per minute ($CV = 0.66$).

Variability of push-up displays

Overall, we observed 14.4 bouts of push-ups per hour (869 bouts/59 hours and 58 minutes). Considering only bouts performed using no legs ($N = 253$, Table 3.1), the mean number of push-ups per bout was 4.85 ± 3.40 (1 – 17), but with a right-skewed distribution (Fig. 3.1, median = 4, mode = 1). We did not observe enough bouts of push-ups per lizard to decide if the number of push-ups per bout within individuals were normally distributed, so for each individual, we calculated the mean, standard deviation, and maximum number of push-ups per bout, the number of push-ups per minute, and the number of bouts of push-ups per minute from raw values. To improve normality of these behaviors, we rank-transformed the number of push-ups per minute and the number of bouts per minute prior to subsequent correlation and regression analyses with other traits. Coefficients of variation varied from 0.47 for the mean number of push-ups per bout and 0.60 for the standard deviation of the number of 0-legged push-ups per bout (Table 3.2).

Considering 2-legged bouts (N = 492), the mean number of push-ups per bout was 7.18 ± 2.62 (1 - 14) with a normal distribution. Coefficients of variation varied from 0.24 for the maximum number of push-ups per bout and 0.32 for the standard deviation of the number of push-ups per bout (Table 3.2). Considering 4-legged bouts (N = 151), the mean of push-ups per bout was 8.01 ± 2.79 (1 - 16), also with a normal distribution. For both 2- and 4-legged push-up bouts, we calculated the same variables as for 0-legged push-up bouts. Coefficients of variation varied from 0.32 for the standard deviation of the number of push-ups per bout and 0.40 for the maximum number of push-ups per bout (Table 3.2). To improve normality of these behaviors, we rank-transformed the number of push-ups per minute and the number of bouts of push-ups per minute.

Repeatability of behavioral traits

Based on 1-way ANOVAs, all behavioral traits differed significantly among individuals (Table 3.1, all $P < 0.001$). Intra-class correlation (ICC) coefficient for the \log_{10} distance travelled per move was only 0.05, whereas the number of push-ups per bout had higher values (push-ups using no legs = 0.15; two legs = 0.19; four legs = 0.31, Table 3.1).

Correlations among behavior traits

After accounting for correlations caused by purely mathematical dependence among traits, we identified several significant correlations among movement

traits and among each type of push-up traits (Table 3.3). Because we calculated a large number of correlations on related data, which inflates the possibility of Type 1 errors, we emphasize correlations for which the significance level was 0.01 or less .

Both movement rates (\log_{10} number of moves per minute and \log_{10} distance moved per minute) were positively correlated with the maximum distance moved and the standard deviation of \log_{10} distance per move showed positive correlation, but lower than expected from simulations (see Table 3.3 for coefficients). Standard deviation of \log_{10} movement distances and \log_{10} moves per minute show negative correlation ($r = -0.295$), also lower than expected ($r = 0.174$) from simulations. The \log_{10} number of moves per minute and the \log_{10} distance moved per minute were also less positively correlated ($r = 0.649$) than expected from simulations ($r = 0.886$).

Mean and standard deviation of the number of push-ups using no legs per bout ($r = 0.767$) show higher than expected ($r = 0.321$) positive correlation. The ranked number of push-ups per minute and ranked number of bouts per minute ($r = 0.805$) show lower than expected ($r = 0.908$) positive correlation. The correlation between mean and maximum number of 4-legged push-ups ($r = 0.892$) was higher than expected ($r = 0.718$). The mean number of 4-legged push-ups and the rank number of bouts per minute ($r = 0.397$), also show higher than expected correlation ($r = 0.044$).

Considering the traits that are mathematically independent, we found significant positive correlations between the distance moved per minute and the rank number of 2-legged bouts per minute ($r = 0.371$), the standard deviation of push-ups using no legs per bout and the \log_{10} standard deviation of 4-legged push-ups per bout ($r = 0.648$). The maximum number of 4-legged push-ups per bout and the ranked number of 2-legged push-ups per minute are positively correlated ($r = 0.397$). Finally, all ranked number of 2- and 4- legged push-ups per minute and bouts of push-ups per minute are positively correlated (not shown here).

Variability of locomotor performances

Considerable variation existed in maximal sprint speed (average = 2.27 m/s \pm 0.26 S.D.) and maximal aerobic capacity (average = 23.63 ml O₂ per hour \pm 4.36 S.D. - Table 3.4). Sprint speed was not significantly affected by body mass ($P = 0.89$), whereas $\dot{V}O_{2\max}$ significantly scaled as $M^{0.70}$ (Table 3.5). After removing the effect of body mass, the highest $\dot{V}O_{2\max}$ value was approximately 2 times the lowest and the fastest lizard was 1.63 times faster than the slowest. The coefficient of variation for residual sprint speed was 15% and for $\dot{V}O_{2\max}$ it was 13%.

Repeatability and correlation of locomotor performances

Sprint speed was repeatable ($r = 0.70$, $P < 0.0001$), with no significant difference between the fastest trials from the two days (paired t-test $P = 0.16$).

Because the relationship of the highest sprint speed trial with body mass was close to significant ($P = 0.077$), we also calculated repeatability of speed residuals from regressions on body mass, using the fastest trials from each day ($r = 0.70$, $P < 0.0001$). $\dot{V}O_{2\max}$ values were highly repeatable ($r = 0.94$, $P < 0.0001$) with a small but significant ($P = 0.011$), increase of 3.09% on the second day. $\dot{V}O_{2\max}$ residuals from regressions on body mass were also correlated between the first and second trials ($r = 0.88$, $P < 0.0001$), showing significant repeatability of mass-independent $\dot{V}O_{2\max}$. Residual sprint speed and residual $\dot{V}O_{2\max}$ were uncorrelated ($r = 0.07$, $P = 0.46$).

Variability of lower-level traits

All organ masses and external morphology measures showed significant positive relationships with body mass (Tables 3.4, 3.5); therefore, we used residuals from regressions on body mass in subsequent analyses. All mass-specific enzyme activities had a positive, but non-significant, relationship with body mass, except for heart lactate dehydrogenase, which scaled negatively as $\text{body mass}^{-0.28}$ ($P = 0.0003$). The coefficient of variation for anatomical and physiological traits varied from 0.02 for hind limb span to 0.16 for hemoglobin concentration. Enzyme assays had higher CV, ranging from 0.08 for heart lactate dehydrogenase activity to 0.25 for liver lactate dehydrogenase activity.

Correlations between replicates

Both hematocrit and hemoglobin had high Pearson correlations for their replicate measures. The coefficient for hematocrit was 0.935 ($P < 0.0001$) with raw values and 0.955 ($P < 0.0001$) with residuals from regressions on body mass. The coefficient for hemoglobin concentration was 0.941 ($P < 0.0001$) with raw values and 0.937 ($P < 0.0001$) with residuals from regressions on body mass.

Correlations among lower-level traits

We found several significant correlations among performances and lower level traits . Hematocrit and hemoglobin levels were positively correlated ($r = 0.90$, $P < 0.001$). To avoid using redundant information and causing multicollinearity issues during regressions (Slinker & Glantz, 1985), we avoided using both traits in subsequent analysis. Residual hindlimb and fore limb span were also positively correlated ($r = 0.74$, $P < 0.001$).

We found positive relations between heart CS and thigh CS ($r = 0.46$, $P = 0.002$), heart CS and thigh LDH ($r = 0.33$, $P = 0.03$), thigh CS and thigh LDH ($r = 0.42$, $P = 0.005$), and a negative correlation between heart LDH and liver LDH ($r = -0.49$, $P = 0.001$). Correlations using residuals from body mass regressions provided similar results (not shown) .

Effects of parasites

We found no evidence of *P. mexicanum* infection. Multiple regressions revealed no significant effect of the number of ticks, intracellular parasites in red

blood cells, and free-living nematodes in the blood, stomach, or intestines on hematocrit, blood hemoglobin levels, sprint speed, $\dot{V}O_{2\max}$, or any of the behavioral traits.

Multiple regressions: morphology to performance

Multiple regressions of residual performance traits on residual lower-level morphological, physiological, and biochemical traits revealed residual calf muscle mass ($B = 0.53$, $P = 0.0022$) and residual thigh muscle mass ($B = -0.37$, $P = 0.027$) as predictors of residual sprint speed ($R^2 = 0.28$), and residual blood hemoglobin concentration ($B = 0.42$, $P = 0.009$) as a significant predictor of residual $\dot{V}O_{2\max}$ ($R^2 = 0.17$).

Multiple regressions: performance to behavior

Residual sprint speed was negatively related to all two-legged push-up traits, but never statistically significant. Residual $\dot{V}O_{2\max}$ predicted the average number of 2-legged ($r = 0.32$, $P = 0.044$) and 4-legged ($r = 0.36$, $P = 0.033$) push-ups per bout, as well as the maximum number of 4-legged push-ups in one bout ($r = 0.39$, $P = 0.018$). Multiple regressions of each of the behavioral traits on both residual sprint speed and $\dot{V}O_{2\max}$ did not reveal any cases in which both independent variables were significant predictors.

Path models: morphology, performance, and behavior

The starting model (model 1.00) included the significant relationships found in multiple regressions between lower-level traits and performance, and between performance and behavior (Fig. 3.2). However, model 1.00 excluded the mean number of 4-legged push-ups per bout due to the high correlation with the maximum number of 4-legged push-ups per bout ($r = 0.89$, $P < 0.0001$, respectively). We included the correlation paths between each pair of lower level traits (hemoglobin, thigh and calf muscle masses) on model 1.00 and we did not remove them in subsequent alterations of the model (Petraitis *et al.*, 1996). We also included the correlation between residual sprint speed and residual $\dot{V}O_2\text{max}$ because we were specifically interested in testing for possible trade-offs between these two performance measures. Model 1.00 had a log likelihood of 218.375 and did not exhibit lack of fit ($\chi^2 = 3.915$, restricted d.f. = 11, $P = 0.97$). We subtracted or added paths, one at a time, to create a total of 12 additional models (Table 3.6) and performed likelihood ratio tests (critical χ^2 with 1 d.f. = 3.841) between each and the starting model (Fig. 3.2). Based on chi-square tests, none of these additional models lacked significance, but removing any causal path from model 1.00 significantly reduced model fit according to likelihood ratio tests. Removing the correlation between the average number of 2-legged push-ups and the maximum number of 4-legged push-ups did not affect significance. Adding paths from lower-level traits directly to behavior did not improve model fit.

Overall, results of the path analysis supported the bivariate and multiple regression analyses described above. In model 1.00, all causal paths would be considered different from zero, based on twice their estimated standard errors. The same is not true for all correlation paths. Twice the standard error of the residual thigh muscle mass and residual calf muscle mass does not overlap zero. However, twice the standard error of the correlations between residual calf muscle mass and residual hemoglobin concentration, between residual sprint speed and residual $\dot{V}O_{2\max}$, between mean number of 2-legged push-ups per bout and maximum number of 4-legged push-ups per bout, and between residual thigh and residual hemoglobin concentration all overlap zero (Fig. 3.2).

Discussion

Variation and repeatability

Variability of the measured traits followed this general rule: behavior in the field showed larger amounts of individual variation, performances showed an intermediate level, and morphological traits were the least variable, as indicated by coefficients of variation (Tables 3.2 and 3.4). However, we note a few exceptions to this pattern. Among behavioral traits, the mean numbers of 2- and 4-legged push-ups are less variable than are push-ups using no legs or mean movement distances. Push-up displays in iguanids are "stereotyped" behaviors (Carpenter & Ferguson, 1978) and have a recognizable repeated sequential pattern (Tinbergen & Iersel, 1947; Pantin, 1996). As stereotyped behaviors,

push-up displays would be expected to vary less than movement traits, which can be strongly influenced by environmental conditions (e.g., home range physical characteristics and resource quantity or quality).

The mean number of push-ups using no legs may be more variable because this behavior is more likely to be used during broadcast displays (Martins, 1993; Decourcy & Jenssen, 1994; Baird, 2013), which are used in the presence of an unknown audience, not during direct conflicts. When no direct dispute is taking place, the consequences of “imperfect” performances during displays should be less severe. In contrast, 2- and 4-legged push-ups are more frequently used during courtship or male-male disputes (Martins, 1993), which determine access to important resources such as territories and their associated basking sites, food, and access to females. Further studies should explicitly compare displays performed during courtships or male-male disputes with broadcast displays to determine where each falls on the spectrum ranging from highly stereotyped to highly variable (Wainwright *et al.*, 2008).

Secondly, hemoglobin concentration showed higher CV than all other traits, except for some enzyme activities (Table 3.4). Perhaps the high variability of hemoglobin concentration compared to that of other traits involved in the oxygen transport pathway can explain why that is the only significant predictor of $\dot{V}O_2\text{max}$. In *Amphibolurus nuchalis*, hemoglobin concentrations also have higher CV than $\dot{V}O_2\text{max}$ and other lower-level traits, except for liver mass, (Garland & Else, 1987) but not in *Ctenosaura similis*, which has higher CV for $\dot{V}O_2\text{max}$ and

liver (Garland, 1984). Both studies found hemoglobin concentration as a significant predictor of $\dot{V}O_2\text{max}$.

Finally, most mass-specific enzyme activities also showed high CV compared to other lower-level traits (Table 3.4). Citrate synthase and lactate dehydrogenase activities measured with similar methods in other lizard species showed similar, or even higher, variation (Garland, 1984; Garland & Else, 1987). Liver wet mass can vary due to differences in glycogen or lipid storage, which could inflate variation in enzyme activities expressed per unit mass of wet tissue. Mixed thigh muscles contain multiple separate muscles, with variable fiber type composition (Gleeson *et al.*, 1980). Even considering a single muscle, the iliofibularis, we can find very high coefficients of variation in the proportion of fast glycolytic, fast oxidative glycolytic, and slow oxidative fiber types [0.39 in *Sceloporus* species and up to 0.62 in other lizards, calculated directly from Table 2 in Bonine (2005)]. Each muscle fiber type has somewhat different contractile properties and mass-specific enzyme activities (Peter *et al.*, 1972), which would tend to increase variability among individuals.

Repeatability generally showed the opposite pattern to CV. Traits with high CV (e.g., behavioral traits, Table 3.1) had lower repeatability, whereas traits with relatively lower CV (e.g., performance traits) had higher repeatability. Among behavioral traits, movement distances had the lowest repeatability, measured as the intraclass correlation coefficient (ICC). Movement distances should be more affected by specific environmental circumstances, such as

ambient temperature or the intensity of the stimulus from a conspecific. For example, we can expect male lizards to move longer distances per move when they are chasing intruders than when they are foraging. Because we did not make any distinction between moves performed under different circumstances (e.g., running to chase another male vs. to catch prey), we expected movement distances to be more variable than 2- and 4-legged push-ups, which are used in specific situations.

Consistent with previous studies of lizard exercise physiology (e.g., Garland, 1985; Garland & Else, 1987; Albuquerque *et al.*, 2015a), we observed significant repeatability in both maximal sprint speed and maximal aerobic capacity using either raw values or residuals from regressions with body mass. The high repeatability indicates consistent variation in locomotor performance among individuals. As a result, locomotor performance traits can be used to test hypotheses related to the effects of maximal performances on individual variation in ecology, behavior, reproductive success, or other direct or indirect correlates of fitness. Repeatability of $\dot{V}O_2\text{max}$ ($r = 0.94$) was considerably higher than that of sprint speed ($r = 0.68$), but both values are consistent with the repeatability range found in the literature (e.g., Garland, 1985; John-Alder *et al.*, 1986; Garland & Else, 1987).

Correlations among traits within levels of biological organization

Considering only push-ups using no legs, lizards with higher means also had higher standard deviations of the number of push-ups per bout, suggesting that individuals that do many push-ups per bout still perform some bouts with few push-ups. Additionally, the lower than expected positive correlation between push-ups per minute and bouts of push-ups per minute suggest that lizards are not performing consistent numbers of push-ups per bout, so push-ups per minute and bouts per minute are not as tightly correlated as expected under the null hypothesis calculated by simulations (Table 3.3).

The higher than expected positive correlation between mean number of 4-legged push-ups per bout and number of 4-legged push-ups per minute suggests that for 4-legged push-ups, lizards that do more push-ups in one bout also engage in more frequent bouts. Because lizards use 4-legged pushups during intense interactions (Carpenter & Ferguson, 1978; Martins, 1993), this correlation might reflect variation in physiological factors (e.g., circulating hormone levels) that affect aspects of "personality," with some individuals consistently showing more aggressive behavior and reacting more readily to conspecifics (Careau *et al.*, 2008; Réale *et al.*, 2010; Careau & Garland, 2012). Such a set of relationships could also help explain the positive correlations found between 2- and 4-legged behavioral traits. These two displays are more frequently used during conspecific interactions, and more aggressive lizards would likely increase frequency and intensity of both behaviors. Future studies should investigate

possible costs of high levels of aggressiveness and the relative reproductive success of possible alternative "strategies" (e.g., Husak *et al.*, 2008).

Among lower-level traits, the high correlation between hematocrit and hemoglobin levels ($r = 0.90$) suggests that little variation exists in the average size of red blood cells or in mean corpuscular hemoglobin content. The positive correlation ($r = 0.74$) between residual fore and hindlimb lengths indicate that even after the effect of body size is removed, limb lengths are still positively associated. Similar results have been observed in several lizard species (Jaksić *et al.*, 1980; Garland, 1985; Christian & Garland, 1996), and those authors suggested that independent development of hindlimbs and forelimbs might be somewhat constrained during ontogeny. The significant positive correlation between thigh CS and thigh LDH ($r = 0.42$) has also been observed in *Ctenosaura similis* (Garland, 1984). In *Amphibolurus nuchalis*, a similar relationship was observed but between CS and pyruvate kinase, which is also involved in anaerobic metabolism (Garland & Else, 1987). These relationships may arise from variation in muscle fiber type composition (e.g., Bonine *et al.*, 2001, 2005), and suggest a general pattern in thigh muscles in which both aerobic and anaerobic capacities increase together.

Relationships with body mass

Behavioral traits were not significantly related to body mass during the breeding-season in adult male *S. occidentalis* lizards at our study site (e.g., we

found no evidence for differential levels of activity in relation to body size). Among species of lizards (and among species of mammals), both home range area and daily movement distances increase with body size, as would be expected, but these comparisons obviously involve a much larger range of body sizes than represented by the lizards studied here (Perry & Garland, 2002; Albuquerque *et al.*, 2015b; Garland & Albuquerque, 2017).

Geometric similarity is often used as a null model for animal scaling relationships (e.g., Hill, 1950; Schmidt-Nielsen, 1984). For geometrically similar animals, linear dimensions should scale as mass to the power of 0.33, organ masses and metabolic rates should scale as mass to the power of one (at least for small ectotherms because the differences in the necessity to lose metabolic heat caused by differences in body size are probably negligible), and mass or volume specific measurements (e.g., hemoglobin and enzyme activities) should have no significant relation with mass (mass exponent = 0). If twice the standard error of the slope of regression lines of \log_{10} traits on \log_{10} body mass did not include those values, then we considered the trait to deviate from isometry. We recognize, however, that measurement error present in the independent variable (body mass) causes the slopes of least-squares regressions to underestimate the true functional relations. This underestimation is best dealt with through measurement-error models (e.g., see Ives *et al.*, 2007), but we lacked comparable estimates of error variance for many of our traits.

Under geometric similarity, A. V. Hill argued that "similar animals of different size should be able to run or swim at the same linear speed" (Hill 1950, p. 215). Since Hill's classic paper, numerous theoretical and empirical studies have addressed the scaling of maximal sprint speed with body size (Garland, 1983; Kram & Roberts, 2016). Among species of lizards and mammals, the scaling of sprint speed does not follow a simple pattern: speed is related to body mass in a curvilinear fashion, with the fastest species being of intermediate body size (Van Damme & Vanhooydonck, 2001; Garland & Albuquerque, 2017; references therein). In the present sample of adult male *S. occidentalis*, maximal sprint speed was positively but not significantly related to body mass with a shallow slope of 0.179 and P-value equal 0.076, so perhaps a wider range of body masses would have revealed a significant relation. The ontogenetic allometry or static allometry of sprint speed has been reported for several other species of lizards, with some showing no statistical relationship with body size (Garland, 1984; Losos, 1990; Peterson & Husak, 2006), and many others a positive relationship (references in Garland & Losos, 1994). Additionally, another lizard species, *Varanus panoptes*, show a negative quadratic relationship with body mass (Clemente *et al.*, 2012). In those cases, speed is highest at an intermediate body size. If sprint speed in *S. occidentalis* from our study site is affected by body mass in a similar manner, then we may have observed no correlation because we included only adult male lizards in our study, and the observed range in body size (10 to 22 grams) may correspond approximately to

the optimal size range for maximal sprint speed. The same explanation might apply to *Crotaphytus collaris* studied by Peterson and Husak (2006), but not to *Amphibolurus nuchalis* or *Ctenosaura similis* studied by Garland (1984, 1985), as the latter studies included juveniles and therefore covered a much wider range of body masses (1.3 to 48 and 12 to 866 grams, respectively).

$\dot{V}O_2$ max showed negative allometry. The $\dot{V}O_2$ max scaling exponent we found (0.71) is lower than the intraspecific exponent for other lizard species (scaling = 0.90 in Garland, 1984; 0.80 and 0.95 in John-Alder, 1984b; a; and 0.96 in Garland & Else, 1987), but some of those studies had a wider range of body sizes and/or used considerably larger-bodied species.

Among lower-level traits, hematocrit and hemoglobin concentrations had positive allometry. Tail length, liver, lung, thigh muscle, calf muscle, upper arm muscle, and forearm muscle masses scaled isometrically. Heart lactate dehydrogenase, heart mass, SVL, and hind- and forelimb spans, had negative allometry (Table 3.2). Some of these results are different from other studied lizard species. For example, *Ctenosaura similis* does not show positive allometry of hematocrit or hemoglobin concentration, negative allometry of hindlimb span, and positive allometry for heart mass (Garland, 1984). On the other hand, *Amphibolurus nuchalis* shows positive allometry of thigh muscle mass and of all mass specific enzyme activities measured here (Garland & Else, 1987).

Predictors of sprint speed and $\dot{V}O_2\text{max}$

Residual calf muscle mass was a positive predictor of residual sprint speed, whereas residual thigh muscle mass had a negative effect. Reilly (1995) studied the contribution of hindlimb muscles to locomotion in *Sceloporus clarki*. He measured the activity of four muscles in the thigh and three in the calf during locomotion at constant speed (0.83 m/s). He showed these seven muscles had high peaks in activity during the stance phase (propulsion phase), but most of them were also active during the swing phase (limb recovery phase), contributing to limb cycling and acting as co-contracting antagonists keeping the joints fixed as the limb travelled in the air. When comparing the electromyography results to the videotaped movement of the lizard, Reilly concluded that plantar flexion (which is created by the contraction of calf muscles) plays a major role in generating thrust. Years later, Higham et al. (2011) showed that individual variation in maximal sprint speed and acceleration in *Sceloporus woodi* is positively correlated with the percentage and diameter of fast glycolytic fibers in the gastrocnemius, a propulsion-generating calf muscle, but not with fiber composition in the iliofibularis, a small thigh muscle active during the swing-phase. If this pattern also holds in *S. occidentalis*, then relatively larger calf muscles would allow lizards to generate greater force exertion (and consequently higher speed), whereas relatively small thigh muscles could reduce leg mass, thus allowing them to move the limb faster during the swing-phase (assuming that the power available for recovery was not compromised).

According to our results, blood hemoglobin concentration is the only significant predictor of $\dot{V}O_2\text{max}$, suggesting that blood oxygen carrying capacity is the limiting factor for maximal aerobic capacity in this population during the breeding season. Hemoglobin (or hematocrit) level is also a significant predictor of $\dot{V}O_2\text{max}$ in *Amphibolurus nuchalis* and *Ctenosaura similis* (Garland, 1984; Garland & Else, 1987). However, contrary to what we found, those studies also reported other lower-level traits as significant predictors of $\dot{V}O_2\text{max}$ in those species. In *Amphibolurus nuchalis*, liver mass and thigh pyruvate kinase (an enzyme not measured in our study) are negative predictors, whereas in *Ctenosaura similis* CS thigh, CS liver, and LDH heart are positive predictors of $\dot{V}O_2\text{max}$. Note that when the same dataset obtained for *C. similis* was analyzed using path models, including all significant relationships as causal paths, CS thigh was not a significant predictor (Garland & Losos, 1994). Blood O₂ carrying capacity (measured as hematocrit or hemoglobin content in blood) is also a significant predictor of lizard endurance capacity (Garland, 1984; Garland & Else, 1987), a trait intimately related with $\dot{V}O_2\text{max}$ in *Cnemidophorus tigris* and *Ctenosaura similis* (Garland, 1993; Garland & Losos, 1994) and shown to be correlated among lizard species (Bennett *et al.*, 1984; Clemente *et al.*, 2009).

Predictors of behavior

Sprint speed is positively correlated with social dominance in paired laboratory tests of males in two species of lizards, *Sceloporus occidentalis* and

Urosaurus ornatus (Garland *et al.*, 1990a; Robson & Miles, 2000; but see Perry *et al.*, 2004), but not in the distantly related *Anolis cristatellus* (Perry *et al.* 2004). Male sprint speed is also positively associated with territory size and the number of offspring sired in *Crotaphytus collaris* (Husak *et al.*, 2006; Peterson & Husak, 2006). In the present study, sprint speed showed no statistically significant relationship with any of the movement or push-up behavioral traits when analyzed with multiple regressions. Perhaps higher sprint speed allows lizards to chase after intruders or cover the territorial area more effectively (cf. Peterson and Husak 2006), supporting the “better defenders” hypothesis (Husak *et al.*, 2008), but it plays no evident role in the individual capacity to perform displays in our study population of *S. occidentalis*. The social dominance test used by Garland *et al.* (1990a) was performed with size-matched pairs of lizards in small enclosures for one hour with one basking source (similar methods were used by Robson and Miles 2000 and Perry *et al.* 2004). This type of setup is useful to determine an immediate winner, but a much more complex scenario exists in nature where lizards can have multiple basking sites to thermoregulate until they are ready to fight, or they can retreat during the fight and come back at later time, increasing the chance of gaining territorial space (Stamps & Krishnan, 1995). Additionally, combats in *Sceloporus occidentalis* can involve multiple individuals (Fitch, 1940).

In this study $\dot{V}O_2\text{max}$ was an important predictor of the maximum number of 4-legged push-ups per bout, suggesting that it affects (and possibly limits) the

effort that lizards can sustain during 4-legged displays. Push-ups using two and four legs are frequently observed during conspecific interactions, especially during male-male territorial disputes (Fitch, 1940; Martins, 1993; Sheldahl & Martins, 2000). Even though these interactions can be brief (e.g., when one lizard is much larger than the other), sometimes they can last for long periods (Stamps & Krishnan, 1997) and escalate to long and intense fights, as once seen during our field work (see beginning of Results section). During that 24-minute long combat, rest periods between movements and attacks were rarely longer than 30 seconds. In the experiment performed by Perry et al. (2004), which found a positive correlation between dominance, assertive displays, and endurance, lizard interactions lasted up to 8 hours. Fitch (1940) also reported a 45-minute long interaction and added comments about individuals “showing evidence of fatigue at later stages of the fight.” Both observations suggest that aerobic and anaerobic capacities were involved during these disputes.

Additionally, the frequency and intensity of push-up displays increase considerably during the breeding season when males establish their territories (Stamps & Krishnan, 1997; Sheldahl & Martins, 2000), which is when we conducted field observations. Therefore, lizards may use these displays frequently throughout the day at that time of the year, instead of only during rare and short bursts of intense display activity. In fact, the frequency of bouts of 2- and 4-legged push-ups during our observations was as high as once every two minutes (2-legged) or once every five minutes (4-legged), respectively (Table

3.2). If *S. occidentalis* are under similar selection favoring higher frequency of push-up displays and territorial defense as *Crotaphytus collaris* (Baird *et al.*, 2003, 2007; Baird, 2013), then it is possible that frequent repetition of this behavior, especially during the hours of high activity, increases its dependency on aerobic metabolism.

Biro *et al.* (2018) suggest that the variability of behavioral activity should be positively associated with aerobic scope (the difference between minimal and maximal metabolic rates). We did not measure standard or resting oxygen consumption, and so cannot calculate aerobic scope *per se*, but we did not find any relationship between $\dot{V}O_2\text{max}$ and the variability (SD) of any behavioral trait.

Complete path models

Path analysis indicated that when the three levels of biological organization (lower-level traits, performance, and behavior) are evaluated simultaneously, the significant relations found between each pair separately (e.g., from lower-level traits to performance) still hold. Removing any of the causal paths found from multiple regressions reduced the overall fit of the model, showing that all the variables included in the models are contributing to explain the variation in the observed traits.

Additionally, including direct paths from any of the significant lower-level traits (residual calf muscle mass, residual thigh muscle mass, or residual hemoglobin concentration) to the maximum number of 4-legged push-ups did not

increase model fit. Our results suggest that, when the appropriate performances are measured, lower-level traits do not have a direct effect on behavior.

However, we did not test whether large differences in body size would affect the decision to display or fight, as suggested by Garland and Losos (1994).

Concluding Remarks

Our results show that aspects of individual variation in field behaviors can be related to whole-organism performance abilities, which in turn reflect differences in morphology and physiology, although not parasite load, at least for this sample of lizards. Moreover, the high variability of behavior in the field might reduce the statistical power to detect correlations with performance and therefore, some of the relationships between behavior and performance may be stronger than suggested by our results (Adolph & Hardin, 2007). One improvement for future studies would be to obtain more fine-grained data on field behaviors, such as rates of movement during specific activities (e.g., foraging or moving near conspecifics) or push-ups directed to males and females separately. Greater durations of observations for each individual would also improve accuracy and precision of estimates of individual differences in means and variabilities. New measures of performance that might directly relate to the capacity to do push-ups should also be included, such as clinging capacity.

Figure Legends

Figure 3.1 – Histograms of behavior traits calculated from each event independently.

Figure 3.2 – Starting path model (model 1.00) including significant variables from multiple regressions from morphological and physiological traits to performance and from performance to behavior traits. * indicates 2 X S.E. does not overlap zero.

Figures

Figure 3.1

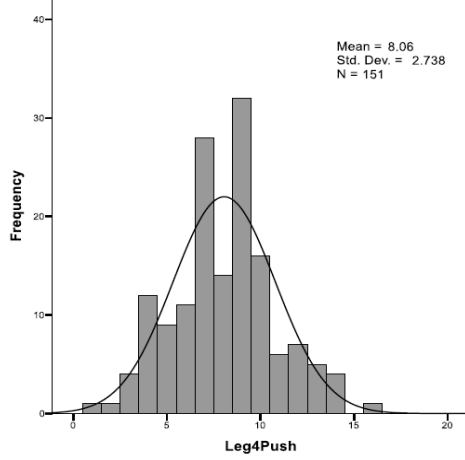
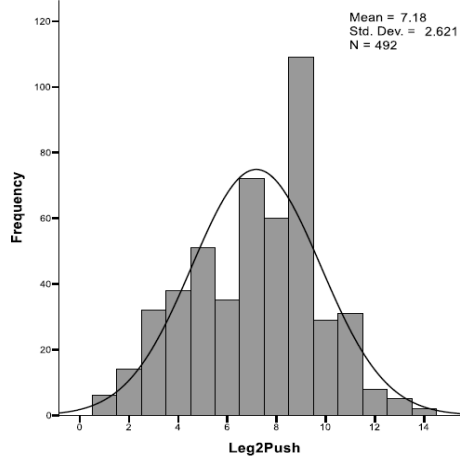
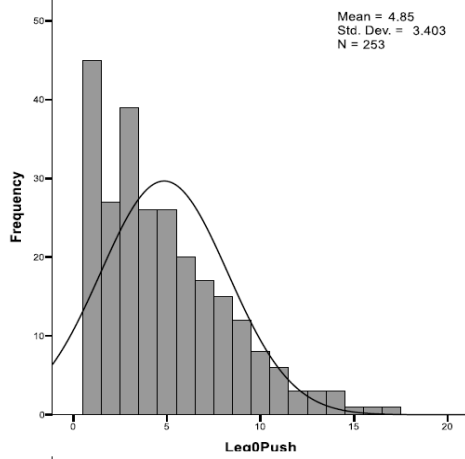
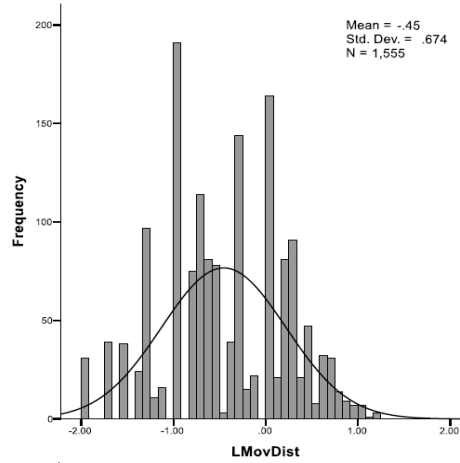
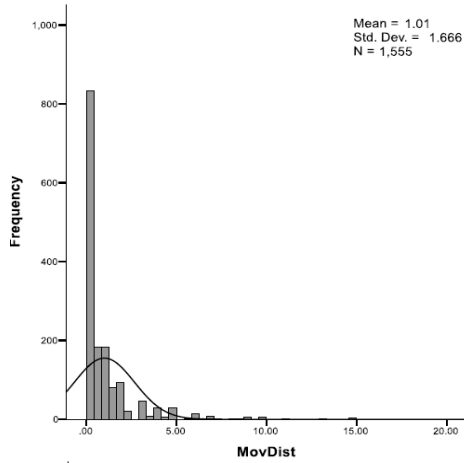


Figure 3.2

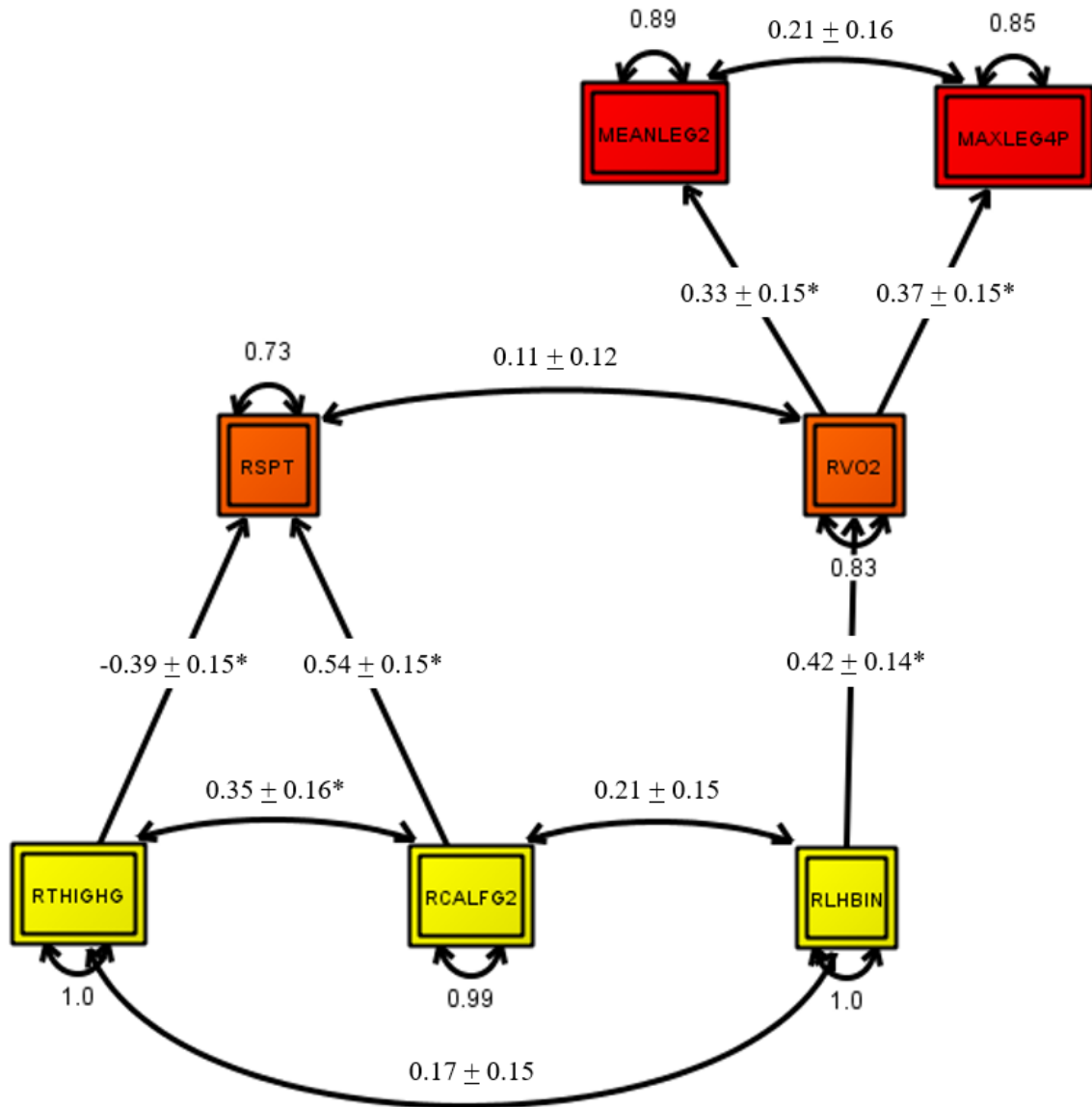


Table 3.1. Descriptive statistics and repeatability (ICC) of movement distances, \log_{10} movement distances, (\log_{10})number of push-ups per bout using no legs [(L)leg0Push], two legs (Leg2Push), and four legs (Leg4Push) in *Sceloporus occidentalis* from Hampton Buttes, OR. One event = one move or one bout of push-ups.

Variable name	N Events	min N events /lizard	max N events /lizard	N events /lizard	Event Min	Event Max
MovDist	1555	2	97	27.28	0.01	15
LMovDist	1555	2	97	27.28	-2	1.18
Leg0Push	253	1	18	5.06	1	17
LLeg0Push	253	1	18	5.06	0	1.23
Leg2Push	492	1	41	9.65	1	14
Leg4Push	151	1	15	3.68	1	16

Variable name	Event Mean	Event S.E.	Event S.D.	Event Skew	ICC	One-way ANOVA P
MovDist	1.01	0.04	1.67	3.58	-	-
LMovDist	-0.45	0.02	0.67	-0.06	0.05	0.00022
Leg0Push	4.85	0.21	3.40	0.99	-	-
LLeg0Push	0.57	0.02	0.34	-0.33	0.15	<0.0001
Leg2Push	7.18	0.12	2.62	-0.22	0.19	<0.0001
Leg4Push	8.06	0.22	2.74	0.13	0.31	<0.0001

Table 3.2. Descriptive statistics of number of moves (MovesNum), distance per move (MovDist), and bouts of push-ups using no legs (Leg0), two legs (Leg2) and four legs (Leg4) among individual lizards of *S. occidentalis* from Hampton Buttes, OR. Coefficients of variation (C.V.) were calculated as the standard deviation of residuals from regressions on body mass multiplied by 2.3026, see methods section for details. Variable names meaning: “Tot” = total, “Max” = maximum, “R” = residual from regressions on body mass using log₁₀ transformed data (base 10), “SD” = standard deviation of movement distances or number of push-ups per bouts within individual lizards, “Rank” = rank transformed data, “BPmin” = bouts per minute.

Variable Name	N	Min	Max	Means	S.E.	S.D.	Skew	C.V.
TotFocNum	57	1	5	3.26	0.16	1.19	-0.67	-
FocDurTot	57	20	100	63.11	3.01	22.76	-0.64	-
MovesNum	57	2	97	27.28	2.40	18.11	1.48	-
MovesPMin	57	0.08	1.20	0.45	0.04	0.27	1.11	-
RMovesPMin	44	-0.64	0.52	0	0.04	0.25	-0.40	0.58
MeanMovDist	55	0.25	2.38	1.04	0.07	0.50	0.75	-
MeanLMovDist	55	-0.81	-0.06	-0.46	0.03	0.19	0.16	-
RMeanLMovDist	43	-0.39	0.36	0	0.03	0.20	0.03	0.46
MaxMovDist	57	1	15	5.82	0.45	3.41	1.11	-
RMaxMovDist	44	-0.67	0.42	0	0.04	0.24	-0.54	0.56
SDMovDist	55	0.20	3.87	1.53	0.11	0.84	1.09	-
SDMovDist	55	0.29	0.95	0.66	0.02	0.13	-0.25	-
RSDMovDist	43	-0.32	0.30	0	0.02	0.11	-0.25	0.26
DistPMin	57	0.09	1.06	0.43	0.03	0.25	0.73	-
RDistPMin	44	-0.59	0.48	0	0.04	0.28	-0.24	0.66
TotLeg0Bouts	57	0	18	4.44	0.59	4.44	1.48	-
MeanLeg0PerBout	50	1	9	4.45	0.30	2.10	0.29	0.47
MaxLeg0PerBout	50	1	17	7.24	0.57	4	0.45	0.55
SDLeg0PerBout	41	0	5.66	2.48	0.23	1.50	0.17	0.60
Leg0PMin	57	0	2.35	0.34	0.06	0.47	2.48	-
RankLeg0PMin	57	4	57	29	2.20	16.58	0.01	-
Leg0BPMin	57	0	0.40	0.07	0.01	0.08	2.13	-
RankLeg0BPMin	57	4	57	29	2.20	16.58	0.01	-

Table 3.2 (cont.)

Variable Name	N	Min	Max	Means	S.E.	S.D.	Skew	C.V.
TotLeg2Bouts	57	0	41	8.63	1.08	8.15	1.69	-
MeanLeg2PerBout	50	3.80	11	7.06	0.24	1.71	-0.03	0.24
MaxLeg2PerBout	50	5	14	10	0.32	2.23	-0.44	0.2231
SDLeg2PerBout	47	0.55	4.15	2.29	0.11	0.74	0.27	0.32
Leg2PMin	57	0	4.18	0.95	0.12	0.87	1.54	-
RankLeg2PMin	57	3.50	57	29	2.20	16.59	0.01	-
Leg2BPMIn	57	0	0.51	0.13	0.01	0.11	1.16	-
RankLeg2BPMIn	57	3.50	57	29	2.20	16.59	0.01	-
TotLeg4Bouts	57	0	15	2.65	0.46	3.44	2.02	-
MeanLeg4PerBout	41	1	12.50	7.46	0.41	2.60	-0.35	0.35
MaxLeg4PerBout	41	1	16	9.02	0.57	3.65	-0.24	0.40
SDLeg4PerBout	26	0.71	5.69	2.03	0.23	1.17	1.33	-
RLSDLeg4PerBout	23	-0.26	0.26	0	0.03	0.14	-0.01	0.32
Leg4PMin	38	0.00	1.28	0.37	0.06	0.38	1.09	-
RankLeg4PMin	38	3.00	38.00	19.50	1.80	11.10	0.01	-
Leg4BPMIn	38	0.00	0.19	0.05	0.01	0.05	1.37	-
RankLeg4BPMIn	38	3.00	38.00	19.50	1.80	11.10	0.01	-

Table 3.3. Correlation coefficients (Pearson's r) and 95% confidence intervals (CI) for behavioral traits that can be related simply by mathematical interdependence (see Methods). Bold values indicate cases in which the 95% CIs for the real data did not include the mean correlation coefficient from simulated data (see Methods). Such cases are deemed to be biologically significant, rather than simply reflecting mathematical relationships.

Behavior traits	Observed r	Observed lower CI	Observed Upper CI	Simulated r
MeanLMovDistPL by LMaxMovDistPL	0.447	0.170	0.614	0.391
MeanLMovDistPL by SDLMovDistPL	0.093	-0.179	0.352	-0.002
MeanLMovDistPL by LMovesPMinPL	-0.072	-0.372	0.152	0.039
MeanLMovDistPL by LDistPMinPL	0.552	0.341	0.716	0.354
MaxMovDistPL by SDLMovDistPL	0.501	0.186	0.624	0.569
LMaxMovDistPL by LMovesPMinPL	-0.033	-0.344	0.173	0.520
LMaxMovDistPL by LDistPMinPL	0.529	0.326	0.702	0.814
SDLMovDistPL by LMovesPMinPL	-0.295	-0.543	-0.065	0.174
SDLMovDistPL by LDistPMinPL	0.059	-0.233	0.297	0.418
LMovesPMinPL by LDistPMinPL	0.649	0.349	0.715	0.886
Leg0 Mean vs Max	0.799	0.669	0.881	0.676
Leg0 Mean vs SD	0.767	0.601	0.869	0.321
Leg0 Mean vs RankPM	0.509	0.194	0.646	0.418
Leg0 Mean vs RankBPM	-0.046	-0.323	0.238	0.045
Leg0 Max vs SD	0.831	0.704	0.907	0.706
Leg0 Max vs RankPM	0.810	0.592	0.849	0.772
Leg0 Max vs RankBPM	0.427	0.166	0.632	0.551
Leg0 SD by RankPM	0.520	0.158	0.661	0.222
Leg0 SD by RankBPM	0.128	-0.192	0.422	0.095
Leg0 RankPM by RankBPM	0.805	0.614	0.860	0.908

Table 3.3 (cont.)

Behavior traits	Observed r	Observed lower CI	Observed Upper CI	Simulated r
Leg2 Mean vs Max	0.684	0.501	0.809	0.607
Leg2 Mean vs SD	0.138	-0.155	0.409	0.087
Leg2 Mean vs RankPM	0.346	0.077	0.571	0.209
Leg2 Mean vs RankBPM	0.079	-0.204	0.350	-0.036
Leg2 Max vs SD	0.531	0.288	0.710	0.599
Leg2 Max vs RankPM	0.633	0.370	0.744	0.616
Leg2 Max vs RankBPM	0.431	0.173	0.633	0.445
Leg2 SD by RankPM	0.028	-0.322	0.252	0.046
Leg2 SD by RankBPM	-0.024	-0.309	0.265	0.020
Leg2 RankPm by RankBPM	0.919	0.886	0.962	0.924
Leg4 Mean vs Max	0.892	0.805	0.941	0.718
Leg4 Mean vs LogSD	0.047	-0.331	0.441	-0.047
Leg4 Mean vs RankPM	0.705	0.270	0.723	0.394
Leg4 Mean vs RankBPM	0.397	0.062	0.652	0.044
Leg4 Max vs LogSD	0.560	0.252	0.791	0.508
Leg4 Max vs RankPM	0.852	0.567	0.856	0.787
Leg4 Max vs RankBPM	0.657	0.405	0.816	0.602
Leg4 LogSD by RankPM	0.168	-0.157	0.577	0.065
Leg4 LogSD by RankBPM	0.092	-0.224	0.571	0.074
Leg4 RankPM by RankBPM	0.918	0.888	0.972	0.936

Table 3.4. Descriptive statistics of sprint speed (m/s), $\dot{V}O_2$ max (ml O₂ per hour), hemoglobin concentration (mg/ml), organ masses (g), external morphology (mm), and tissue specific enzyme activities (micromoles per gram of wet tissue) of *S. occidentalis* from Hampton Buttes, OR. Variables starting with “R” refers to residuals calculated from regressions on body mass using log transformed data (base 10).

Trait	N	Min	Max	Mean	SE	SD	Var	Skew	C.V.
Body mass (sprint speed)	44	10.300	22.100	14.861	0.422	2.798	7.828	0.42	
Sprint speed	41	1.768	3.113	2.312	0.053	0.342	0.117	0.45	14.81
Residual Sprint speed	41	-0.112	0.134	0.000	0.010	0.064	0.004	0.15	14.64
$\dot{V}O_2$ max	44	15.852	33.671	23.666	0.652	4.326	18.713	0.00	
Residual $\dot{V}O_2$ max	44	-0.128	0.150	0.000	0.009	0.058	0.003	-0.06	13.31
Hemoglobin	44	60.889	140.082	99.709	2.639	17.507	306.488	-0.04	
Residual Hemoglobin	44	-0.187	0.125	0.000	0.011	0.073	0.005	-0.68	16.71
Heart mass	44	0.022	0.043	0.032	0.001	0.006	0.000	0.11	
Residual Heart mass	44	-0.081	0.077	0.000	0.006	0.037	0.001	-0.25	8.61
Liver mass	44	0.107	0.287	0.177	0.006	0.041	0.002	0.66	
Residual Liver mass	44	-0.084	0.089	0.000	0.007	0.047	0.002	0.38	10.79
Thigh muscle mass	44	0.231	0.508	0.367	0.011	0.073	0.005	0.02	
Residual Thigh muscle mass	44	-0.064	0.045	0.000	0.004	0.025	0.001	-0.45	5.80
Calf muscle mass	42	-1.004	-0.664	-0.844	0.013	0.086	0.007	-0.06	
Residual Calf muscle mass	42	-0.042	0.061	0.000	0.004	0.025	0.001	0.43	5.83
Upper arm muscle mass	44	0.015	0.039	0.025	0.001	0.006	0.000	0.29	
Residual Upper arm muscle mass	44	-0.110	0.095	0.000	0.007	0.047	0.002	0.07	10.74
Forearm muscle mass	43	-2.022	-1.654	-1.824	0.014	0.091	0.008	-0.27	
Residual Forearm muscle mass	43	-0.075	0.060	0.000	0.005	0.032	0.001	-0.63	7.33
Lung mass	42	0.048	0.139	0.085	0.003	0.020	0.000	0.63	
Residual Lung mass	42	-0.129	0.092	0.000	0.008	0.051	0.003	-0.08	11.72
Fore limb span	44	83.000	99.000	88.909	0.524	3.476	12.085	0.65	
Residual Fore limb span	44	-0.016	0.023	0.000	0.001	0.009	0.000	0.48	2.12
Hind limb span	42	109.000	126.000	116.262	0.584	3.787	14.344	0.18	
Residual Hind limb span	42	-0.020	0.019	0.000	0.001	0.009	0.000	0.06	1.96

Table 3.4 (cont.)

Trait	N	Min	Max	Mean	SE	SD	Var	Skew	C.V.
Heart LDH actv.	40	442.430	724.847	575.487	9.046	57.213	3273.312	0.53	
Residual Heart LDH actv.	40	-0.095	0.066	0.000	0.006	0.036	0.001	-0.29	8.28
Thigh LDH actv.	44	390.876	678.564	550.690	9.358	62.076	3853.406	-0.18	
Residual Thigh LDH actv.	44	-0.134	0.081	0.000	0.007	0.049	0.002	-0.49	11.33
Liver LDH actv.	44	117.335	360.768	236.657	8.659	57.436	3298.855	0.01	
Residual Liver LDH actv.	44	-0.263	0.205	0.000	0.017	0.111	0.012	-0.53	25.48
Heart CS actv.	44	58.789	138.287	93.696	2.572	17.060	291.055	0.65	
Residual Heart CS actv.	44	-0.188	0.170	0.000	0.012	0.077	0.006	0.21	17.79
Thigh CS activ	44	4.735	10.842	7.710	0.217	1.439	2.072	-0.08	
Residual Thigh CS activ	44	-0.198	0.159	0.000	0.012	0.082	0.007	-0.34	18.91
Liver CS activ	44	3.826	8.326	5.854	0.135	0.897	0.805	0.27	
Residual Liver CS activ	44	-0.160	0.152	0.000	0.010	0.066	0.004	-0.18	15.27

Table 3.5. Allometric equations for maximal sprint speed, maximal oxygen consumption ($\dot{V}O_2\text{max}$), organ masses, external morphology, and lactate dehydrogenase (LDH) enzyme activity in the heart, for *Sceloporus occidentalis* from Hampton Buttes, OR.

Trait	N	Intercept	Intercept 95% C.I. lower	Intercept 95% C.I. upper	Slope	Slope 95% C.I. lower	Slope 95% C.I. upper	R ²	SEE	P
Sprint speed	41	0.146	-0.085	0.378	0.179	-0.020	0.379	0.082	0.048	0.0770
$\dot{V}O_2\text{max}$	44	-1.206	-1.450	-0.961	0.699	0.484	0.913	0.506	0.058	<0.0001
Hematocrit	44	-0.946	-1.195	-0.697	0.307	0.087	0.526	0.163	0.059	0.0073
Hemoglobin	44	1.539	1.230	1.848	0.401	0.128	0.673	0.173	0.073	0.0049
Heart mass	44	-2.410	-2.569	-2.251	0.806	0.666	0.946	0.762	0.038	<0.0001
Liver mass	44	-1.950	-2.149	-1.750	1.051	0.875	1.227	0.776	0.047	<0.0001
Thigh muscle mass	44	-1.614	-1.721	-1.507	1.035	0.940	1.130	0.921	0.025	<0.0001
Calf muscle mass	42	-1.938	-2.046	-1.830	0.968	0.873	1.063	0.913	0.026	<0.0001
Upper arm dry muscle mass	44	-2.853	-3.051	-2.654	1.096	0.921	1.271	0.792	0.047	<0.0001
Forearm dry muscle mass	43	-2.987	-3.123	-2.850	1.027	0.907	1.148	0.879	0.032	<0.0001
Lung mass	42	-2.273	-2.491	-2.055	1.054	0.862	1.246	0.755	0.052	<0.0001
Snout-vent length	44	1.606	1.557	1.655	0.256	0.212	0.299	0.771	0.012	<0.0001
Fore limb span	44	1.757	1.718	1.796	0.169	0.135	0.204	0.699	0.009	<0.0001
Hind limb span	42	1.907	1.869	1.945	0.140	0.107	0.174	0.637	0.009	<0.0001
Tail length	23	1.685	1.571	1.799	0.293	0.195	0.390	0.650	0.017	<0.0001
Heart LDH	40	3.078	2.913	3.243	-0.283	-0.429	-0.137	0.289	0.036	0.0003
Thigh LDH	44	2.598	2.388	2.807	0.124	-0.061	0.309	0.042	0.050	0.1824
Liver LDH	44	2.039	1.569	2.510	0.284	-0.131	0.700	0.043	0.112	0.1747
Heart CS	44	1.880	1.551	2.209	0.075	-0.215	0.365	0.006	0.078	0.6042
Thigh CS	44	0.610	0.261	0.960	0.238	-0.070	0.547	0.055	0.083	0.1267
Liver CS	44	0.615	0.332	0.897	0.131	-0.118	0.380	0.026	0.067	0.2950

Table 3.6. Number of estimated parameters (E.P.), chi-square (χ^2), log likelihood, and AICc for 8 path models connecting size corrected lower-level traits [thigh muscle mass (ThighG), blood hemoglobin content (Hb), calf muscle mass (Calf)], performance [sprint speed (SPT) and maximal aerobic capacity ($\dot{V}O_2\text{max}$)], and behavior traits [maximum number of push-ups using four legs (MaxLeg4) in *Sceloporus occidentalis* from OR. N = 44. P-values refer to comparisons between each model and model 1.00 using log likelihood ratio tests according to a chi-square distribution. All variables are residuals calculated from regressions on body mass, except for MaxLeg4 and MeanLeg2.

Model	Altered path	E.P.	D.f.	χ^2	LL	P	AICc
1.00	-	17	11	3.92	218.38	-	-402.75
Removing causal paths							
0.99	MeanLeg2 <-> MaxLeg4	16	12	5.81	217.43	0.1683	-402.85
0.98	R $\dot{V}O_2$ -> MaxLeg4	16	12	9.91	215.38	0.014	-398.75
0.97	R $\dot{V}O_2$ -> MeanLeg2	16	12	8.48	216.10	0.033	-400.19
0.96	RCALFG -> RSPT	16	12	15.92	212.37	0.001	-392.74
0.95	RTHIGHG -> RSPT	16	12	10.64	215.01	0.009	-398.02
0.94	RLHBIN -> R $\dot{V}O_2$	16	12	12.46	214.10	0.003	-396.20
Adding causal paths							
1.01	RTHIGHG -> MaxLeg4	18	10	3.39	218.64	0.469	-401.27
1.02	RCALFG -> MaxLeg4	18	10	3.82	218.42	0.754	-400.85
1.03	RLHBIN -> MaxLeg4	18	10	3.77	218.45	0.699	-400.90
1.04	RTHIGHG -> MeanLeg2	18	10	3.30	218.68	0.434	-401.36
1.05	RCALFG -> MeanLeg2	18	10	3.88	218.39	0.850	-400.79
1.06	RLHBIN -> MeanLeg2	18	10	3.88	218.39	0.858	-400.78

SUMMARY AND CONCLUSIONS

My goal with this dissertation was to improve our understanding of the ecological and evolutionary importance of maximal oxygen consumption (a measure of aerobic capacity) in lizards and mammals. Most previous efforts studying whole-animal performances related to locomotion in these groups focused on maximal sprint speed capacity (Garland, 1983; Van Damme & Vanhooydonck, 2001; Garland & Albuquerque, 2017) and, for lizards, on maximal endurance capacity (Garland, 1999; e.g., Albuquerque *et al.*, 2015a). These two performances are ‘fueled’ via different metabolic pathways (crudely, anaerobic versus aerobic, respectively) and should be important for the ability to engage in different types of behavior (e.g., escaping predators, foraging, defending territories) that – intuitively, at least – seem likely to be ecologically relevant. However, relatively few studies focused on direct measures of maximal oxygen consumption during exercise ($\dot{V}O_2\text{max}$), possibly due to the logistical difficulties involved in the method (see Seeherman *et al.*, 1981).

Maximal aerobic capacity, measured as the maximum rate of oxygen consumption during forced exercise (Seeherman *et al.*, 1981), sets the upper limit to the rate of work that can be sustained over relatively long periods (more than a minute or so) (Seeherman *et al.*, 1981; Jones & Lindstedt, 1993; Levine, 2008; Spurway *et al.*, 2012). It directly affects maximal sustainable speed and endurance, and hence may potentially limit activity levels during ecologically relevant tasks (e.g., patrolling a territory, fighting, courting, foraging). Among

mammal species, $\dot{V}O_2\text{max}$ is significantly higher in canids and in *Equus caballus*, *Antilocapra americana*, and *Phyllostomus hastatus* (Dlugosz *et al.*, 2013) and is positively correlated with home range size (chapter 1, also published as Albuquerque *et al.*, 2015b). Among lizard species, $\dot{V}O_2\text{max}$ is significantly higher in some evolutionary lineages such as varanid and helodermatid lizards (Beck *et al.*, 1995; Clemente *et al.*, 2009) and is related to foraging mode in lizards (Bennett *et al.*, 1984; Clemente *et al.*, 2009), but apparently not to nocturnality (Autumn, 1999). Within species of lizards, $\dot{V}O_2\text{max}$ covaries positively with activity (and circulating hormone levels) levels (John-Alder, 1984b; John-Alder *et al.*, 2009) and appears to be limited most frequently by blood oxygen-carrying capacity, but also by enzyme activities in specific tissues (Garland, 1984; Garland & Else, 1987; Garland & Losos, 1994).

In this dissertation, I expand the knowledge about $\dot{V}O_2\text{max}$ for lizards and mammals. Chapters one and two focus on evolutionary correlates of $\dot{V}O_2\text{max}$ among species of mammals and lizards, whereas the third chapter examines among-individual variation and the correlates of $\dot{V}O_2\text{max}$ (and sprint speed) at the suborganismal and behavioral levels of biological organization simultaneously (Garland & Kelly, 2006).

In the first chapter, I show that $\dot{V}O_2\text{max}$ is weakly but positively correlated with home range size among species of mammals. This positive correlation suggests that both traits might have evolved concomitantly, presumably because they were under correlated selection (coadaptation). All else being equal, larger

home range areas imply longer distances travelled per day on average. Moving faster is relatively cheaper for terrestrial animals (Taylor *et al.*, 1982; Rezende *et al.*, 2009) and allows them to quickly cover a longer distance, saving energy and time (Kenagy & Hoyt, 1989). Higher $\dot{V}O_2\text{max}$ would allow animals to maintain higher speeds without fatiguing when traveling around their home range.

However, the correlation is not significant when phylogenetic statistical methods are used. We offer two non-mutually exclusive explanations for this result that would also explain the weak positive correlation found when using regular statistics. First, when analyzing the data for this chapter, we immediately noticed that home range area varies much more than $\dot{V}O_2\text{max}$. The calculation of home range areas might include considerable amounts of measurement error (Ives *et al.*, 2007), increasing its variability, reducing its repeatability and, consequently, reducing the correlation coefficient with any other biologically related trait. Second, home range areas can be significantly affected by sex, seasons, or environmental conditions, such as food availability and predator density, creating considerable variation among populations. The mismatch between the populations for which $\dot{V}O_2\text{max}$ and home range data were available in the literature might have introduced variation caused by different environmental (and researcher) conditions for each population. Considering these two explanations, the 'real' correlation coefficient between home range area and $\dot{V}O_2\text{max}$ is probably higher than the one observed.

In the second chapter, I correlated $\dot{V}O_2\text{max}$ data for 58 lizard species (seven of those included two populations, creating a total of 65 data points) with diet, climate, nocturnality, viviparity, and phylogenetic lineage, while controlling for body mass and temperature. The evolutionary allometric slope of $\dot{V}O_2\text{max}$ among lizards was 0.803, which is very similar to the 0.839 slope reported previously for mammals (Dlugosz *et al.*, 2013). Both phylogenetic and traditional regressions indicate that viviparous species have lower $\dot{V}O_2\text{max}$ and that varanids, helodermatids, and skinks have higher $\dot{V}O_2\text{max}$ than other lizard species. The reason why viviparous species have lower $\dot{V}O_2\text{max}$ is unclear. Pregnancy imposes negative effects on locomotor capacities in lizards (Garland & Else, 1987; Olsson *et al.*, 2000; Zani *et al.*, 2008), and viviparous species thermoregulate more intensely during a relatively longer gestation period. Therefore, I expected to find higher $\dot{V}O_2\text{max}$ in those species, which would allow them to maintain adequate activity levels even with the negative effects caused by extended pregnancies. Perhaps changes in $\dot{V}O_2\text{max}$ occur only in females during pregnancy (all measurements were from non-pregnant individuals), or changes in behavior that compensate for the locomotor impairment during that period (e.g., Bauwens & Thoen, 1981; Brodie, 1989) reduce the need for adaptive changes in $\dot{V}O_2\text{max}$.

Other studies have shown that varanids and helodermatids have higher $\dot{V}O_2\text{max}$ than other lizards (Beck *et al.*, 1995; Autumn, 1999; Clemente *et al.*, 2009). Clemente *et al.* (2009) also showed that high activity levels, endurance,

and $\dot{V}O_2\text{max}$ are plesiomorphic in varanids and that, within the group, active foragers have higher endurance and $\dot{V}O_2\text{max}$ than sit-and-wait foragers. So, the high oxygen consumption capacity in varanids seems to be related to high activity levels in nature. Similarly, helodermatids have high endurance capacities (see Figure 11.7 in Garland, 1994a) and usually travel long distances during the active season (John-Alder *et al.*, 1983; Jones, 1983). However, Beck *et al.* (1995) also noted that males, which show significantly higher $\dot{V}O_2\text{max}$ and aerobic scope than females, engage in strenuous agonistic interactions, which could also require high aerobic capacity. Finally, I also observed high $\dot{V}O_2\text{max}$ for skinks. Similar to varanid and helodermatids, most skinks are active predators (Huey & Pianka, 1981; Cooper, 1994a; b) and the omnivore and insectivore species quickly respond to chemical cues from their prey (Cooper *et al.*, 2000). All species included in this study are omnivores or insectivores, which probably show similar behavior patterns.

In the third chapter, I investigated the proximate causes and behavioral correlates of variation in sprint speed and $\dot{V}O_2\text{max}$ capacities in adult male *Sceloporus occidentalis* lizards during the breeding season. This is one of few studies in which lower-level suborganismal traits, performance, and behavior have been analyzed in the same individuals. Multiple regressions revealed residual calf muscle mass as a positive predictor of sprint speed and residual thigh muscle mass as a negative predictor of sprint speed, whereas residual hemoglobin concentration in blood was the only significant (positive) predictor of

residual $\dot{V}O_2\text{max}$. Reilly (1995) showed that both thigh and calf muscles are active during the propulsion phase of the stride but that “plantar flexion” (which is created by the contraction of calf muscles) “plays a major role in generating thrust” in *Sceloporus clarki*. Years later, Higham et al. (2011) showed that individual variation in maximal sprint speed and acceleration in *Sceloporus woodi* is positively correlated with the percentage and diameter of fast glycolytic fibers in the gastrocnemius, a propulsion-generating calf muscle, but not with fiber composition in the iliofibularis, a small thigh muscle active during the swing-phase. If this pattern also holds in *S. occidentalis*, then relatively larger calf muscles would allow lizards to generate greater force exertion (and consequently higher speed), whereas relatively small thigh muscles could reduce leg mass, thus allowing them to move the limb faster during the swing-phase (assuming that the power available for recovery was not compromised).

Blood oxygen carrying capacity (measured as hematocrit level or hemoglobin concentration) is a common intraspecific predictor of $\dot{V}O_2\text{max}$ in lizards among all studies done so far (Garland, 1984; Garland & Else, 1987; Garland & Losos, 1994). However, these studies also reported other lower-level traits (e.g., liver mass, heart lactate dehydrogenase, liver citrate-synthase activity) as significant. These studies included juveniles and both sexes, whereas in this study I only analyzed adult males collected during the peak of the breeding season. Sexual dimorphism, ontogenetic development, and seasonal changes in physiology can increase variation in performance and lower-level

traits considerably (John-Alder, 1984b; Garland & Else, 1987; Dohm *et al.*, 1998), which might explain the difference in predictors.

When evaluating the effect of performance on behavior, residual sprint speed did not predict any of the behavioral traits measured in breeding-season observations of free-living males from a population in Hampton Butte, Oregon. However, residual $\dot{V}O_2\text{max}$ significantly predicted the average number of 2-legged and 4-legged push-ups per bout and the maximum number of 4-legged push-ups in one bout. In several lizard species, sprint speed is significantly correlated with sexually selected behaviors, such as social dominance and territory size in males (Garland *et al.*, 1990a; Robson & Miles, 2000; Peterson & Husak, 2006). However, even though the push-up displays are used during territorial disputes, sprint speed does not seem to be related to individual display capacities among breeding male *S. occidentalis* from Hampton Butte. The correlation between $\dot{V}O_2\text{max}$ and push-ups using two or four legs per bout, instead of per unit of time, might seem counter intuitive at first. One bout of push-ups does not last more than several seconds and probably does not tax the animal aerobically. However, the antagonistic interactions during which they occur can last for long periods of time in nature. During field work, I observed a 24-minute combat with very brief resting periods (see description in results) and Fitch (1940) also reported a 45-minute long interaction and added comments about individuals “showing evidence of fatigue at later stages of the fight.” Higher aerobic capacities would allow the individual to maintain higher overall

exercise intensity during these interactions. Additionally, field observations took place during the peak of the breeding season, when the frequency and intensity of push-up displays increase considerably (Stamps & Krishnan, 1997; Sheldahl & Martins, 2000). During that period, lizards may use these displays frequently throughout the day, instead of only during rare and short bursts of intense display activity.

Finally, I built a path model including all the significant causal relations from the multiple regressions. Adding or removing any of the causal paths significantly reduced model fit and adding paths from lower-level traits directly to behavior did not improve model fit. Therefore, results of the path analysis including all levels of biological organization supported the results obtained from the multiple regression analyses.

In this dissertation, I have attempted to expand our knowledge of both the ultimate (Bennett *et al.*, 1984; Autumn *et al.*, 1999; Clemente *et al.*, 2009; Killen *et al.*, 2016) and proximate (Garland, 1984; Garland & Else, 1987) causes of variation in $\dot{V}O_2\text{max}$ of lizards and mammals. At the ultimate level, ecological correlates offer insights about how natural and sexual selection might have shaped aerobic capacity according to how it interacts with behavior, life history, and the environment. My results confirm previous findings of high $\dot{V}O_2\text{max}$ in some phylogenetic lineages (varanids and helodermatids) and expand the list of significantly correlated traits to include viviparity for lizards and home range size for mammals. Additionally, I also identified skinks as a group of lizards with high

$\dot{V}O_2\text{max}$. These results reinforce the importance of $\dot{V}O_2\text{max}$ as a trait related to the evolution of high activity levels, but also suggest new possibilities for investigation, such as the unexpected negative association with viviparity in lizards.

At the proximate level, I provide additional evidence that blood oxygen carrying capacity is a common limiting factor for $\dot{V}O_2\text{max}$ among individual lizards. Previous studies included juveniles and/or both sexes, which may have expanded the range of variation, but in my study of only adult males collected during a specific time of the year (the peak of the breeding season), hemoglobin concentration or hematocrit were also significant predictors of individual variation in $\dot{V}O_2\text{max}$. Additionally, I show that behaviors related to territorial defense and courtship might be limited by $\dot{V}O_2\text{max}$ in these lizards, suggesting that these behaviors are costly and that $\dot{V}O_2\text{max}$ can influence reproductive success in iguanid lizards.

Future studies among lizard species should test for correlations with foraging mode and home range area, whereas future studies among individuals within a population should focus on quantifying the duration and intensity of territorial behaviors in nature or perhaps try to measure the energetic cost of these behaviors in the laboratory. These studies should also investigate the seasonality of $\dot{V}O_2\text{max}$ and related traits (hematocrit, hemoglobin, organ masses, and tissue-specific enzyme activities) (see also John-Alder, 1984b; Garland & Else, 1987).

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