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RESEARCH ARTICLE

Lower-level predictors and behavioral correlates of maximal aerobic capacity and sprint speed among individual lizards

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ABSTRACT

The standard paradigm of organismal biology views lower-level traits (e.g. aspects of physiology) as determining organismal performance ability (e.g. maximal sprint speed), which in turn constrains behavior (e.g. social interactions). However, few studies have simultaneously examined all three levels of organization. We used focal observations to record movement behaviors and push-up displays in the field for adult male *Sceloporus occidentalis* lizards during the breeding season. We then captured animals, measured aspects of their physiology, morphology and performance, and counted ectoparasites and endoparasites as potential predictors of sprint speed and maximal oxygen consumption ($\dot{V}_{O_2,max}$). Field behaviors were statistically repeatable, but not strongly so. Sprint speed and $\dot{V}_{O_2,max}$ were repeatable using residuals from regressions on body mass (speed: $r=0.70$; $\dot{V}_{O_2,max}$: $r=0.88$). Both calf [standardized partial regression (path) coefficient $B=0.53$] and thigh [$B=-0.37$] muscle mass (as residuals from regressions on body mass) were significant predictors of sprint speed; hemoglobin concentration ($B=0.42$) was a predictor of $\dot{V}_{O_2,max}$. In turn, $\dot{V}_{O_2,max}$ predicted the maximum number of four-legged push-ups per bout ($B=0.39$). In path analysis, log likelihood ratio tests indicated no direct paths from lower-level traits to behavior, supporting the idea that morphology, in the broad sense, only affects behavior indirectly through measures of performance. Our results show that inter-individual variation in field behaviors can be related to performance ability, which in turn reflect differences in morphology and physiology, although not parasite load. Given the low repeatability of field behaviors, some of the relationships between behavior and performance may be stronger than suggested by our results.

KEY WORDS: Behavior, Locomotion, Parasites, Path analysis, Performance, Social displays

INTRODUCTION

Modern organismal biology is founded upon integration across disciplines and levels of biological organization (e.g. Dalziel et al., 2009; Galván et al., 2022; Mykles et al., 2010; Schwenk et al., 2009; Zamer and Scheiner, 2014). A key organizing principle for research in organismal biology is that lower-level subordinate traits (e.g. morphology, physiology) affect organismal performance ability (e.g. maximal sprint speed), which in turn both enable and constrain

behaviors (Arnold, 1983; Biro et al., 2018; Garland and Losos, 1994; Husak, 2015; Lailvaux and Husak, 2014; Storz et al., 2015). Many whole-organism performance traits, such as maximal sprint speed and endurance capacity, are potentially relevant for various natural behaviors, including foraging, territorial defense, courtship and mating. Accordingly, studies in a variety of organisms have been motivated by the 'morphology–performance–behavior–fitness' paradigm (see Dantzer et al., 2016, on squirrels; Le Roy et al., 2019, on butterflies; McCormick et al., 2018, on fishes; Violle et al., 2007, on plants). Many such studies have used lizards (e.g. John-Alder et al., 2009) for both historical and practical reasons. The original paper that formalized the paradigm in the context of path analysis was written by a herpetologist (Arnold, 1983), and colleagues who worked with lizards (Bennett, 1987; Bennett and Huey, 1990; Bonine, 2007) quickly noted the relative ease with which locomotor performance, exercise physiology and limb dimensions could be measured in lizards, many of which are diurnal, locally abundant and easy to observe in the wild (Huey et al., 1983; Reilly et al., 2007; Vitt and Pianka, 1994).

Two key measures of whole-animal performance are maximal sprint speed and maximal oxygen consumption ($\dot{V}_{O_2,max}$) (Albuquerque et al., 2015a,b; Dlugosz et al., 2013b; Garland and Losos, 1994). 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 (Husak and Lailvaux, 2019; Irschick et al., 2008). Additionally, given the possibility of functional conflicts among some of the underlying subordinate traits, one might expect trade-offs between performance ability or behaviors that require burst versus sustained activity (Garland et al., 2022). Studies of locomotor trade-offs at the level of individual variation have identified them in some cases, such as between speed and endurance in human athletes (Careau and Wilson, 2017), and between terrestrial stamina and aquatic burst performance in frogs (Herrel and Bonneaud, 2012), but not in others (e.g. Bennett et al., 1989, on salamanders). In lizards, which are the subject of the present report, several studies have found little or no evidence for such trade-offs either within or among species (Albuquerque et al., 2015a; Garland, 1984; Garland and Else, 1987; Lailvaux et al., 2019).

On the anaerobic end, 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 western fence lizards, *Sceloporus occidentalis*, the lizard species studied here, maximal sprint speed is positively correlated with dominance during staged territorial disputes in the laboratory (Garland et al., 1990). If that pattern holds for other territorial iguanians, then it could explain why faster eastern collared lizards, *Crotaphytus collaris*, are better at defending their territories and also sire more offspring (Husak et al., 2006, 2008).

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On the aerobic end, maximal aerobic capacity 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 (Autumn, 1999; Clemente et al., 2009; Garland and Albuquerque, 2017; Garland and Losos, 1994). In male lizards of two species (desert iguanas, *Dipsosaurus dorsalis*, and eastern fence lizards, *Sceloporus undulatus*), $\dot{V}_{O_2,max}$ increases during the breeding season, when activity levels are also at their peak (John-Alder, 1984; John-Alder et al., 2009). During the breeding season, males in these and other species of lizards (Baird et al., 2001; Ruby, 1978) 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 relationship between $\dot{V}_{O_2,max}$ and activity levels (e.g. Bennett et al., 1984), and varanids, many of which are highly active, have a relatively high $\dot{V}_{O_2,max}$ (Clemente et al., 2009, and references therein). More generally, $\dot{V}_{O_2,max}$ is related to home-range size at the level of comparing mammals with lizards (Garland and Albuquerque, 2017) and among species of mammals (Albuquerque et al., 2015b).

In some organisms, behaviors related to sexual selection (e.g. male–male disputes or courtship) can be energetically costly and/or physically demanding (Brandt, 2003; Daly, 1978; Höglundi et al., 1992; Marler et al., 1995; Mowles and Jepson, 2015). For example, territorial disputes among *S. occidentalis* males can be long and apparently strenuous, with durations of up to 45 min (Fitch, 1940). During these disputes, lizards perform push-up displays, head bobs and other associated displays such as arched back and lateral flattening. 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 and Ferguson, 1978; Stamps, 1978). The number of push-up displays performed in sequence (hereafter called a display or bout of push-ups), the number of legs flexed (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 and 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 (Baird et al., 2003; Carpenter, 1962; Martins, 1994), but that seems to occur very rarely (Sheldahl and Martins, 2000). Thus, maximal sprinting ability 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 *C. collaris* lizards. In further studies, Baird and colleagues also observed that display frequency, intensity and duration are correlated with reproductive success in males (Baird, 2013; Baird et al., 2007).

Finally, locomotor performance and behavioral traits can also be affected by parasite infection (Herbison, 2017; Klein, 2003; Lehmann, 1993; Timi and Poulin, 2020). For example, in *S. occidentalis*, infection by *Plasmodium mexicanum* reduces hematocrit and hemoglobin concentration in the blood, leading to reduced aerobic capacity and running stamina (Schall et al., 1982). Non-infected males display more often to other males and to females, and they are dominant during social interactions in seminatural outdoor enclosures (Schall and Dearing, 1987). In addition to being submissive, infected lizards are less active and

have reduced home ranges in nature (Schall and Houle, 1992). Infestation by ticks also reduces hematocrit and aggressive behavior (Lanser et al., 2021) and alters thermoregulatory behavior in *S. occidentalis* (Megía-Palma et al., 2020). Therefore, in this study, we also scored the presence of ectoparasites and endoparasites.

The first goal of the present study was to test whether movement rates or aspects of the push-up display (behavior), measured in the field reflect individual variation in maximal sprint speed and/or $\dot{V}_{O_2,max}$ (performance), measured in the lab, 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 (morphology/physiology, e.g. blood hematocrit and hemoglobin levels; heart and calf muscle mass; citrate synthase and lactate dehydrogenase activity in thigh muscle, heart and liver). Few studies have been able to measure the relationship among traits at all three of these hierarchical levels for the same set of individuals (e.g. Goodman et al., 2008). Third, we tested whether organismal performance might be associated with parasite count (number of ticks, intracellular parasites in red blood cells and free-living nematodes in blood) (Binning et al., 2014; Dlugosz et al., 2013a; Lanser et al., 2021; Sorci et al., 1994). Finally, we used path-analytic models to test two (non-exclusive) alternatives in the morphology/physiology–performance–behavior framework: (1) morphology/physiology influences behavior directly, without acting through performance; and/or (2) morphology/physiology influence behavior indirectly, as mediated by a path through performance.

MATERIALS AND METHODS
Field site and behavior observations
During the spring breeding season (from 24 May to 29 June 2017), we conducted field work in a sagebrush–juniper woodland near the unincorporated community of Hampton, OR, USA, taking advantage of long-term field sites developed by P.A.Z. The landscape was dominated by small and medium-sized bushes (*Artemisia tridentata*, *Ribes cereum*, *Ericameria nauseosa*, *Bromus tectorum* and *Achnatherum hymenoides*). The field site included three rock cliffs of approximately 20 m height and spanning over approximately 1500 m in length in total. A population of *Sceloporus occidentalis* Baird and Girard 1852 inhabits rock outcrops and boulder field 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 08:00 h and 15:00 h Pacific Time from 26 May to 29 June 2017. The first observation each day started at least 1 h after we encountered the first lizard, to avoid observing non-active lizards or lizards primarily thermoregulating at the start of each day. We gave each lizard an identification number represented by a unique combination of toe clips (Tinkle, 1967) and wrote the unique portion of that number on the animal's back with Painters® acrylic paint markers to allow identification from a distance. Animals were marked at least 1 week prior to the start of observations. We attempted to observe each lizard for 20 min from at least 5 m with binoculars. If the lizard moved out of sight for less than 5 min, we extended the observation period to obtain a total of about 20 min. If the lizard was out of sight for more than 5 min, we ended the focal observation period. The minimum length of focal observations included in this study was 20 min. We used a digital voice recorder to register the number of

moves, distance moved, number of head bobs and the number of two- and four-legged push-ups during each bout, as described in several ethogram studies for the genus (e.g. Carpenter and Ferguson, 1978; Martins, 1994). We estimated lizard movement distance visually. During training, we projected our hands forward and observed how much of our vision field was covered by the width of our fingers and correlated that with objects in the environment that were separated by known distances. All observers were trained by P.A.Z. 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 a bout of push-ups to be any continuous sequence of push-ups (even if they included only one push-up). If the lizard stopped for more than 3 s after the last push-up or if it altered its position (e.g. performing 180 deg turn or moving a few centimeters), then we considered that as the end of the bout. Because push-ups on different numbers 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 four-legged push-ups per minute. As four-legged push-ups were relatively rare, and some individuals were observed for as little 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 min or more of total observation time, the relationship was not statistically significant, so we excluded lizards with fewer than three focal observations (60 min 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 or changing display intensity). Therefore, we excluded those bouts when calculating the number of push-ups and bouts of push-ups per minute to ensure statistical independence.

We performed an average of 3.26 (1–5) focal observations on 57 adult male fence lizards. From those, we found and captured 44 by string snare, 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 (~38 l) terraria and gave them a 2 day acclimation period with *ad lib.* access to water, plus misting with water on the first day, but no food. We performed sprint-speed trials on days 3 and 4 and $\dot{V}_{O_2,max}$ trials on days 7–10 (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 >24 h 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 (Albuquerque et al., 2015a; Garland, 1985) 4 times per individual lizard (twice per day with at least 4 h between runs) on a 6.5 m racetrack with 12 photocell sensors spaced every 0.5 m. We used the fastest consecutive 1 m 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 in opaque cloth bags for at least 2 h 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 and Else, 1987). Prior to each trial,

animals were maintained for at least 2 h 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 conduced 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 LabHelper software (WarthogSystems, https://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 oxygen consumption. When we observed no increase in oxygen consumption despite increases in speed for at least 60 s, we stopped the trial. Because $\dot{V}_{O_2,max}$ trials take longer than sprint-speed trails, 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 33.7–35.5°C for sprint speed trials (only one trial was lower than 34°C) and within 34.5–36.9°C for $\dot{V}_{O_2,max}$ trials (only four trials were higher than 35.9°C). Regressions of each performance measure on body mass and body temperature revealed no significant effect of temperature on either performance measure, 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 euthanized lizards by decapitation and obtained blood samples directly from the neck into a weigh boat containing sodium heparin powder. From the weigh boat, we collected two 75 µl microcapillary tubes for hematocrit measures and pipetted two 20 µl samples into 5 ml of Drabkin's reagent (Sigma, D5941-6VL) for hemoglobin measures. The microcapillary tubes were centrifuged at 11,700 rpm for 5 min and hematocrit data consisted of the volume 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 min at room temperature and measured absorbance at 540 nm wavelength after we finished dissections. The absorbance was used to calculate hemoglobin levels based on a regression using known hemoglobin concentrations (Fisher Scientific 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 (to 0.0001 g) and then immediately froze tissues in liquid nitrogen. Heart, liver and thigh were dissected and frozen within 10 min to minimize the chance of enzyme degradation. We then stored all tissues in a −80°C freezer for subsequent enzyme analysis.

Parasite inspection

Because malaria parasites (*Plasmodium mexicanum*) were shown to affect behavior, blood hemoglobin level and potentially maximal aerobic capacity in a *S. occidentalis* population from northern California (Schall and Dearing, 1987; Schall and Sarni, 1987; Schall et al., 1982), 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 a similar density of cells on each blood smear at ×1000 magnification and counted all free-living nematodes by scanning the entire blood smear at ×10 magnification. We also counted the number of ticks attached to the skin. Ticks may affect many aspects of their host's physiology and behavior, including aggressive behavior in staged contests (Lanser et al., 2021), thermoregulatory behavior and body mass in the laboratory (Megía-Palma et al., 2020), and hematocrit (Dunlap and Mathies, 1993). Therefore, our parasite variables consisted of three counts of parasites: intracellular parasites in red blood cells, free-living nematodes in blood, and ticks attached to the skin.

Enzyme assays

Before enzyme assays, we removed tissues from the −80°C 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 s continuously to avoid buildup of heat or frothing. Each 10 s cycle was repeated until no solid particles were visible. The homogenization buffer consisted of 100 mmol l^{-1} potassium phosphate and 5 mmol l^{-1} EDTA buffer containing 0.1% Triton X-100, 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 3 times. After the third freeze–thaw cycle, we sonicated homogenates for 10 s 3 times, with a 10 s interval in between, using a Fisher Scientific Sonic Dismembrator model 100, also keeping samples on ice. Finally, we vortexed homogenates, aliquoted them to final-assay dilution, centrifuged the aliquot at 15,000 rpm for 5 min at 2°C and used a sample of the supernatant for assays (Garland, 1984; Garland and Else, 1987; Suarez et al., 1986, 1990).

We determined citrate synthase (CS) and lactate dehydrogenase (LDH) activity in liver, heart and mixed thigh muscle tissues spectrophotometrically, following the procedures described by Srere (1969) and Somero and Childress (1980), respectively. Assays were performed at 35°C with non-limiting concentrations of substrates and co-factors. The CS assay starting solutions contained 50 mmol l^{-1} Tris-HCl buffer pH 8.0, 0.3 mmol l^{-1} acetyl-CoA (Sigma A2181), 0.1 mmol l^{-1} DTNB and the tissue sample. We measured the increase in absorption at 412 nm wavelength for 3 min 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 mmol l^{-1} oxaloacetate (Sigma O4126) and followed the increase in absorption for 7 min.

We measured LDH activity in the pyruvate reductase direction (production of lactate) by following the decrease in absorption at 340 nm wavelength for 7 min. The assay mixture contained 50 mmol l^{-1} Tris-HCl buffer pH 7.5, 100 mmol l^{-1} KCl,

150 mmol l^{-1} NADH and 2 mmol l^{-1} 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 activity is 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), 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 coefficient (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 analysis of variance (ANOVA) (Lessells and 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 randomized (or simulated) data and compare them with the coefficients obtained from the observed data.

To check whether any of the correlations among the behavioral traits were purely a function of mathematical relationships, we bootstrapped the observed behavior events (movement distances or bouts of push-ups) 1000 times. For example, from the 1555 observed movement events, we resampled another 1555 by randomly choosing one movement distance value at a time, with replacement (i.e. each time a value was sampled, it was also replaced in the pool of observed values, so it had a chance to be picked again). During this process, we retained the same number of moves per lizard as in the original dataset. Once we had resampled 1555 values, the bootstrapping ended. Then, we repeated this entire process 999 times, so we had 1000 resampled datasets (including the original) with identical sample sizes per lizard to the observed dataset. We repeated the process for head bobs, two-legged push-ups and four-legged push-ups (Table S3). We applied the same transformations to the bootstrapped datasets as used for the observed data (log and rank when necessary for some traits). The resampling procedure and data transformation described above were performed in R version 3.4.3.

We used the mean correlation coefficients from the bootstrapped 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 with the correlation coefficients obtained from the bootstrapped data. If the 95% confidence interval of the correlations from the observed lizards did not include the correlation coefficient from the bootstrapped data, then we considered the observed correlations to be biologically significant.

For laboratory measures of performance ($\dot{V}_{O_2,max}$, sprint speed), and associated body mass, 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 activity, organ mass and external morphology) and parasite counts for whole-animal performance ($\dot{V}_{O_2,max}$, sprint speed). The multiple regression predicting $\dot{V}_{O_2,max}$ included the three parasite counts (intracellular parasites in red blood cells, free-living nematodes in blood, and ticks) along with heart, liver and lung mass, thigh and calf muscle mass, and upper and forearm dry muscle mass, hemoglobin concentration in blood, and CS activity in the heart, liver and thigh muscle. We used canonical correlation to test for relationships 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 hindlimb 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, eight had broken but fully regenerated tails, five had partially regenerated tails, and eight had 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; Jagnandan et al., 2014; McElroy and Bergmann, 2013), we used dummy variables coding for each tail condition listed above. The final dataset used in the multiple regressions predicting sprint speed included the three parasite counts, calf and thigh muscle mass, upper and forearm dry muscle mass, hindlimb span, LDH activity in the thigh muscle and heart, CS activity in the thigh (all residuals from regressions with body mass) and three 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 its 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 and Else, 1987).

We used multiple regressions to measure the predictive ability of sprint speed and $\dot{V}_{O_2,max}$ 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, head bobs, two-legged push-ups and four-legged push-ups 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 ($P<0.050$) relationships identified from lower-level traits to the two measures of organismal performance, and from performance to behavior. 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 (von Oertzen et al., 2015). Then, we tested whether 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 and Losos, 1994).

RESULTS
Behavior in the field
We observed a total of 57 lizards for a grand total of 59 h and 58 min. Pooling all observations and considering each move or bout of push-ups as one independent event yielded 2424 total events (=rows in the dataset). The total observation time per lizard averaged 63.1 min (range 20–100 min). During these observations, we recorded 1555 moves (Fig. 1A,B); and 869 displays (Fig. 1C–E). The majority of the displays were two-legged push-ups (53.4%), followed by head bobs (29.1%) and four-legged push-ups (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 min long intense combat between two males that were already interacting when we first saw them. We performed 20 min focal observations on each, then moved on to find other lizards before the combat was resolved, so we do not know the total length of their interaction. During the fight, the lizards would align their bodies side-to-side with their snouts pointing in opposite directions and try to bite each other on the tail, 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 s and most were shorter than 30 s. Both focal observations were included in the analyses described below, and values for these two observations included the 1st and 4th most extreme number of moves per minute (2.68 and 1.50).

Variability and repeatability of movements and push-up displays
Overall, we observed 14.4 displays per hour (869 bouts/59 h and 58 min). The mean (±s.d.) number of head bobs per bout was 4.85±3.40 (1–17, $N=253$), but with a right-skewed distribution (Fig. 1C; median=4, mode=1). The mean number of two-legged push-ups per bout was 7.18±2.62 (Fig. 1D; 1–14, $N=492$) with a normal distribution, and the mean number of four-legged push-ups per bout was 8.01±2.79 (Fig. 1E; 1–16, $N=151$), also with a normal distribution (Table S1). For more details on the variability of behavior traits, see Supplementary Materials and Methods.

Based on one-way ANOVA, all behavioral traits differed significantly among individuals (Table S1; all $P<0.001$). The ICC for the $\log_{10}$ distance traveled per move was only 0.05, whereas the number of head bobs and push-ups per bout had higher values (head bobs ICC=0.15; two-legged push-ups ICC=0.19; four-legged push-ups ICC=0.31; Table S1).
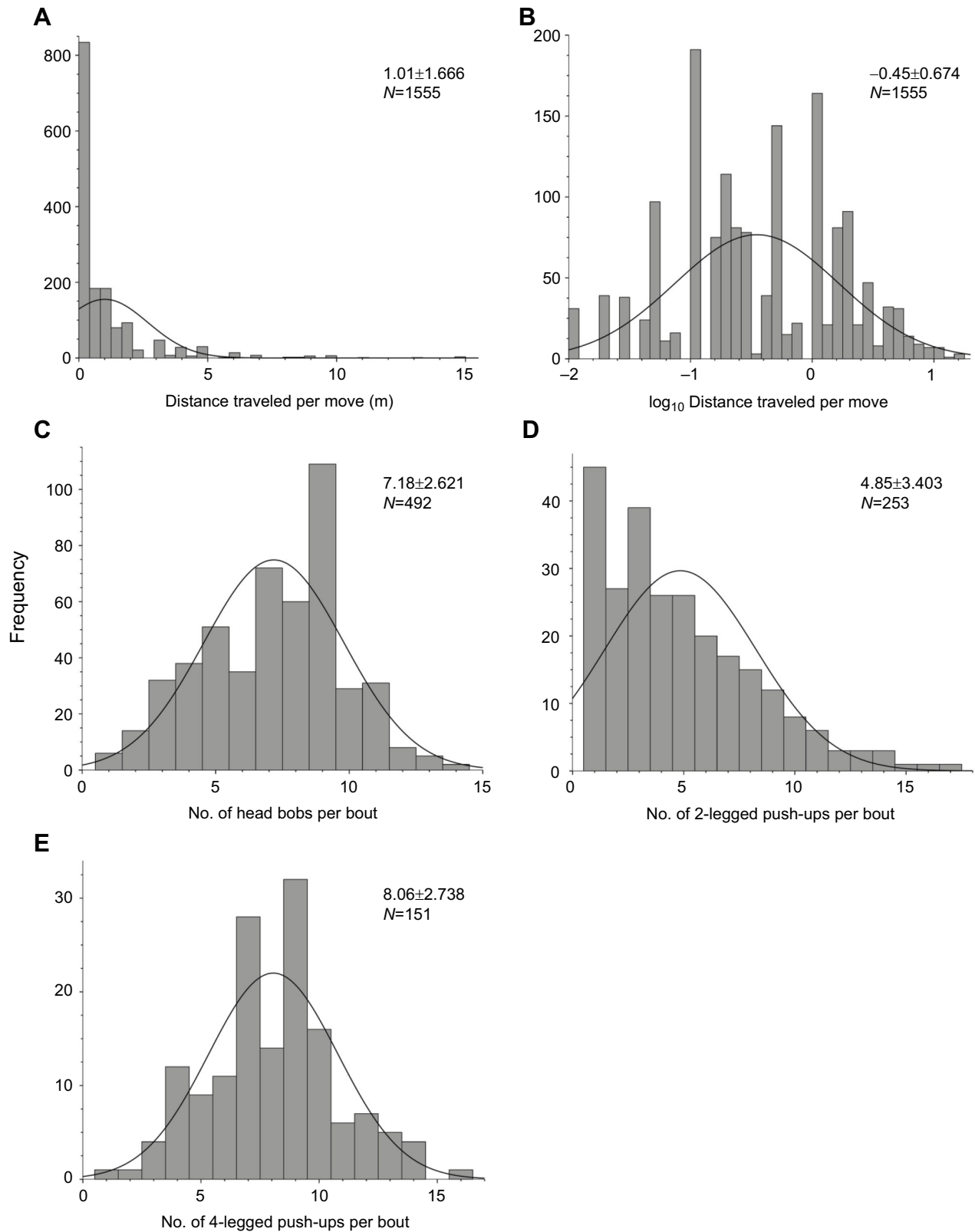
Fig. 1. Histograms of behavior traits. (A) Distance traveled per move, (B) $\log_{10}$ distance traveled per move, (C) number of head bobs per bout, (D) number of two-legged push-ups (forelimbs) and (E) number of four-legged push-ups (forelimbs and hindlimbs) per bout. Data were calculated independently for each event. Means±s.d. and number of observations (N) are shown.

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 trait (Table S3). 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 (Table S4).

Four of the movement traits were significantly more strongly correlated than expected from bootstrapping (see Table S3 for coefficients): $\log_{10}$ maximum movement distance versus $\log_{10}$ distance moved per min ($r=0.529$, lower than expected); s.d. of $\log_{10}$ movement distance versus $\log_{10}$ number of moves per minute ($r=-0.295$, lower than expected); s.d. of $\log_{10}$ movement distance versus $\log_{10}$ distance moved per min ($r=0.059$, lower than expected); $\log_{10}$ number of moves per minute versus $\log_{10}$ distance moved per minute ($r=0.649$, higher than expected).

Various aspects of display behavior were significantly intercorrelated, as judged against bootstrapped values. For example, mean and standard deviation of the number of head bobs per bout ($r=0.767$) showed higher than expected ($r=0.542$) positive correlation. The $\log_{10}$ number of head bobs per minute and the ranked number of bouts of head bobs per minute ($r=0.787$) showed lower than expected ($r=0.892$) positive correlation. The correlation between mean and maximum number of four-legged push-ups ($r=0.892$) was higher than expected ($r=0.717$). The mean number of four-legged push-ups and the rank number of bouts per minute ($r=0.397$) also showed higher than expected correlation ($r=-0.001$).

Considering the traits that are mathematically independent, we found significant positive correlations between the distance moved per minute and the rank number of two-legged bouts per minute ($r=0.371$), the standard deviation of head bobs per bout and the $\log_{10}$ standard deviation of four-legged push-ups per bout ($r=0.648$). The maximum number of four-legged push-ups per bout and the ranked number of two-legged push-ups per minute were positively correlated ($r=0.397$). Finally, all ranked number of two- and four-legged push-ups per minute and bouts of push-ups per minute were positively correlated (see Table S4 for coefficients).

Performance in the laboratory

Variability of locomotor performance

Considerable variation existed in maximal sprint speed (range 1.8–3.1 m s^{-1}, mean±s.d. 2.31±0.34 m s^{-1} and maximal whole-animal aerobic capacity (23.63±4.33 ml O$_2$ h^{-1}; Table 1). Sprint speed was not significantly related to body mass ($P=0.890$), whereas $\dot{V}_{O_2,max}$ significantly scaled as body mass$^{0.70}$ (Fig. 2 and Table 2). After removing the effect of body mass, the highest $\dot{V}_{O_2,max}$ value was approximately 2 times the lowest value and the fastest lizard was 1.72 times faster than the slowest one. The CV for residual sprint speed was 15% and for $\dot{V}_{O_2,max}$ it was 13%.

Repeatability and correlation of locomotor performance

Sprint speed was repeatable ($r=0.70$, $P<0.001$), with no significant difference between the fastest trials from the two days (paired t-test, $P=0.316$). The $\dot{V}_{O_2,max}$ values were highly repeatable ($r=0.94$, $P<0.001$) with a small but significant ($P=0.011$) increase of 3.09% on the second day. The $\dot{V}_{O_2,max}$ residuals from regressions on body mass were also correlated between the first and second trials ($r=0.88$, $P<0.001$), showing significant repeatability of mass-independent $\dot{V}_{O_2,max}$. Residual sprint speed and residual $\dot{V}_{O_2,max}$ were uncorrelated ($r=0.07$, $P=0.663$).

Lower-level traits

Variability of lower-level traits

All organ mass and external morphology measures showed significant positive relationships with body mass (Table 2); 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 LDH, which scaled negatively as body mass$^{-0.28}$ ($P<0.001$). The CV for anatomical and physiological traits varied from 0.02 for hindlimb span to 0.16 for hemoglobin concentration. Enzyme assays had higher CV, ranging from 0.08 for heart LDH activity to 0.25 for liver LDH activity.

Correlations among lower-level traits

We found several significant correlations among performance and lower-level traits (Table S5). 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 and Glantz, 1985), we did not use either trait in subsequent analysis. Residual hindlimb and forelimb span were also positively correlated ($r=0.74$, $P<0.001$).

We found positive relationships between heart CS and thigh CS ($r=0.46$, $P=0.002$), heart CS and thigh LDH ($r=0.33$, $P=0.030$), and 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 (Table S5).

Effects of parasites

We found no evidence of *P. mexicanum* infection. Multiple regressions and canonical correlations revealed no significant effect of the number of ticks, intracellular parasites in red blood cells, or free-living nematodes in the blood on hematocrit, blood hemoglobin levels, sprint speed, $\dot{V}_{O_2,max}$ or any of the behavioral traits.

Multiple regressions and path analysis

Multiple regressions: morphology to performance

Multiple regressions of residual performance traits on residual lower-level morphological, physiological and biochemical traits (Fig. 3C,D) revealed residual calf muscle mass ($B=0.53$, $P=0.002$) and residual thigh muscle mass ($B=-0.37$, $P=0.027$) as predictors of sprint speed ($R^2=0.28$), and blood hemoglobin concentration ($B=0.42$, $P=0.009$) as a significant predictor of $\dot{V}_{O_2,max}$ ($R^2=0.17$).

Multiple regressions: performance to behavior

Residual sprint speed was negatively related to all two-legged push-up traits, but was not statistically significant. Residual $\dot{V}_{O_2,max}$ predicted the average number of two-legged ($r=0.32$, $P=0.044$) and four-legged ($r=0.36$, $P=0.033$) push-ups, as well as the maximum number of four-legged push-ups ($r=0.39$, $P=0.018$) (Fig. 3A,B). 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. 4). However, model 1.00 excluded the mean number of four-legged push-ups per bout because of the high correlation with the maximum number of four-legged push-ups per bout ($r=0.89$,

Table 1. Descriptive statistics for *Sceloporus occidentalis* from near Hampton Buttes, OR, USA

Trait	N	Min.	Max.	Mean	s.e.	s.d.	Variance	Skew	CV (%)
Body mass (sprint speed)	44	10.30	22.10	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	14.8
Residual sprint speed	41	−0.112	0.134	0	0.010	0.064	0.004	0.15	14.6
$\dot{V}_{O_2,max}$	44	15.85	33.67	23.67	0.652	4.326	18.713	0.00	
Residual $\dot{V}_{O_2,max}$	44	−0.128	0.150	0	0.009	0.058	0.003	−0.06	13.3
Hemoglobin	44	6.09	14.01	9.971	0.264	1.751	3.065	−0.04	
Residual hemoglobin	44	−0.187	0.125	0	0.011	0.073	0.005	−0.68	16.7
Hematocrit	43	17.42	34.76	25.43	0.555	3.638	13.235	0.06	
Residual hematocrit	43	−0.130	0.109	0	0.009	0.058	0.003	−0.42	13.4
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	0.006	0.037	0.001	−0.25	8.6
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	0.007	0.047	0.002	0.38	10.8
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	0.004	0.025	0.001	−0.45	5.8
Calf muscle mass	42	0.099	0.217	0.146	0.004	0.029	0.001	0.33	
Residual calf muscle mass	42	−0.042	0.061	0	0.004	0.025	0.001	0.43	5.8
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	0.007	0.047	0.002	0.07	10.7
Forearm muscle mass	43	0.010	0.022	0.015	0.000	0.003	0.000	0.12	
Residual forearm muscle mass	43	−0.075	0.060	0	0.005	0.032	0.001	−0.63	7.3
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	0.008	0.051	0.003	−0.08	11.7
Forelimb span	44	83.0	99.0	88.9	0.524	3.476	12.085	0.65	
Residual forelimb span	44	−0.016	0.023	0	0.001	0.009	0.000	0.48	2.1
Hindlimb span	42	109.0	126.0	116.3	0.584	3.787	14.344	0.18	
Residual hindlimb span	42	−0.020	0.019	0	0.001	0.009	0.000	0.06	2.0
Heart LDH activity	40	442.4	724.8	575.5	9.046	57.213	3273.312	0.53	
Residual heart LDH activity	40	−0.095	0.066	0	0.006	0.036	0.001	−0.29	8.3
Thigh LDH activity	44	390.9	678.6	550.7	9.358	62.076	3853.406	−0.18	
Residual thigh LDH activity	44	−0.134	0.081	0	0.007	0.049	0.002	−0.49	11.3
Liver LDH activity	44	117.3	360.8	236.7	8.659	57.436	3298.855	0.01	
Residual liver LDH activity	44	−0.263	0.205	0	0.017	0.111	0.012	−0.53	25.5
Heart CS activity	44	58.8	138.3	93.7	2.572	17.060	291.055	0.65	
Residual heart CS activity	44	−0.188	0.170	0	0.012	0.077	0.006	0.21	17.8
Thigh CS activity	44	4.74	10.84	7.71	0.217	1.439	2.072	−0.08	
Residual thigh CS activity	44	−0.198	0.159	0	0.012	0.082	0.007	−0.34	18.9
Liver CS activity	44	3.83	8.33	5.85	0.135	0.897	0.805	0.27	
Residual liver CS activity	44	−0.160	0.152	0	0.010	0.066	0.004	−0.18	15.3

Data are given for sprint speed (m s^{-1}), maximal oxygen consumption ($\dot{V}_{O_2,max}$, ml O$_2$ h^{-1}), blood hemoglobin concentration (g 100 ml^{-1}), hematocrit (%), organ mass (g), external morphology (mm) and tissue-specific enzyme activity (μmol g^{-1} wet tissue). N, number of lizards; CV, coefficient of variation; LDH, lactate dehydrogenase; CS, citrate synthase.

$P<0.001$). We included the correlation paths between each pair of lower-level traits (hemoglobin, and thigh and calf muscle mass) on model 1.00 and we did not remove them in subsequent iterations of the model (Petraitis et al., 1996). We also included the correlation between residual sprint speed and residual $\dot{V}_{O_2,max}$ 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) and performed likelihood-ratio tests (critical χ^2 with 1 d.f.=3.841) between each and the starting model (Fig. 4). 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 two-legged push-ups and the maximum number of four-legged push-ups did not affect significance. Adding paths from lower-level traits directly to behavior did not improve model fit.

Overall, the 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 two-legged push-ups per bout and maximum number of four-legged push-ups per bout, and between residual thigh and residual hemoglobin concentration all overlap zero (Fig. 4).

DISCUSSION

Our results show that aspects of individual variation in field behaviors can be related to whole-organism performance ability, which in turn reflects differences in morphology and physiology, although not parasite load, at least for this sample of lizards. This is one of the few studies to attempt to make connections across all levels in the standard morphology–performance–behavior paradigm of organismal biology. Below, we first consider the relative variability and repeatability of traits at different levels of
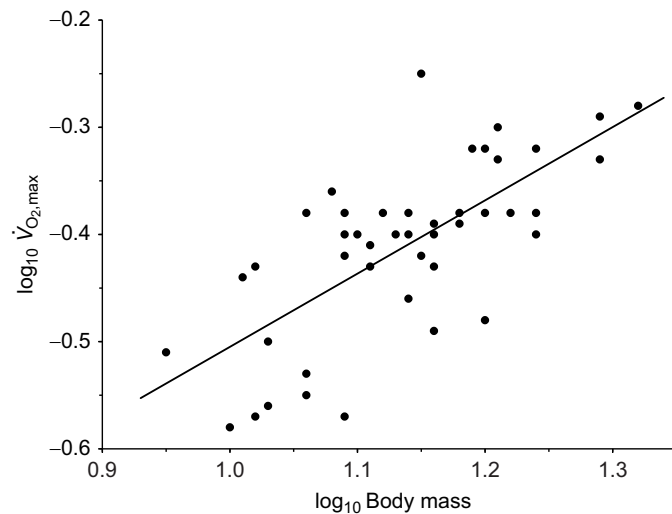
Fig. 2. Scaling of maximal aerobic capacity with body mass. Maximum aerobic capacity ($\dot{V}_{O_2,max}$) was measured in ml O_2 h^{-1}; body mass was measured in g. The solid line is the least-squares linear regression with a slope of 0.70 and a 95% confidence interval of 0.48–0.91.

biological organization, which has implications for statistical power. Next, we discuss correlations among traits of a given kind, including the possibility of a trade-off between sprint speed and aerobic capacity. We then discuss the success of attempts to predict variation at a higher level (e.g. behavior) from variation at a lower level (e.g. performance ability). We close with a consideration of path analysis, which provides a unified statistical frame of reference. Importantly, path analysis allows us to reject the possibility of direct paths from morphology/physiology to behavior. Thus, as the standard paradigm suggests, whole-organism performance is the crucial level of organization that transduces the effects of lower-level traits on behavior (Arnold, 1983; Biro et al., 2018; Garland and Losos, 1994; Husak, 2015; Lailvaux and Husak, 2014; Storz et al., 2015).

Variation and repeatability

Variability of the measured traits followed this general rule: behavior in the field showed larger amounts of individual variation, performance showed an intermediate level, and morphological traits were the least variable, as indicated by the CV (Tables S1 and S2). However, we note a few exceptions to this pattern. Among behavioral traits, the mean number of two- and four-legged push-ups was less variable than the number of head bobs or mean movement distance. Push-up displays in iguanians are 'stereotyped' behaviors (Carpenter and Ferguson, 1978) and have a recognizable, repeated, sequential pattern (Pantin, 1950; Tinbergen and Van Iersel, 1947). 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) (e.g. Wainwright et al., 2008).

The mean number of head bobs may be more variable because this behavior is more likely to be used during broadcast displays (Baird, 2013; Decourcy and Jenssen, 1994; Martins, 1993), 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' performance during displays should be less severe. In contrast, two- and four-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 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).

Mass-specific enzyme activity in most cases had a relatively high CV compared with other lower-level traits (Table 1). CS and LDH activity measured with similar methods in other lizard species showed similar, or even higher, variation (Garland, 1984; Garland and Else, 1987). Liver wet mass can vary as a result of differences in glycogen or lipid storage, which could inflate variation in enzyme activity expressed per unit mass of wet tissue. Mixed thigh muscles

Table 2. Allometric equations for maximal sprint speed, $\dot{V}_{O_2,max}$, organ mass, external morphology and LDH activity in the heart for *S. occidentalis*

Trait	N	Intercept	Intercept 95% CI		Slope	Slope 95% CI		R^2	SEE	P
			Lower	Upper		Lower	Upper			
Sprint speed	41	0.339	0.036	0.642	0.018	−0.242	0.278	0.001	0.048	0.890
$\dot{V}_{O_2,max}$	44	−1.206	−1.450	−0.961	0.699	0.484	0.913	0.506	0.058	<0.0001
Hematocrit	44	1.054	0.805	1.303	0.307	0.087	0.526	0.163	0.059	0.0073
Hemoglobin	44	.539	0.230	0.848	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
Forelimb span	44	1.757	1.718	1.796	0.169	0.135	0.204	0.699	0.009	<0.0001
Hindlimb 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

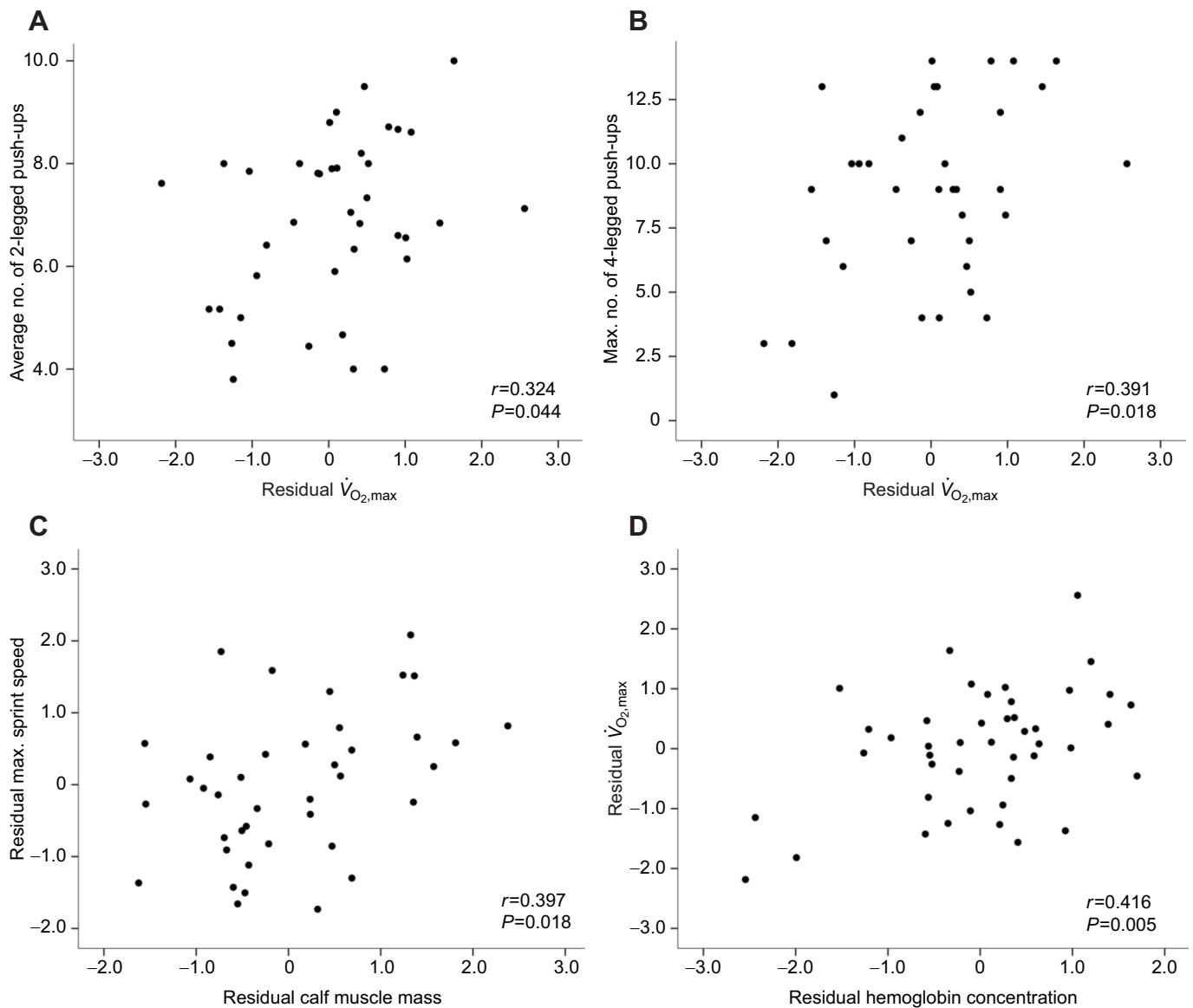CI, confidence interval; SEE, standard error of the estimate.

Fig. 3. Bivariate relationships for residual variables that remained in the final path model. (A) Average number of two-legged push-ups and (B) maximum number of four-legged push-ups against $\dot{V}_{O_2,max}$. (C) Maximal sprint speed against calf muscle mass. (D) $\dot{V}_{O_2,max}$ against blood hemoglobin concentration. Residual variables were standardized (except for behavior variables, which are raw values). All relationships are also statistically significant in the path model, based on log-likelihood ratio tests.

contain multiple separate muscles, with variable fiber-type composition (Gleeson et al., 1980). Even considering a single muscle, the iliofibularis, very high CV in the proportion of fast glycolytic, fast oxidative glycolytic and slow oxidative fiber types can occur (0.39 in *Sceloporus* species and up to 0.62 in other lizards, calculated directly from table 2 in Bonine et al., 2005). Each muscle fiber type has somewhat different contractile properties and mass-specific enzyme activity (Peter et al., 1972), which would tend to increase variability among individuals.

Among behavioral traits, movement distance had the lowest repeatability, measured as the ICC (Tables S1). Movement distance 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 more closely approach maximal performance capacity when they are chasing intruders or escaping from predators than when they are foraging (Irschick and Losos, 1998; Zani, 2001). Because we

did not make any distinction between moves performed under different circumstances (e.g. running to chase another male versus to catch prey), we expected movement distance to be more variable than two- and four-legged push-ups, which are used in specific situations.

Consistent with previous studies of lizard exercise physiology (Albuquerque et al., 2015a; Garland, 1985; Garland and Else, 1987), we observed significant repeatability for both maximal sprint speed and maximal aerobic capacity using either raw values or residuals from regressions with body mass. As a result, locomotor performance traits can be used to test hypotheses related to the effects of maximal performance on individual variation in ecology, behavior, reproductive success or other direct or indirect correlates of fitness. Repeatability of residual $\dot{V}_{O_2,max}$ ($r=0.88$) was considerably higher than that of sprint speed ($r=0.70$), but both values are consistent with values reported in the literature (e.g. Garland, 1985; Garland and Else, 1987; John-Alder et al., 1986).
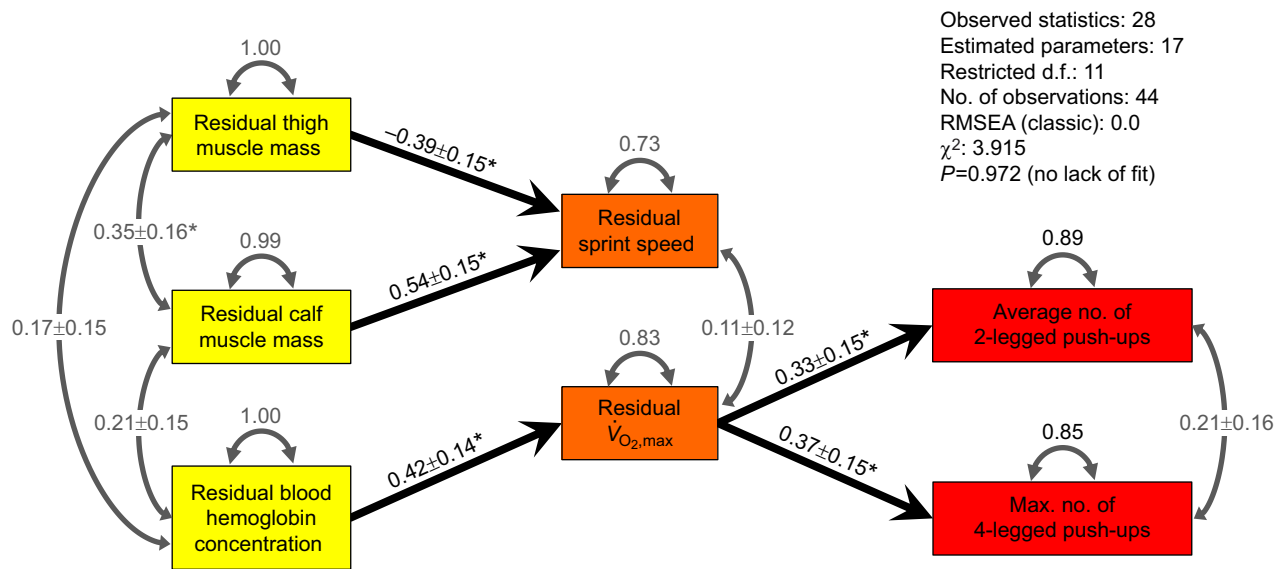
Fig. 4. Starting path model. This 'full' model (model 1.00 listed in Table 3) includes all variables that were statistically significant in multiple regressions from and physiological traits to performance and from performance to behavior traits. By convention, double-headed arrows indicate correlations between traits, single-headed arrows indicate putative causal relationships, and double-headed arrows above a given variable indicate the amount of unexplained variation. *2×s.e. does not overlap zero. RMSEA, root mean square error of approximation. See Table 3 for additional statistical information.

Correlations among traits within levels of biological organization

Considering only head bobs, lizards with higher means also had higher standard deviations of the number of head bobs per bout, suggesting that individuals that do many head bobs per bout still perform some bouts with few head bobs. Additionally, the lower than expected positive correlation between the number of head bobs per minute and the number of bouts of head bobs per minute suggests that lizards are not performing consistent numbers of head bobs per bout, so the number of head bobs per minute and bouts per minute are not as tightly correlated as expected under the null hypothesis calculated by bootstrapping (Table S3).

The higher than expected positive correlation between the mean number of four-legged push-ups per bout and the number of four-legged push-ups per minute suggests that for four-legged push-ups, lizards that do more push-ups in one bout also engage in more frequent bouts. Because lizards use four-legged push-ups during intense interactions (Carpenter and 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 and Garland, 2012; Careau et al., 2008; Réale et al., 2010). Such a set of relationships could also help explain the positive correlations found between two- and four-legged behavioral traits. These two displays are more frequently used during conspecific interactions, and more aggressive lizards would likely increase the frequency and intensity of both behaviors. Future studies should

Table 3. Number of estimated parameters, chi-square, log likelihood and Akaike information criteria for eight path models connecting size-adjusted lower-level traits and behavior traits in *S. occidentalis*

Model	Altered path	EP	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	RVO2→MaxLeg4	16	12	9.91	215.38	0.014	−398.75
0.97	RVO2→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→RVO2	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

Lower level traits were thigh muscle mass (THIGHG), blood hemoglobin content (HB), calf muscle mass (CALF), performance (sprint speed, SPT) and maximal aerobic capacity (VO2); behavior traits were maximum number of four-legged push-ups (MaxLeg4) and mean number of two-legged push-ups (MeanLeg2). *N*=44. EP, estimated parameters; LL, log likelihood; AICc, Akaike information criteria; R, residual. *P*-values refer to comparisons between each model and model 1.00 (shown in Fig. 4) using log likelihood-ratio tests according to a chi-square distribution. All variables are residuals calculated from regressions on body mass (see Materials and Methods), except for MaxLeg4 and MeanLeg2.

investigate possible costs of high levels of aggressiveness and the relative reproductive success of possible alternative 'strategies' (Fernández et al., 2018; Husak et al., 2008; Mills et al., 2008).

Importantly, residual $\dot{V}_{O_2,max}$ and sprint speed and were statistically uncorrelated (r=0.11 in the path analysis; Fig. 4), thus providing no evidence for a trade-off (e.g. Careau and Wilson, 2017; Garland et al., 2022; Lailvaux and Husak, 2014; Scales and Butler, 2016) between these two different types of organismal performance. These results are consistent with those for two other species of lizards (Garland, 1984, 1985; Garland and Else, 1987).

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 hindlimb and forelimb lengths indicates that even after the effect of body size is taken into account, limb lengths are still positively associated. Similar results have been observed within and among other lizard species (Christian and Garland, 1996; Garland, 1985; Jakšić et al., 1980), 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 and 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 aerobic and anaerobic capacities co-vary to some extent.

Predictors of $\dot{V}_{O_2,max}$ and sprint speed
Blood hemoglobin concentration was the only significant predictor of $\dot{V}_{O_2,max}$, suggesting that blood oxygen carrying capacity may be the limiting factor for maximal aerobic capacity (e.g. Hedrick et al., 2015; Scott and Dalziel, 2021) during the breeding season. Hemoglobin (or hematocrit) level is also a significant predictor of $\dot{V}_{O_2,max}$ in *C. similis* and *A. nuchalis* (Garland, 1984; Garland and Else, 1987), but contrary to what we found, CS activity in thigh muscles and liver, and LDH activity in the heart were also significant predictors of $\dot{V}_{O_2,max}$ in *C. similis* (Garland, 1984).

Residual calf muscle mass was a positive predictor of maximal sprint speed, whereas, perhaps surprisingly, residual thigh muscle mass had a negative effect. Based on quantitative electromyography and kinematic data for the lizard *Sceloporus clarkii*, calf muscles are usually active during the stance phase of the stride (when the foot is touching the ground and propulsive retraction occurs during the power stroke), whereas thigh muscles are associated with both the stance phase and swing phase (when the foot is not in contact with the ground and the limb is moving forward to recover before the next power stroke) (Reilly, 1995). Marsh and Bennett (1985, 1986) suggested that the swing phase might be the limiting factor for the sprint speed of *S. occidentalis* and *D. dorsalis*. However, in the lizard *Sceloporus woodi*, individual variation in maximal sprint speed and acceleration are positively associated 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 (Higham et al., 2011). 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 smaller 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).

Predictors of behavior
Sprint speed is positively correlated with social dominance in paired laboratory tests of males in two relatively closely related species of lizards, *S. occidentalis* and *Urosaurus ornatus* (Garland et al., 1990; Robson and Miles, 2000), but not in the more 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 *C. collaris* (Husak et al., 2006; Peterson and Husak, 2006). In the present study, sprint speed was not statistically related to any of the movement or display behavioral traits when analyzed with multiple regressions. Perhaps higher sprint speed allows lizards to chase after intruders or cover the territorial area more effectively (see 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. (1990) was performed with size-matched pairs of lizards in small enclosures for 1 h with one basking source (similar methods were used by Perry et al., 2004; Robson and Miles, 2000). 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 interact, or they can retreat during the combat and return at a later time, increasing the chance of gaining territorial space (Stamps and Krishnan, 1995). Additionally, combats in *S. occidentalis* can involve multiple individuals (Fitch, 1940).

In this study, $\dot{V}_{O_2,max}$ was an important predictor of the maximum number of four-legged push-ups per bout, suggesting that it affects (and possibly limits) the effort that lizards can sustain during four-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 and 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 and Krishnan, 1997) and escalate to long and intense physical fights, as observed during our field work (see Results, 'Behavior in the field'). During that 24 min-long combat, rest periods between movements and attacks were rarely longer than 30 s. 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 h. Fitch (1940) also reported a 45 min-long interaction and noted individuals 'showing evidence of fatigue at later stages of the fight'. Both observations suggest that aerobic and anaerobic capacities are involved during extended disputes.

Additionally, the frequency and intensity of push-up displays increase considerably during the breeding season when males establish their territories (Sheldahl and Martins, 2000; Stamps and Krishnan, 1997), 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 two- and four-legged push-ups during our observations was as high as once every 2 min (two-legged) or once every 5 min (four-legged), respectively. If *S. occidentalis* are under similar selection favoring a higher frequency of push-up displays and territorial defense to *C. collaris* (Baird, 2013; Baird et al., 2003, 2007), 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 $\dot{V}_{O_2,max}$ and the variability (s.d.) 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 relationships 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 four-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). Regardless, our results encourage future use of path analysis to enhance our understanding of relationships among traits at multiple levels of biological organization (Arnold, 1983; Biro et al., 2018; Garland and Losos, 1994; Husak, 2015; Lailvaux and Husak, 2014; Mills et al., 2008; Storz et al., 2015).

Caveats and future directions

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 and Hardin, 2007). That might be particularly relevant for the movement traits, which are more susceptible to external stimuli (presence of prey, variation in basking sites, etc.) and inter-observer error (movement distances are harder to estimate visually than are push-up displays). 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 displays directed to males and females separately. Greater durations of observations for each individual would also improve the 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 (Zani, 2001).

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