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RESEARCH ARTICLE

It's just sand between the toes: how particle size and shape variation affect running performance and kinematics in a generalist lizard

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ABSTRACT

Animals must cope with and be able to move effectively on a variety of substrates. Substrates composed of granular media, such as sand and gravel, are extremely common in nature, and vary tremendously in particle size and shape. Despite many studies of the properties of granular media and comparisons of locomotion between granular and solid substrates, the effects of systematically manipulating these media on locomotion is poorly understood. We studied granular media ranging over four orders of magnitude in particle size, and differing in the amount of particle shape variation, to determine how these factors affected substrate physical properties and sprinting in the generalist lizard *Eremias arguta*. We found that media with intermediate particle sizes had high bulk densities, low angles of stability and low load-bearing capacities. Rock substrates with high shape variation had higher values for all three properties than glass bead substrates with low shape variation. We found that *E. arguta* had the highest maximum velocities and accelerations on intermediate size particles, and higher velocities on rock than glass beads. Lizards had higher stride frequencies and lower duty factors on intermediate particle size substrates, but their stride lengths did not change with substrate. Our findings suggest that sand and gravel may represent different locomotor challenges for animals. Sand substrates provide animals with an even surface for running, but particles shift underfoot. In contrast, gravel particles are heavy, so move far less underfoot, yet provide the animal with an uneven substrate.

KEY WORDS: Granular substrate, Locomotion, *Eremias arguta*, Gravel

INTRODUCTION

Locomotion is an important fitness-related behavior that most animals depend on for predator escape, foraging, finding mates and territory defense (Aerts et al., 2000; Ghalambor et al., 2003; Losos et al., 2006; Scales and Butler, 2016). As such, there is an expectation that natural selection will select for phenotypic traits that maximize locomotor performance (Arnold, 1983; Calsbeek and Irschick, 2007). However, an ever-growing body of research shows weak or non-existent relationships between phenotype and performance (Holzman et al., 2011; Wainwright et al., 2005; Wiens and Rotenberry, 1980; Zaaf and Van Damme, 2001). This paradox may be explained, at least in part, by (1) complex mapping of phenotype on performance, and (2) the ecological context in which an animal carries out a task. First, because phenotypic traits that are involved in locomotion are often involved in carrying out multiple tasks, redundancy and trade-offs exist in most functional systems, obscuring phenotype–performance relationships (Alfaro et al., 2005; Holzman et al., 2012; Wainwright et al., 2005). Second, interactions between the animal's phenotype and the substrate on which it is moving (Hosoi and Goldman, 2015; Mazouchova et al., 2010; Sathe and Husak, 2015) can further obscure these relationships. Most animals live in highly heterogeneous environments, and their locomotor performance frequently differs with the substrate on which they are moving (Kelley et al., 1997; Losos and Sinervo, 1989; Stark et al., 2015), but our understanding of how this plays out is rudimentary.

Animals must often cope with substrate heterogeneity, which represents a challenge for maintaining high locomotor performance. For example, substrates differ in incline, width, complexity, roughness and compliance (Collins et al., 2015; Irschick and Jayne, 1998; Losos and Sinervo, 1989; Qian et al., 2015). Considerable work shows that locomotor velocity, a common measure of performance, decreases on inclines, narrow perches or in complex environments by constraining the animal's movement (Birn-Jeffery and Higham, 2014; Irschick and Jayne, 1998; Irschick and Losos, 1999; Kohlsdorf et al., 2001; Sathe and Husak, 2015). The substrate can also affect locomotion in different ways, depending on the animal's phenotype. For example, many lizards suffer decreased performance on smoother substrates because of decreased friction with their feet (Brandt et al., 2015). However, geckos show the opposite pattern because smoother substrates provide greater contact with their adhesive pads (Vanhooydonck et al., 2005).

Species also employ different strategies for coping with environmental heterogeneity, being either habitat specialists or generalists. Substrate specialists tend to have high sprint sensitivity, meaning that they have good locomotor performance on some substrates but not on others (Greenville and Dickman, 2009; Irschick and Losos, 1999). Hence, they actively select substrates on which they perform well, and often have adaptations for moving on their preferred substrate, such as toe fringes for moving on sand (Carothers, 1986) or short limbs for moving on narrow perches (Losos and Sinervo, 1989). In contrast, generalists tend to have low sprint sensitivity, so can exploit a wide range of substrates while maintaining acceptable locomotor performance (Irschick and Losos, 1999; Qian et al., 2015). Generalists often lack adaptations for particular substrates, and behaviorally modify their kinematics on different substrates to maximize their performance (Brandt et al., 2015).

Granular media are composed of particles and are extremely common in nature, and a major source of substrate variation. They include silt, sand, gravel and mud, which dominate coastal habitats, deserts, savannahs and anywhere that soil is exposed for an animal to move across (Hosoi and Goldman, 2015; Jaeger and Nagel,

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List of symbols and abbreviations

CoM	center of mass
ISO	International Organization for Standardization
LBC	load-bearing capacity
MPD	mean particle diameter
SS_A	sum of squares for the main fixed effect
SS_ε	sum of squares of the residuals
η^2	statistical effect size

1997). At rest, granular media behave as solids, but if a threshold force, called the yield stress, is exceeded, they behave as fluids (Jaeger and Nagel, 1997; Mehta and Barker, 1994). They can also be compressed, which increases their packing, or volume fraction, and the yield stress necessary to fluidize them (Li et al., 2013; Mazouchova et al., 2010). Such compression leads to jamming of the constituent particles, resulting in solid-like behavior (Albert et al., 2001, 2000).

Granular media can vary in the size, shape, density, coefficient of friction of their particles, and the amount of moisture between them (Li et al., 2013), resulting in high substrate heterogeneity in nature (Qian and Goldman, 2015). All of these characteristics affect the physical properties of the medium, some of which are easy to quantify in the laboratory and field, and are ecologically relevant. The load-bearing capacity (LBC), also called surface strength, measures the resistance of the substrate to compression, or when the substrate deforms (Korff and McHenry, 2011). LBC increases with inter-particle friction and shape variation (Stone et al., 2004). Although the depth of penetration of an animal's foot in a substrate is a function of both substrate properties and the animal's weight and foot area (Li et al., 2009; Qian et al., 2015), LBC is useful in understanding the substrate's contribution to this interaction. The angle of stability is the angle at which the medium begins to spontaneously flow downhill (Gravish and Goldman, 2014; Mehta and Barker, 1994). This also depends on volume fraction and inter-particle friction, which is affected by particle shape variation (Brzinski et al., 2013). Finally, bulk density is the weight of the medium per unit volume (Brzinski et al., 2013; Mueth et al., 1998). Under consistent packing and uniform particle material density, bulk density is a relative measure of the amount of interstitial space between particles. When the effects of volume fraction are accounted for, bulk density is affected by particle size and shape.

Animals moving on granular media interact with their substrate in different ways. In some instances, animals exploit the physical properties of granular media to enhance their locomotor performance. For example, many lizards move through granular substrates using lateral undulations (Baumgartner et al., 2008). These undulations fluidize the substrate around the animal, facilitating sand-swimming (Maladen et al., 2016). In contrast, running hatchling sea turtles and jumping robots show that compression of the granular medium without exceeding the yield stress allows them to attain locomotor performance comparable to that on a solid substrate (Aguilar and Goldman, 2015; Mazouchova et al., 2010). Studies of limbed locomotion on granular versus solid media have often shown a lack of effect on locomotor performance (Bergmann and Irschick, 2010; Korff and McHenry, 2011; Mazouchova et al., 2010; Renous et al., 2008). However, findings are mixed, with some lacertid lizards showing decreased acceleration on sand relative to a solid substrate (Vanhooydonck et al., 2015). Acceleration is highly force-dependent, so it is possible that during acceleration animals exceeded the yield stress of the medium. Granular media do not seem to allow increased locomotor performance. Locomotor kinematics are also sometimes unaffected by granular media (Bergmann and Irschick, 2010; Mazouchova et al., 2010), although some small gymnophthalmid lizards achieve similar velocity on granular and solid substrates by taking longer, lower-frequency strides on sand (Renous et al., 2008). Unfortunately, much of the work described here has considered only one or two granular media of arbitrary and often unquantified properties. Although highly detailed work has developed a resistive force theory for modeling locomotion on granular media (Hosoi and Goldman, 2015; Li et al., 2013; Zhang and Goldman, 2014), the only systematic examinations of how varying the characteristics of granular media affects locomotion have manipulated volume fraction in a single medium (Li et al., 2009; Qian et al., 2015).

To address this, we studied the locomotor performance and kinematics of a generalist lizard running on a wide range of level granular substrates, varying in particle size and shape. We used a substrate generalist, the steppe-runner lizard [*Eremias arguta* (Pallas 1773)], because it lacks any special adaptations for running on granular media, yet is able to move effectively on a wide range of substrates (Arnold and Ovenden, 2004). This allowed us to address the question of how an animal maintains high locomotor performance on a variety of granular substrates, differing in particle size over four orders of magnitude, from silt to coarse gravel (50–40,000 µm) (ISO, 2002), and on both natural rock and glass beads. Glass beads provided a consistent, spherical particle shape, while natural rock had irregular and variable particle shape. We also quantified the bulk density, angle of stability and LBC of each substrate to better understand why media affected running performance or kinematics. We tested two main hypotheses. First, we tested how particle size and shape variation affect the physical properties of the substrate. We predicted that smaller particles would have higher bulk densities because they have higher cohesive forces and can be packed more tightly than larger particles, and lower angles of stability and LBC because the particles are lighter and should be less stable (Gravish and Goldman, 2014; Li et al., 2013). We also predicted that all three properties would be greater for natural rock than glass beads because the irregular and more variable shape of natural rock should facilitate packing and increase inter-particle friction (Brzinski et al., 2013; Stone et al., 2004). Second, we tested whether particle size and variation in shape affected the running performance and kinematics of the lizards. We predicted that locomotor performance of *E. arguta* would be unaffected because it is a substrate generalist, but that the kinematics would be affected, meaning that the lizards would behaviorally compensate for variation in substrate. In particular, we predicted that finer particle substrates and those consisting of spherical beads would require considerable kinematic compensation because those particles are more easily moved by the forces of the lizard running on them (Attum et al., 2007).

MATERIALS AND METHODS

Animal husbandry

We obtained 16 captive-bred steppe-runner lizards (*E. arguta*; Fig. 1B) from a commercial breeder. Animals ranged in snout–vent length from 50 to 78 mm (59±6 mm, mean±s.d.). We obtained these lizards in two groups of eight and used the first group for experiments on natural sand substrates in 2013, and the second group for experiments on glass bead substrates in 2014. We housed animals individually in plastic terraria (34×20×21 cm, length×width×height) on a vermiculite substrate, with a hide box and a water dish. We provided UVB lighting on a 12 h:12 h light:dark

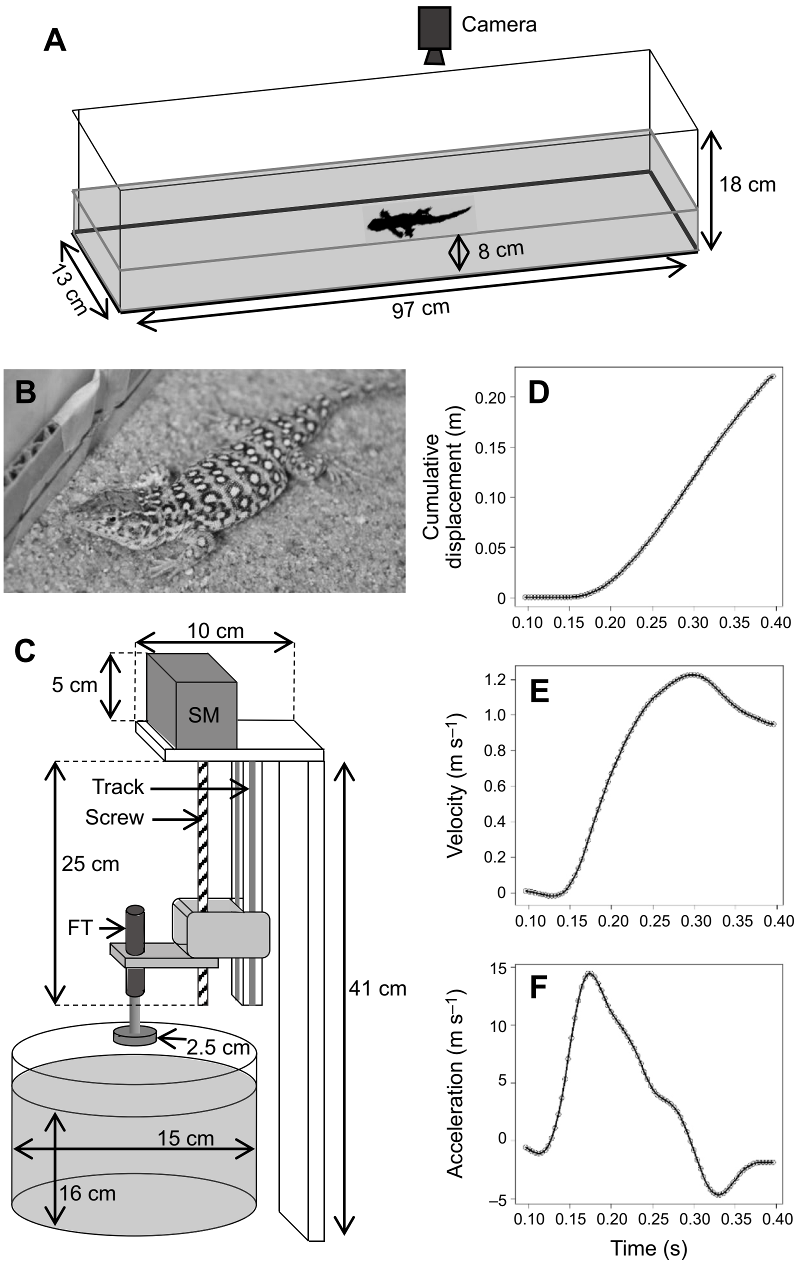

Fig. 1. Experimental setup, subject and performance data for a representative trial. (A,C) Diagrams of experimental materials, including important dimensions. FT, force transducer; SM, stepper motor. The screw is attached to the motor, to make a linear actuator that moves the bracket holding the force transducer along the track. (B) A photograph of *Eremias arguta*. (D–F) Cumulative displacement (D), velocity (E) and acceleration (F) data against time for a sample trial of an animal running on 1250 µm sand.

cycle, and an under-tank heater on one side of the terraria to allow animals to thermoregulate. We maintained ambient temperatures of 25±2°C in the room housing the terraria. We fed animals with two crickets and misted them with water three times per week. We allowed animals 3 weeks after arrival to acclimate to their new conditions before beginning experimental trials. The Clark University Institutional Animal Care and Use Committee approved all husbandry and experimental procedures (protocol 017R).

Experimental substrates

We ran animals on a series of glass bead and natural rock substrates with varying particle sizes in the sand and gravel range (ISO, 2002) (Table 1). We used glass beads and rock to test for the effects of particle shape variation on lizard running; glass beads were spherical and had negligible shape variation, while the rock substrates consisted of particles with irregular shapes and large shape variation. We obtained glass beads commercially (Dragonite Solid Glass Beads, Jaygo Inc., Randolph, NJ, USA) in sizes comparable to natural rock substrates that we used. We also obtained rock substrates commercially as play sand, aquarium gravel, river pebbles and pond pebbles. We sieved 68 kg of oven-dried play sand using a series of screen sieves (10, 35, 60, 120 and 230 ‘openings per linear inch’; Hubbard no. 548, Forestry Suppliers Inc., Jackson, MS, USA) to obtain sufficient amounts of each fraction for running animals. Despite the volume of sand sieved, we were unable to obtain enough 64–125 µm (230 sieve) sand for animal trials, but we included it in the analysis of substrate characteristics for a more comprehensive understanding of how particle size affects the behavior of the substrate. We sorted river and pond pebbles by hand to exclude rocks of unusual size or shape.

To better understand the physical properties of the substrates that we used, we quantified their bulk density, angle of stability and LBC. For the two largest rock substrates (Table 1), we also measured the three major axes of 30 rocks from each substrate to 0.1 mm using a digital caliper (Mitutoyo, Kanagawa, Japan). To calculate the range of particle sizes in each of these substrates, we

Table 1. Substrate mean particle diameter (MPD) and range, and mean (*n*=10) estimates of bulk density, angle of stability and load-bearing capacity (LBC) for glass bead and rock substrates

	Particle size range (μm)	MPD (μm)	Bulk density (g ml^{-1})	Angle of stability (deg)	LBC (N cm^{-2})
Glass beads	0–50	25	1.275	51	0.529
	70–110	90	1.464	34	0.623
	200–300	250	1.466	30	0.552
	500–750	625	1.492	26	0.331
	4000	4000	1.443	29	0.657
	19,000	19,000	1.353	26	–
Natural rock	64–125	94	1.429	41	1.483
	125–250	188	1.463	38	1.288
	250–500	375	1.532	34	1.207
	500–2000	1250	1.518	37	1.471
	3000–5000	4000	1.372	41	3.044
	18,000–28,000	22,000	1.544	51	–
	35,000–49,000	40,000	1.659	58	–

Data for the finest rock substrate are included for completeness, although an insufficient amount of substrate was available for running lizards. Substrates lacking LBC estimates had particles sizes that were too large to take the measurement.

took the minimum and maximum values of the average of the three axes. We then took the average for all 30 rocks, rounded to the nearest 1000 μm, as an estimate of the midpoint diameter.

We measured bulk density of each substrate by measuring a volume of 50 ml using a graduated cylinder, and then weighing the substrate to the nearest 0.1 g using an electronic balance (Scout Pro, Ohaus, Parsippany, NJ, USA) and dividing the mass by the volume. For the 19 mm glass beads and the two largest rock sizes, we needed to use a larger volume to accommodate a sufficient number of particles. For these substrates, we used a 700 ml plastic container and weighed the substrates to the nearest gram on a triple-beam balance (Ohaus). To measure angle of stability, we placed 1000 ml of substrate (1500 ml for the 19 mm beads and two largest particle rock substrates) in a 27×17×12 cm (length×width×height) plastic container, mounted on a sturdy wooden board. We then aligned one end of the board with the center of a vertically mounted protractor, and slowly lifted the other end of the board, tipping the container of substrate until particles began spontaneously flowing, and recorded the angle. We measured bulk density and angle of stability 10 times for each substrate.

We measured the LBC of the substrates using the adaptor foot of a pocket penetrometer (LR-281, Forestry Suppliers), mounted on a Kistler type 9203 force transducer, attached to a Type 5995 Charge Amplifier (Kistler Instrument Corp., Amherst, NY, USA) to maximize accuracy. We attached the force transducer and adaptor foot to a custom-built linear actuator, run by a stepper motor (HT23-394, 2.8 V, 2.0 A/phase), with a 3540i Driver and PS150A24 power supply (Applied Motion Products Inc., Watsonville, CA, USA), and controlled from a PC (Fig. 1C). This allowed us to ensure that the adaptor foot penetrated each substrate at a uniform angle (normal to the substrate) and at a uniform speed (50 mm s^{-1}) to the desired depth (level with the top of the adaptor foot). We converted measurements to N cm^{-2} and took 10 measurements per substrate. For LBC measurements, we used a cylindrical container, 15 cm in diameter, with substrate 16 cm deep. These dimensions are sufficient to negate edge effects (Stone et al., 2004). We did not measure LBC for the 19 mm glass beads or the two largest particle rock substrates because they were too coarse. These particles did not move when lizards ran over them. We did not account for volume fraction in our experiments on the physical properties of the media. Instead, we used a uniform filling method, gently pouring and smoothing the media in the containers, ensuring that the volume fractions that we used were always relatively densely packed and above the granular critical state (Gravish and Goldman, 2014).

Lizard running trials

We ran lizards on a level racetrack (97×13 cm, length×width) with substrate to a depth of 8 cm, from a standstill, which allowed us to measure acceleration as well as velocity (Fig. 1A). We ran each animal three to four times on each substrate, and recorded trials from dorsal view using a high-speed video camera (LE500ME, Fastec Imaging Corp., San Diego, CA, USA) at 250 Hz. We included a 2×2 cm scale object in each video for later conversion of pixels to meters. Prior to trials, we painted a series of nine 5 mm diameter dots on the lizards using a non-toxic white paint pen. Dots were placed at the occiput, pectoral girdle, mid-dorsum [corresponding to the center of mass (CoM)], pelvic girdle, level of the cloaca, and on both elbows and knees. We incubated lizards for 30 min prior to each trial at 30°C. To avoid biasing the results owing to digestion or fatigue, we did not feed animals for 1 day prior to trials, conducted a maximum of two trials per animal per day, separated by at least 30 min, and conducted trials during a maximum of three non-consecutive days per week. We also randomized the order in which substrates were used and randomized the order that lizards ran in each trial. We saved trials in which the lizard ran without touching the walls of the track and remained quadrupedal. We recorded the surface body temperature of each lizard immediately at the conclusion of each trial to 0.2°C using an infrared thermometer (MT6, Raytek, Santa Cruz, CA, USA) (Bergmann and Irschick, 2010).

Quantifying sprint performance and kinematics

We digitized the CoM point in all the videos that we collected using DLTdv5 (Hedrick, 2008) in MATLAB 2010b (MathWorks, Natick, MA, USA), which provided us with *xy* coordinates for the point in each frame. We used the ‘Set Scale’ function in ImageJ (http://imagej.nih.gov/ij/) to calculate the number of pixels per meter, and then used that number and the frame rate to calculate the cumulative distance that the lizard moved by each frame in meters. We also converted frame number to time in seconds. We used the curve-fitting toolbox for MATLAB to fit a quantic spline to the cumulative displacement and time data (Fig. 1D), adjusting the smoothing of the spline until secondary oscillations in the second derivative were removed and magnitudes of the maxima were stabilized (Umberger et al., 2013). We exported the splined cumulative distance data, and first and second derivatives, which represented instantaneous velocity and acceleration data (Fig. 1E,F). From these data, we calculated average and maximum velocity and maximum acceleration. We then selected the trial with the highest acceleration for each individual on each substrate for further analysis, to avoid pseudoreplication and to work with the highest performance trials (Bergmann and Irschick, 2006). All analyses presented herein use data only from this subset of videos.

We also collected kinematic data for these trials, including stride frequency, stride length, duty factor, and the angle of maximal limb protraction and retraction for both the front and hind limbs. We defined a stride as from foot up to foot up (Bergmann and Irschick, 2006). We first noted the frame numbers for each foot up and foot down in the video. We then calculated stride frequency as the inverse of the duration of the stride in seconds (number of frames divided by the frame rate), and duty factor as the duration of stance (foot in contact with the ground) divided by the duration of stride. We calculated stride length as the cumulative distance moved during a stride. Finally, we used the angle tool in ImageJ to measure limb angles. The complete dataset is available as supplementary material (Dataset 1).

Statistical analysis

We carried out all analyses in R v.3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). We conducted analyses for glass bead and rock substrates separately because particle sizes between the two types of substrate did not directly coincide and different animals were used for each experiment. We tested whether substrates of different particle sizes differed in density, angle of stability and LBC using an ANOVA. For all analyses, we tested the assumptions of normality and homoscedasticity of the residuals using Shapiro–Wilk and Bartlett tests, respectively. Because these assumptions were violated in a number of cases, we used randomization ANOVAs, which take into account the distribution of the response variable and residuals (Mitchell and Bergmann, 2016). We used two-sample *t*-tests as *post hoc* tests in the case of a significant ANOVA to tease apart which substrates differed. We corrected for multiple comparisons using the Benjamini–Hochberg method (Benjamini and Hochberg, 1995), which controls for both type I error and false discovery rate, while having a higher power than other options (Williams et al., 1999). We also calculated effect size for the ANOVA to understand how much of the variance explained in the response variable was due to substrate particle size using η^2, which we calculated as $\eta^2=[SS_A/(SS_A+SS_\varepsilon)]$, where SS_A is the sum of squares of the treatment and SS_ε is that for the residuals, and with values >0.25 considered large effects (Cohen, 1973).

We used mixed-effects models, as implemented in the ‘lme4’ and ‘lmertest’ packages for R, to analyze our repeated-measures locomotor data (Bolker et al., 2009; Crawley, 2012). We chose mixed effects models over repeated-measures ANOVA because we were missing data for a few individuals for some substrates and variables (see sample sizes in Table 3), and the latter analysis option cannot handle missing data. Mixed-effects models also quantify the amount of variance explained by specified random factors (Crawley, 2012). We ran mixed effects models with individual and body temperature as random effects, and substrate as a fixed effect. When the fixed effect was significant, we used pairwise generalized linear hypothesis testing with the Benjamini–Hochberg correction for our *post hoc* tests (Bretz et al., 2010). Finally, we compared locomotor performance variables attained on glass bead versus sand substrates using two-sample *t*-test that accounted for heteroscedasticity.

RESULTS

Substrate physical properties

Both glass bead and rock substrates significantly differed in their physical characteristics with particle size, and effect sizes were very large (Table 2). Bulk density increased with particle size to a maximum for 625 and 375 µm mean particle diameter (MPD) for glass and rock substrates, respectively, and then decreased as particle size continued to increase (Fig. 2A,B). For glass beads, bulk density continued to decrease even as MPD increased to 19 mm (Fig. 2A). However, for rock substrates, density reached a minimum at particle sizes of 4 mm and then increased considerably as particle sizes increased (Fig. 2B). Bulk densities were approximately comparable between glass and rock substrates. The angle of stability decreased as particle size increased, leveling off at a minimum of approximately 26 deg for glass beads, as particle sizes went above MPDs of 625 µm (Fig. 2C). For the rock substrates, angle of stability decreased with increasing particle size to a minimum of ~34 deg at MPDs of 375 µm, and then increased steadily as particle sizes increased (Fig. 2D). Angle of stability was consistently higher for rock substrates than glass bead substrates, and rock substrates with large particles had angles of stability as high as 60 deg. For glass bead substrates, LBC increased with particle size for fine beads, then decreased to a minimum for the 625 µm MPD beads, and then increased for the 4 mm substrate (Fig. 2E). For the rock substrates, LBC decreased gradually with increasing particle size to the 325 µm MPD substrate, and then increased with further increases in particle size (Fig. 2F). LBC was much higher for rock than glass bead substrates, but the general pattern between the two types of substrate coincided because the finest rock substrate is actually most comparable to the second finest glass bead substrate (Fig. 2E,F).

Effects of substrate on locomotion

Mixed-effects modeling allowed us to quantify and differentiate the random effects of body temperature and individual variation from substrate particle size fixed effects (Table 3). In general, lizard body temperature across trials explained little variation (4.0±9.1%, mean ±s.d.) in locomotor variables, with the exception of hind limb stride frequencies on rock substrates and hind limb angles on glass substrates. Individual variation played a larger role in explaining locomotor variables (16.5±17.0%), with 17 out of 25 variables having at least 10% of variation explained by individual on glass bead substrates and 13 out of 25 on the rock substrates (compared with two and four variables, respectively, for temperature; Table 3).

Substrate type and particle size also affected running performance and kinematics of *E. aruta* (Table 3). Maximum velocity and acceleration were affected by particle size, with the highest levels of performance most often at intermediate particle size substrates (Fig. 3). This pattern was not observed for maximum (Fig. 3A) and average (see Fig. S1) velocity on glass substrates. Although maximum acceleration (t_{69}=0.34, P=0.738) and average velocity (t_{91}=1.08, P=0.282) were comparable across glass and rock substrates, maximum velocity was higher on rock substrates (t_{92}=7.83, P<0.001; Fig. 3).

Lizards responded to changes in substrate particle size primarily by modulating their stride frequencies (Fig. 4), but not their stride lengths (Figs S1 and S2), and qualitatively similar patterns were

Table 2. Results for randomization ANOVAs testing for differences in bulk density, angle of stability and LBC for glass bead and rock substrates listed in Table 1

	Response	d.f.$_s$	d.f.$_e$	MS$_s$	MS$_e$	F	P_{rand}	η^2
Glass	Bulk density	5	54	0.0704	0.0003	201.5	<0.0001	0.949
	Angle of stability	5	54	902.67	3.81	237.1	<0.0001	0.956
	LBC	4	44	0.1613	0.0013	126.0	<0.0001	0.920
Rock	Bulk density	6	60	0.0731	0.0007	107.6	<0.0001	0.915
	Angle of stability	6	63	746.22	5.16	144.7	<0.0001	0.932
	LBC	4	43	5.2771	0.0241	218.9	<0.0001	0.953

d.f. and MS are degrees of freedom and mean squares for substrate effects (s) and error/residuals (e). P_{rand} is the probability calculated based on 10,000 randomizations of the data. η^2 is a measure of effect size.

Table 3. Sample sizes and percent variance explained by the random effects body temperature and individual in mixed-effects models of various locomotor variables for *Eremias arguta* running on six glass bead and six rock substrates

	Glass bead				Rock			
Locomotor variable	*n*	Temperature	Individual	Residual	*n*	Temperature	Individual	Residual
Average velocity	48,8	0.0	0.0	100.0	48,8	0.0	4.9	**95.1**
Max. velocity	48,8	0.0	1.6	**98.4**	48,8	7.9	10.4	**81.7**
Max. acceleration	48,8	1.6	17.8	**80.6**	48,8	0.0	16.5	**83.5**
Stride frequency – hind 1	48,8	0.0	22.5	**77.5**	48,8	4.5	15.0	80.4
Stride frequency – hind 2	47,8	0.0	29.0	**71.0**	44,8	29.0	12.9	58.1
Stride frequency – hind 3	43,8	0.0	61.2	**38.8**	31,8	45.7	19.5	**34.8**
Stride frequency – front 1	48,8	0.0	41.2	**58.8**	48,8	3.8	0.0	96.2
Stride frequency – front 2	46,8	0.0	51.8	**48.2**	43,8	0.0	22.1	**77.9**
Stride frequency – front 3	42,8	0.0	53.0	**47.0**	27,8	4.3	76.4	**19.3**
Stride length – hind 1	48,8	2.0	24.8	73.1	48,8	0.0	5.2	94.8
Stride length – hind 2	46,8	0.0	18.7	81.3	44,8	0.0	11.9	88.1
Stride length – hind 3	41,8	0.0	0.3	99.7	30,8	22.4	3.5	74.1
Stride length – front 1	48,8	0.0	33.0	67.0	48,8	0.0	21.4	78.6
Stride length – front 2	46,8	0.0	16.6	**83.4**	43,8	14.5	0.0	85.5
Stride length – front 3	42,8	0.0	11.9	**88.1**	26,7	0.0	0.0	100.0
Duty factor – hind 1	48,8	0.0	0.5	**99.5**	48,8	0.0	3.5	96.5
Duty factor – hind 2	47,8	0.0	0.7	**99.3**	44,8	4.3	0.0	95.7
Duty factor – hind 3	43,8	0.0	15.6	**84.4**	31,8	0.0	2.7	97.3
Duty factor – front 1	48,8	0.0	5.9	**94.1**	48,8	0.0	18.1	**81.9**
Duty factor – front 2	46,8	0.0	23.3	**76.7**	43,8	0.0	7.9	92.1
Duty factor – front 3	42,8	0.0	38.3	**61.7**	27,8	4.7	0.0	95.3
Hind max. protraction	48,8	23.2	19.7	57.2	48,8	0.0	3.3	96.7
Hind max. retraction	48,8	24.2	0.8	75.1	48,8	0.0	19.1	80.9
Front max. protraction	48,8	0.0	10.0	90.0	48,8	0.0	20.8	79.2
Front max. retraction	48,8	6.9	2.6	90.5	48,8	0.0	29.3	70.7

n includes the total number of observations and the number of individuals for each analysis. Residual variation is that not accounted for by random effects. Significant differences between substrates (the fixed effect) are indicated with bold font for residual variance. Specific differences are shown in the figures. Hind and front refer to the limb considered for each kinematic variable, and numbers refer to the stride from a standstill.

apparent for both front and hind limbs (Fig. 4; Fig. S2). Stride frequency was highest at intermediate particle sizes, on which the lizards moved fastest. This pattern was better defined on rock substrates and during later strides, particularly stride 3 (Fig. 4; Figs S2 and S3). Duty factors tended to decrease for higher particle sizes on both glass bead and rock substrates and for both front and hind limbs (Fig. 5; Fig. S4). Coinciding with our stride length results, we also found that front and hind limb protraction and retraction angles were not affected by substrate type or particle size (Fig. S5).

DISCUSSION

Glass bead and rock granular media differ quantitatively and qualitatively

We compared some basic physical properties of a wide range of granular media differing in particle size and variation in shape. We found that both glass bead and rock media with intermediate particle sizes had the highest bulk densities, low angles of stability and lowest LBCs (Fig. 2), meaning that these media are unstable but dense. We also found that media composed of glass beads had qualitatively different patterns for angle of stability, which decreased as particle size increased, while rock media had a minimum angle of stability at an intermediate MPD of ∼375 µm (Fig. 2, Table 1). These findings were unexpected, as one would expect finer particles to pack more closely together, resulting in more stable substrates (Li et al., 2013; Mehta and Barker, 1994).

Some of our findings may be explained by different behaviors exhibited by silt, sand and gravel (Fig. 2) (ISO, 2002). In general, dry granular media lack cohesion, instead interacting through dispersive forces that repel adjacent particles (Li et al., 2013). However, fine powders, with particles in the silt range, are cohesive (Li et al., 2013). In contrast, gravel is composed of large particles that weigh much more than sand particles, and so gravity may play a more important role in individual inter-particle interaction (Brzinski et al., 2013). This may explain the high angles of stability and LBC, as well as greater bulk density of gravels. The patterns of substrate physical properties that we observed (Fig. 2) may have implications for animal locomotion, because as individual particles approach the size of the animal moving on them, the locomotor strategies the animal adopts often change (Hosoi and Goldman, 2015; Li et al., 2013).

Differences in particle shape variation may also explain the different physical properties between glass beads and rock media that we observed. Spherical particle media are composed of 26% voids when maximally packed, and these voids increase in size with MPD (Jaeger and Nagel, 1992). In contrast, irregular and variable particle shapes, as seen in rock media, act to fill these voids, leading to higher bulk densities and promoting particle jamming (Stone et al., 2004). Therefore, one would expect spherical particles to be less stable, especially when composed of large particles, owing to relatively lower contact areas between particles and lower inter-particle friction (Brzinski et al., 2013; Hosoi and Goldman, 2015). Indeed, rock media had higher angles of stability and LBCs (Mueth et al., 1998; Stone et al., 2004; present study). The differences in properties of glass bead and rock media are not fully understood, as others have suggested that media composed of irregular and less spherical particles may actually have larger voids and be less prone to jamming (Cho et al., 2006). The qualitative difference between glass bead and rock media is also reflected in that resistive force theory makes poorer predictions of locomotion on irregularly shaped particles than on uniform spheres (Li et al., 2013; Zhang and Goldman, 2014).

Unlike some previous studies of granular medium properties, we did not account for the volume fraction (packing) of our substrates when measuring their physical properties. Instead, we used a

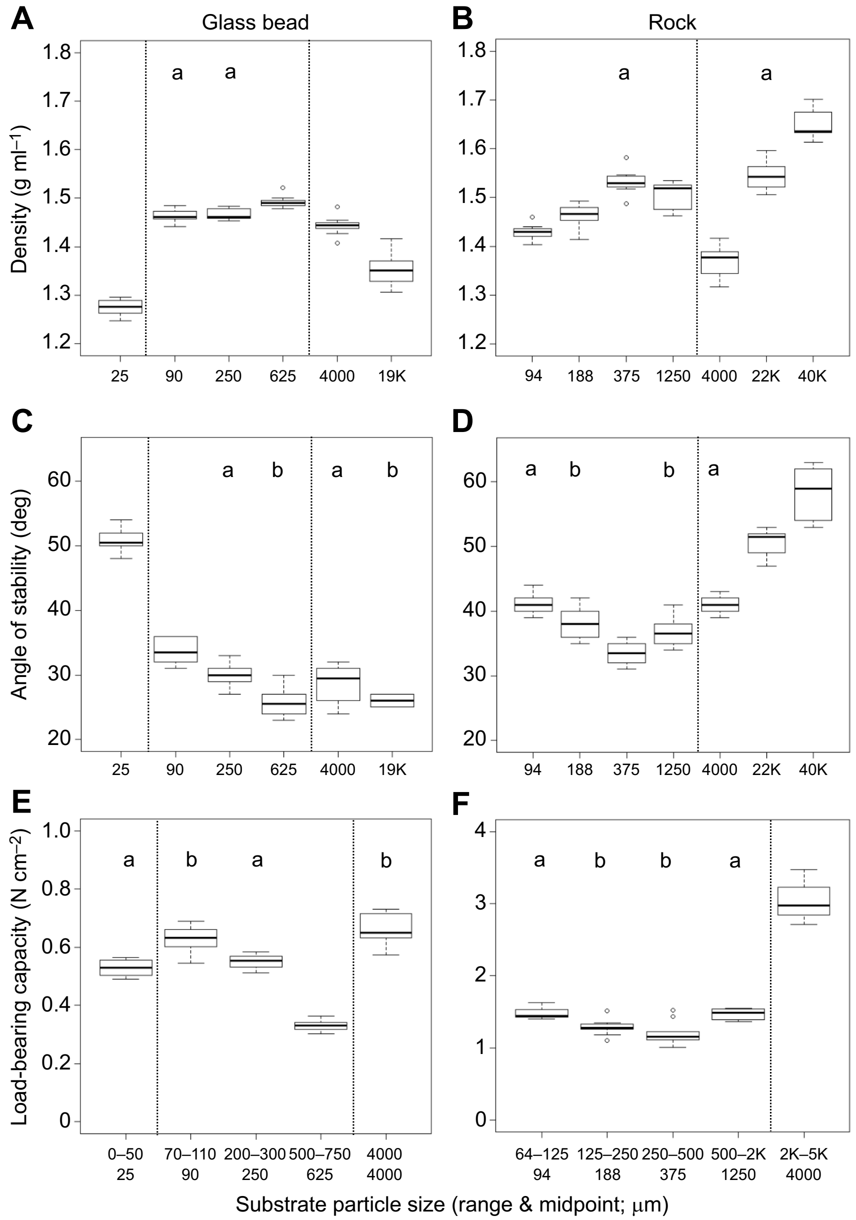

Fig. 2. Physical properties of experimental substrates. Box plots for bulk density (A,B), angle of stability (C,D) and load-bearing capacity (E,F) for glass bead (A,C,E) and rock (B,D,F) substrates. Different lowercase letters indicate which substrates within each plot are significantly different from one another. Box plots with no lowercase letter are significantly different from all other substrates. Vertical lines separate silt, sand and gravel substrates (none of the rock substrates was in the silt fraction). Significant *P*-values for pairwise *post hoc* tests by panel were: (A) <0.001–0.012; (B) <0.001–0.009; 375 µm versus 1250 µm, 0.041; (C) a versus b, <0.001–0.013; 25 µm versus all others, <0.001; (D–F) all differences, <0.001–0.003. *n*=10 replicates per treatment.

uniform method of filling containers with media, and so expected our media to be relatively densely packed, and our results are thus reproducible (Goldman and Umbanhowar, 2008; Vanel and Clément, 1999). Not controlling for volume fraction might increase the variance in our data for each medium, but this did not appear to have a major effect on our results because all documented patterns were very pronounced and highly significant (Fig. 2, Table 2). Furthermore, our data were consistent with those published by others, finding that LBCs for rock substrates were approximately two times those for glass beads (Stone et al., 2004), and that bulk density and angle of stability for ~300 µm glass beads were ~1.47 g ml^{-1} and 30 deg, respectively (Brzinski et al., 2013; Gravish and Goldman, 2014).

Generalists can be specialists, and specialists can be generalists

Because *E. arguta* are substrate generalists (Arnold and Ovenden, 2004), we expected their sprinting performance to be unaffected by substrate. However, we found that maximum velocity and acceleration were both significantly affected by particle size on both glass bead and rock substrates (Fig. 3), suggesting some level of specialization for coarse sand substrates. We ran the lizards on substrates ranging in particle size over four orders of magnitude, from silt and fine sand to rocks larger than the lizards' feet (Table 1). Whether a substrate is silt, sand or gravel may represent different challenges for running in similar sized animals. Particle sizes that include silt and sand result in a substrate that is even, but the particles move underfoot (Greenville and Dickman, 2009; Li et al., 2012). In contrast, particle sizes that include gravel, particularly our coarsest media, result in substrates that are highly uneven, but are less likely to move underfoot (Collins et al., 2013; Li et al., 2013). Coarse sand (1250 µm MPD), where *E. arguta* sprinted best, may represent an optimum between these two challenges, with particles that allow for an even surface, yet are heavy enough to limit their displacement when an animal runs over them. Although these intermediate particles can be displaced, the substrate often also acts as a solid because of jamming, even when small animals move on them (Aguilar and Goldman, 2015; Mazouchova et al., 2010).

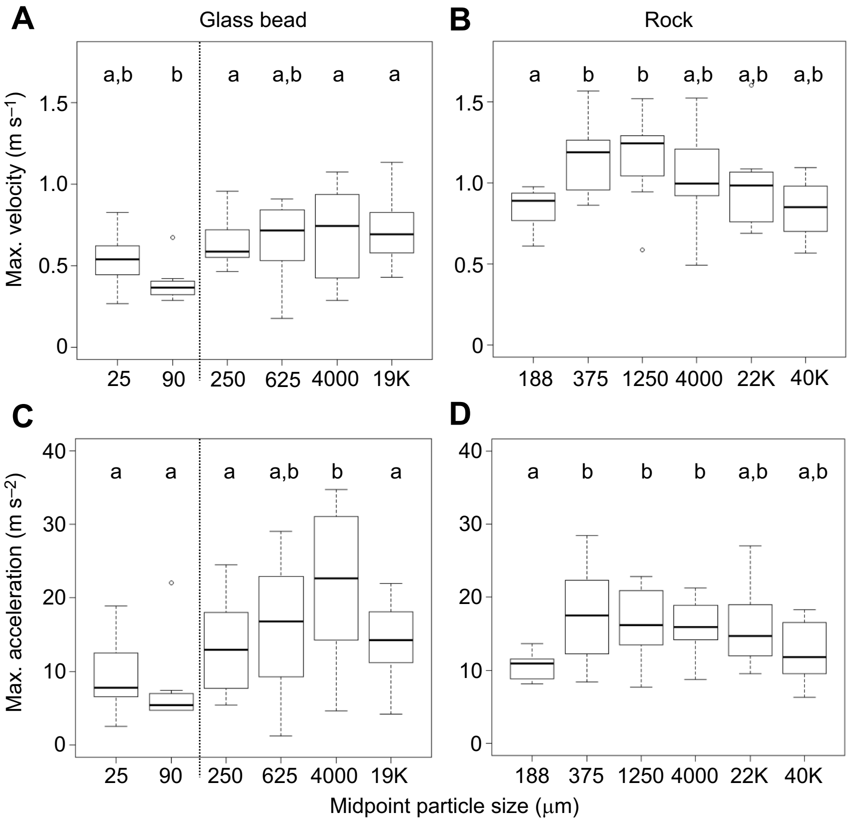

Fig. 3. Lizard running performance on different substrates. Box plots for maximum velocity (A,B) and acceleration (C,D) for *Eremias arguta* running on different glass bead (A,C) and rock (B,D) substrates. Different lowercase letters indicate which substrates within each plot are significantly different from one another. Substrates to the right of the vertical lines in plots for glass beads include the range of rock substrates sampled. Silt substrate was 25 μm, sand substrates were 90–1250 μm and gravel substrates were >2000 μm. Significant *P*-values for pairwise *post hoc* tests by panel were (a versus b in each case): (A) 0.029–0.030; (B) 0.041; (C) <0.001–0.027; (D) 0.023–0.050. *n*=8 animals per treatment.

Although most studies have not found differences in locomotor performance between loose sand and solid substrates (Bergmann and Irschick, 2006; Korff and McHenry, 2011; Li et al., 2012; Mazouchova et al., 2010), some have. The small agamid lizard *Phrynocephalus frontalis* also achieved the highest velocities on intermediate particle size substrates (500–1000 μm; Li et al., 2011). For *P. frontalis*, this substrate represented that which the animals most frequently exploited in nature (Li et al., 2011). *Eremias arguta*

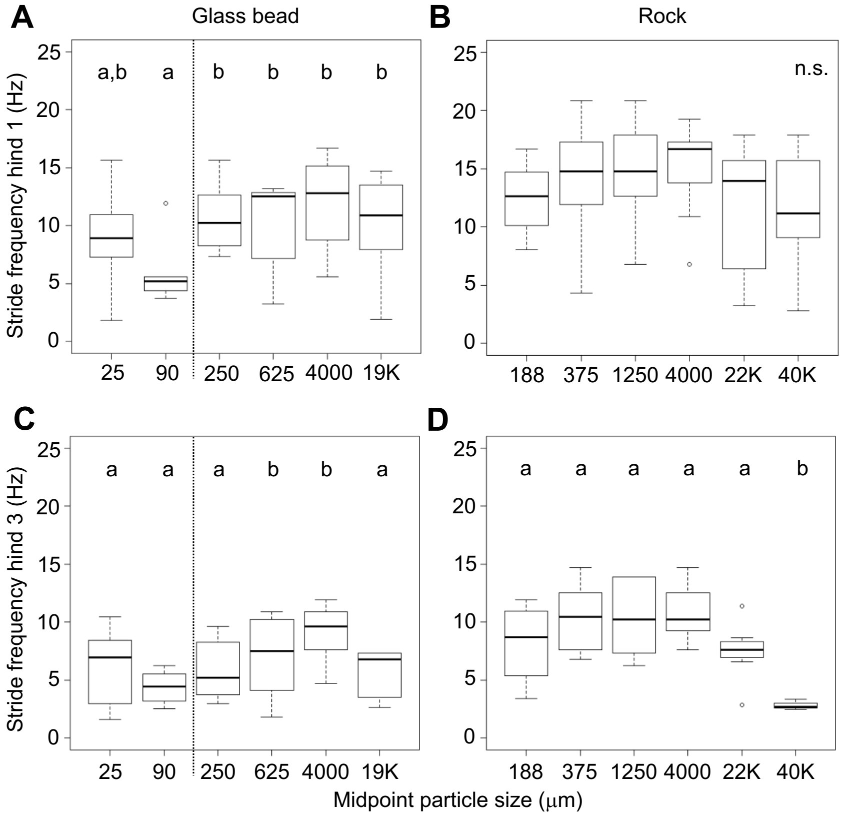

Fig. 4. Lizard running performance on different substrates. Box plots for frequency of hind limb stride 1 (A,B) and stride 3 (C,D) for *Eremias arguta* running on different glass bead (A,C) and rock (B,D) substrates. Different lowercase letters indicate which substrates within each plot are significantly different from one another. Substrates to the right of the vertical lines in plots for glass beads include the range of rock substrates sampled. Silt substrate was 25 μm, sand substrates were 90–1250 μm and gravel substrates were >2000 μm. Significant *P*-values for pairwise *post hoc* tests by panel were (a versus b in each case): (A) 0.002–0.025; (B) not significant; (C) <0.001–0.009; (D) <0.001. *n*=8 animals per treatment.

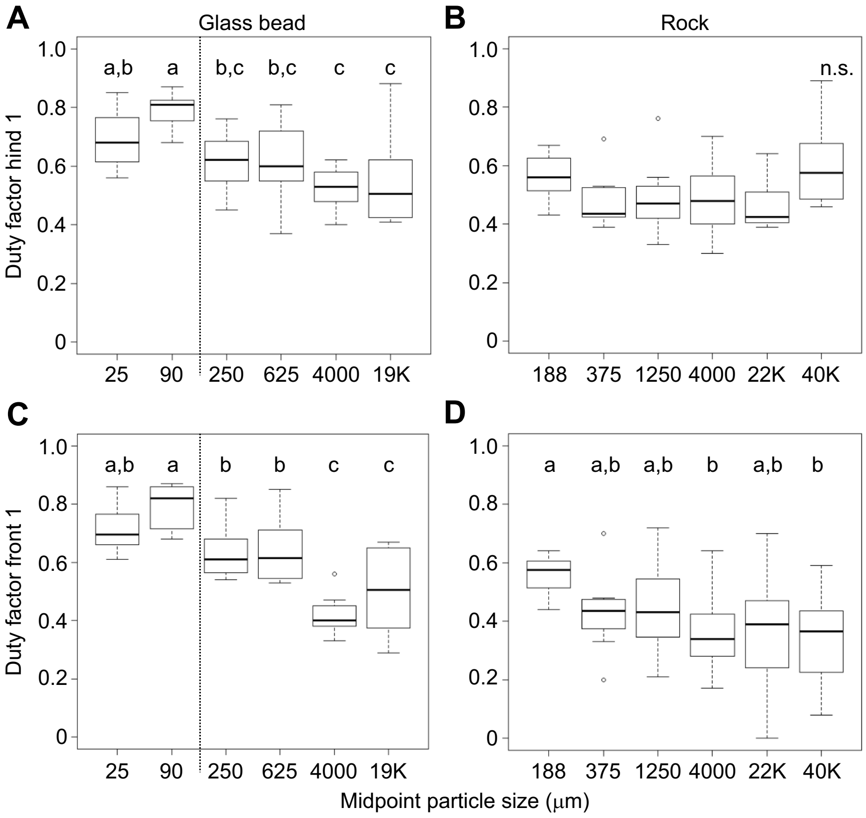

Fig. 5. Duty factors for running on different substrates. Box plots for duty factor for hind limb stride 1 (A,B) and front limb stride 1 (C,D) for *Eremias arguta* running on different glass bead (A,C) and rock (B,D) substrates. Different lowercase letters indicate which substrates within each plot are significantly different from one another. Substrates to the right of the vertical lines in plots for glass beads include the range of rock substrates sampled. Silt substrate was 25 μm, sand substrates were 90–1250 μm and gravel substrates were >2000 μm. Significant *P*-values for pairwise *post hoc* tests by panel were: (A) a versus b, 0.006; a versus c, <0.001, b versus c, 0.009–0.028; (B) not significant; (C) a versus b, 0.003–0.005; a versus c, <0.001; b versus c, <0.001–0.014; (D) a versus b, 0.004. *n*=8 animals per treatment.

inhabit a wide range of sparsely to moderately vegetated sandy habitats (Arnold and Ovenden, 2004), and it is possible that substrates they use in nature match those on which they performed maximally in the laboratory. This may be further supported by the observations that very fine sand can inhibit the growth of vegetation (Attum et al., 2007; Greenville and Dickman, 2009), and that *E. arguta* exploit vegetation as refuges from predators (Arnold and Ovenden, 2004).

Our findings bring to question what a habitat generalist or specialist is and why. We argued that *E. arguta* are substrate generalists, lacking conspicuous adaptations for running on sand and inhabiting a range of habitats, yet we found that their locomotor performance was affected by substrate. This may, at first, suggest that they actually are substrate specialists, but a more nuanced exploration of this issue is informed by studies of the North American sand lizards *Callisaurus draconoides* and *Uma scoparia*. Both of these species are sprinters, but while *C. draconoides* is a relative generalist, inhabiting habitats from sand to dry gravel creek beds and lacking adaptations for a specific substrate, *U. scoparia* is a sand dune specialist and has evolved fringes on its toes that are thought to enhance sprint performance on fine sand (Brennan and Holycross, 2006; Irschick and Jayne, 1998; Korff and McHenry, 2011). Although one study found that amputation of toe fringes in *U. scoparia* decreased sprint velocity on sand but not on a solid substrate (Carothers, 1986), several others have found that the sprint speed does not differ between these two species or between substrates (Bergmann and Irschick, 2010; Korff and McHenry, 2011; Li et al., 2012). In contrast, Qian et al. (2015) found that as substrate resistance decreased, *C. draconoides* suffered no loss in performance, while *U. scoparia* did. This supports a generalist categorization for *C. draconoides*, but is the opposite result from that expected for *U. scoparia*, being a fine sand specialist. Interestingly, species along a generalist–specialist spectrum are all subject to a universal model that relates sprint velocity to leg penetration depth, the latter being subject to the weight of the lizard, the area of its feet and the resistance to penetration of the substrate (Qian et al., 2015).

Paradoxically, *E. arguta* is considered a substrate generalist, but our data suggest it is better at running on some substrates than others, while *U. scoparia* is considered a substrate specialist, but its sprint performance may not be affected by substrate. These findings relate to the underlying reasons for these lizards generalizing or specializing on certain substrates. It is possible that *E. arguta* does run on a range of substrates, as exemplified by similar velocities on many of the intermediate particle sizes that we used (Fig. 3), but that it does not encounter the most extreme substrates that we considered in nature. Behaviorally, *U. scoparia* is a substrate specialist (Brennan and Holycross, 2006; Korff and McHenry, 2011; P.J.B., personal observation), but this may not be for the reason of maximizing sprinting performance (this is strongly supported by the findings of Qian et al., 2015). It may exploit fine sand habitats and use its toe fringes for burial to escape predators and extreme heat instead (Arnold, 1995; Attum et al., 2007).

Why are phenotype–function relationships often weak?

Many studies that have shown that relationships between phenotype and function are surprisingly weak have focused on the complexity of those relationships as an explanation (Alfaro et al., 2005; Bergmann and McElroy, 2014; Holzman et al., 2011). For example, phenomena such as functional redundancy, trade-offs and facilitation are ubiquitous when multiple phenotypic traits carry out multiple functions. Their ubiquity makes the existence of straightforward, direct relationships between phenotype and function the exception rather than the rule (Wainwright et al., 2005).

However, even when phenotype–function relationships are direct, they can be obscured by the diversity of ecological contexts that an animal finds itself in. It is now understood that environmental factors can affect patterns of genetic (co)variation (Cano et al., 2004), and the timing and rate of developmental processes (Parsons et al., 2016; Touchon and Warkentin, 2010). The same can be expected and has been shown for phenotype–function relationships, which can differ by ecological situation. Therefore, the phenotypic traits that are important in determining locomotor performance can differ from one substrate to another, and so natural selection will act on different traits, depending on the substrate on which they are used (Irschick and Losos, 1999; Sathe and Husak, 2015).

Here, we found that in *E. arguta*, both locomotor performance and kinematics were affected by substrate, and this might obscure phenotype–function relationships. We also found that sprint velocity on different substrates was modulated through changes in stride frequency, but not stride length or angles of limb protraction and retraction. Therefore, we might expect stride frequency to have weak relationships with phenotypic traits in general, but that stride length would have more consistent and stronger relationships with the phenotype. Indeed, a general finding of studies of locomotion is that limb length is a strong predictor of stride length (Bonine and Garland, 1999; Irschick and Jayne, 1999; Losos, 1990). Hence, an important implication of our work is that locomotor variables will differ in how they are affected by ecological context and that we can predict which ones will be involved in stronger phenotype–function relationships.

We also found that duty factor increased on finer particle substrates (Fig. 5), and this may be because lighter particles are more prone to displacement (Mehta and Barker, 1994), as exemplified by lower LBC values for sand compared with gravel substrates (Fig. 2). If the substrate moves underfoot, then one would expect that the foot would be in contact with the substrate for a longer proportion of the stride cycle. In such a case, it may be that under challenging locomotor situations, animals increase duty factor to allow for more time to generate the propulsive force needed for locomotion. The evidence for larger duty factors on granular media relative to solid substrates is weak, but some lizards appear to modulate velocity through changes in both stride length and stride frequency on these media, often with lower stride lengths (Li et al., 2012; Qian et al., 2015; Renous et al., 2008). However, these studies compared a single granular substrate with a solid one, as opposed to a range of granular substrates. Running up inclines is another challenging locomotor task, and a diversity of animals often deal with it by increasing duty factor and decreasing stride length in response (reviewed by Birn-Jeffery and Higham, 2014). Clearly, there are many kinematic or behavioral strategies for coping with various locomotor challenges, and this is also likely to obscure phenotype–function relationships. Further detailed studies that take a systematic approach to manipulating the substrate are needed for a better understanding of how ecological context affects phenotype–function relationships.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
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