Microgeographic variability in locomotor traits among lizards in a human-built environment

Colin Donihue

Microgeographic variability in fitness-relevant traits may be more common than previously appreciated. The fitness of many vertebrates is directly related to their locomotor capacity, a whole-organism trait integrating behavior, morphology, and physiology. Because locomotion is inextricably related to context, I hypothesized that it might vary with habitat structure in a wide-ranging Greek lizard, *Podarcis erhardii*. I compared lizard populations living on human-built rock walls, a novel habitat with hard vertical structure, with nearby populations naive to human-built infrastructure that live in flat, loose-substrate habitat. I tested for differences in morphology, behavior, and performance. Lizards from built sites were larger and had significantly (and proportionately) longer arms and legs. The differences in leg morphology were especially pronounced for distal components, the foot and longest toe. These morphologies facilitated a significant behavioral shift to jumping across the rocky experimental substrate. I found no difference in maximum velocity between these populations, however females originating from wall sites potentially accelerated faster over the rocky experimental substrate. The variability between these closely neighboring populations suggests that the lizards inhabiting walls have experienced a suite of trait changes enabling them to take advantage of the novel habitat structure created by humans.

Microgeographic variability in locomotor traits among lizards in a human-built environment

Colin M. Donihue[1]*

[1] Yale University, School of Forestry and Environmental Studies, New Haven CT, USA
*Correspondence: colin.donihue@yale.edu

Abstract:

Microgeographic variability in fitness-relevant traits may be more common than previously appreciated. The fitness of many vertebrates is directly related to their locomotor capacity, a whole-organism trait integrating behavior, morphology, and physiology. Because locomotion is inextricably related to context, I hypothesized that it might vary with habitat structure in a wide-ranging Greek lizard, *Podarcis erhardii*. I compared lizard populations living on human-built rock walls, a novel habitat with hard vertical structure, with nearby populations naive to human-built infrastructure that live in flat, loose-substrate habitat. I tested for differences in morphology, behavior, and performance. Lizards from built sites were larger and had significantly (and proportionately) longer arms and legs. The differences in leg morphology were especially pronounced for distal components, the foot and longest toe. These morphologies facilitated a significant behavioral shift to jumping across the rocky experimental substrate. I found no difference in maximum velocity between these populations, however females originating from wall sites potentially accelerated faster over the rocky experimental substrate. The variability between these closely neighboring populations suggests that the lizards inhabiting walls have experienced a suite of trait changes enabling them to take advantage of the novel habitat structure created by humans.

Keywords:

locomotion, morphometrics, context-dependence, local adaptation, *Podarcis erhardii*

Introduction:

Locomotor performance integrates a suite of morphological, behavioral, and physiological attributes and impacts an individual's fitness (Irschick and Garland 2001, Irschick et al. 2008). Furthermore, locomotor mode and performance is of necessity closely tied to and individual's immediate ecological setting. Other studies have demonstrated that habitat substrate and structure are consistently related to a lizard species' behavior, morphology, and/or performance (Vanhooydonck and Van Damme 2003, Losos 2011). Emerging evidence suggests however that microgeographic variability in ecological context can result in more intraspecific variability in traits and fitness than previously appreciated (Richardson et al. 2014).

Tests of lizard locomotor performance typically employ a single experimental substrate. Moreover, the types of substrates used may (*e.g.* sand) or may not (*e.g.* cork or sandpaper) reflect naturally occurring substrates that have given rise to different adaptations for locomotion. Comparing lizard locomotion across multiple substrates is increasingly the focus of new studies (Tulli et al. 2012, Vanhooydonck et al. 2015), but studies have yet to investigate performance of individuals from populations living in different habitats.

Humans are ecosystem engineers, shaping habitat structure across landscapes (Jones et al. 1994). In the Greek islands, stone walls and terraces crisscross the landscape, and the eponymous Aegean Wall Lizard, *Podarcis erhardii,* can readily be found throughout (Valakos et al. 2008). However, *P. erhardii*, can also commonly be found in nearby wall-less habitats with sand or loose-soil substrates. Based on other research showing that lizards may change their traits to accommodate new demands for locomotor performance (e.g. Losos 2011), I hypothesized that human alteration of the landscape should affect morphological traits associated with locomotion as well as performance itself. I tested for inter-population differences in maximum velocity and acceleration over sandy and rocky experimental substrates, and informing differences in behavior and relative limb shape, between lizards living on loose, flat substrates, or rocky, vertical structures. The research provides insight into how human alteration of the environment causes species to respond on the Greek island of Naxos.

Methods:

I collected 324 *P. erhardii* from 10 sites within 15 km of each other on Naxos. Five sites had built stone walls, the other five were characterized by sandy substrate with interspersed *Juniperus oxycedrus* shrubs or a loose jumble of soil and Mediterranean phrygana (Fig. 1). All sites were selected for having a high density of lizards, and wall-less sites for being more than 200 m from the nearest built stone structure. For all lizards, I recorded sex and measured snout-to-vent length (SVL), and the length of each segment of the right fore and hind limb (Fig. 1, Table 1) using digital calipers (Frankford Arsenal 672060).

I constructed two tracks for assessing lizard locomotion using heavy-duty plastic sheeting. Each track was 50 cm wide and 2 m long. One track had a sandy substrate (5 cm depth) reflecting the homefield of the five no-wall lizard populations, and the other was paved with large flagstones (averaging approximately 20 cm in diameter) from nearby walls. These flagstones did not move for all trials and were placed so each abutted the next, mimicking the position and spacing of stones on top of rock walls and preventing escape of the lizard under rocks during the sprint trial. Before each trial, all lizards were allowed to thermoregulate at will for at least 30 minutes along a temperature gradient radiating from a suspended lamb (sand temperature 45C to 25C). Immediately before running the lizard, I recorded their temperature using a cloacal thermometer (Miller and Webber T6000). The sprinting temperatures selected by males and females between wall and no-wall sites did not significantly differ (Males: $\chi^2_{(1, N=172)}=1.6895$, $p=0.1937$; Females: $\chi^2_{(1, N=145)}=0.2531$, $p=0.6149$). Lizards were stationary in the same position at the start of each trial, and the entirety of each sprint was recorded with a video camera (Sony HDRPJ260V; 1920 x 1080 px; 50 FPS) suspended directly over the track using a ~~large~~ tripod. The camera~~'s~~ field of view encompassed the first 1.5 m of track and had a full dorsal perspective of the running lizard. I calculated the position of the lizard frame-by-frame, scaled to mm relative to a tape measure in the field of view, using a custom-built JavaScript program (code: https://github.com/bkazez/savra). ~~In order~~ to calculate velocity and acceleration, I fit a quintic spline to the position data (Walker 1998) with the SPAPI function in MatLab (MathWorks Inc., 2014). Finally, I watched each stone-substrate trial and counted the number of times the lizards jumped (body and all limbs simultaneously in the air) from rock to rock. The Yale IACUC office approved all experiments involving animals (permit: 2013-11548). All work was conducted with permission from the Greek Ministry of Environment, Energy, and Climate Change (Permit 11665/1669).

Statistical analyses

To test for differences in morphology between populations I used linear mixed effects models, evaluated using the lme command within the nlme (v3.1-121; 2015) package in R (v3.1.2; 2014). Each morphometric was treated as a response variable with presence or absence of wall as fixed effects and with site of origin as a random effect. I tested for relative morphological differences by adding SVL as a covariate of the wall/no wall model. To test for differences in performance response variables – maximum velocity and acceleration over each substrate, and number of jumps in the rocky experimental track – I again used wall presence or absence as a fixed effect and site identity as a random effect with sprint temperature as an additional random effect. Count variables (such as number of jumps in this analysis) are often non-normal, however a Shapiro-Wilk normality test found that jump counts in this dataset are normally distributed (W=0.9435, P<0.0001) enabling analysis using LME models. When these performance analyses were repeated to control for differences in body size, SVL was added as a covariate. Whenever body size or temperature was used in a model, they were standardized to have a mean of zero so as to make the estimates of each response variable directly interpretable (standardized value = initial value – global mean value). In all cases, males and females were analyzed independently to reduce interactions in the models. Finally, I used a type II ANOVA (CAR package, v2.0-25) to calculate Wald chi-square values for the model fixed effects and assign *p*-values appropriate for the unbalanced design (Langsrud 2003).

Results:

Lizards, both males and females, from wall sites had larger SVLs than lizards at no-wall sites (Males: $\chi^2_{(1, N=175)}=10.13$, $p=0.0015$; Females: $\chi^2_{(1, N=149)}=4.74$, $p=0.0294$; Fig. 1; Table 1). This pattern was consistent across both sexes for multiple limb measurements (Fig. 1; Table 1). In particular, the distal portions of the hindlimbs – the length between the ankle joint and the tip of the longest toe, and the longest toe itself – were significantly longer among wall populations (ankle to tip: Males: $\chi^2_{(1, N=175)}=14.77$, $p=0.0001$; Females: $\chi^2_{(1, N=149)}=23.87$, $p<0.0001$; longest toe: Males: $\chi^2_{(1, N=175)}=27.85$, $p<0.0001$; Females: $\chi^2_{(1, N=149)}=34.28$, $p<0.0001$; Fig. 1; Table 1). Furthermore, the relative length of these limb segments, standardized by SVL, was larger for lizards from wall than no-wall sites (ankle to tip of toe: Males: $\chi^2_{(1, N=175)}=5.05$, $p=0.025$; Females: $\chi^2_{(1, N=149)}=7.64$, $p=0006$; longest toe: Males: $\chi^2_{(1, N=175)}=5.60$, $p=0.018$; Females: $\chi^2_{(1, N=149)}=19.68$, $p<0.0001$; Table 1). All together, lizards living on walls had proportionally longer hind limbs than lizards in no-wall habitats (Males: $\chi^2_{(1, N=175)}=9.69$, $p=0.0019$; Females: $\chi^2_{(1, N=149)}=15.17$, $p<0.0001$; Fig. 2a).

I found no difference in maximum velocity among lizards from either habitat of origin across either experimental substrate (maximum velocity on rock: Males: $\chi^2_{(1, N=171)}=0.79$, $p=0.37$; Females: $\chi^2_{(1, N=143)}=0.91$, $p=0.34$; maximum velocity on sand: Males: $\chi^2_{(1, N=171)}=0.72$, $p=0.396$; Females: $\chi^2_{(1, N=143)}=0.786$, $p=0.375$). While I found no difference in either population's acceleration capacity over sand (Males: $\chi^2_{(1, N=165)}=0.678$, $p=0.41$; Females: $\chi^2_{(1, N=141)}<0.001$, $p=0.98$), I found that females originating from wall sites accelerated over the rocky experimental substrate faster than lizards from no-wall populations (corrected for SVL: $\chi^2_{(1, N=143)}=5.84$, $p=0.016$; Fig 2b). Both males and females from wall populations exhibited a strong behavioral shift: the lizards accustomed to walls consistently traversed the rocky experimental substrate by jumping rock-to-rock. No-wall lizards jumped significantly fewer times crossing the same experimental track (Males: $\chi^2_{(1, N=172)}=6.08$, $p=0.0137$; Females: $\chi^2_{(1, N=144)}=3.648$, $p=0.056$; adjusted for SVL: Males: $\chi^2_{(1, N=172)}=6.317$, $p=0.012$; Females: $\chi^2_{(1, N=144)}=4.078$, $p=0.043$; Fig. 2c).

Discussion:

I found consistent differences between close-proximity populations of *P. erhardii* inhabiting different habitat-structure contexts. Lizards originating on sites with walls were larger than lizards from no-wall sites. Furthermore, the absolute length of each component of the hind limbs, and the relative length of the leg as a whole was proportionally larger among wall populations of both sexes (Fig. 1a). The difference in relative leg length was driven by proportional differences in the foot of wall-inhabiting lizards (Table 1).

Morphological differences between lizard populations sometimes result in local, habitat-specific performance advantages (*e.g.* limb length determining motility across branches of different diameters in *Anolis*; Losos 2011). Long limbs in Lacertids often correspond to fast sprints over loose substrates (Bauwens et al. 1995, Bonine and Garland Jr. 1999), however, I found the opposite trend in limb length according to habitat substrate, and no inter-population differences in sprinting ability across sand. Alternatively, long hind limbs are also associated with jumping ability (Toro et al. 2004). Indeed, ~~I found that~~ lizards from wall sites (with longest limbs) jump 1.5 times more often than non-wall populations on the same experimental track (Fig. 2c). ~~I did not detect a difference in the~~ maximum sprint velocity ~~of either~~ population ~~across either~~ substrate. ~~I did find that the~~ females from rock wall populations accelerated more quickly than those from the no-wall habitats over the rocky experimental substrate (Fig. 2b).

Other authors have demonstrated that slow video frame rates are prone to considerable error in estimating acceleration of fast-moving animals (Walker 1998). A 50 Hz camera was the maximum speed available for this field study, and, although my calculated values (Table 2) are commensurate with published values for closely related species (Vanhooydonck et al. 2015), further work with high-speed cameras (exceeding 250 Hz) is necessary to conclusively test the locomotion implications of these observed morphological differences.

Few studies investigate relative lizard locomotion capacity over multiple experimental substrates (Vanhooydonck et al. 2015). Studies that have, found little advantage in performance among species racing on an experimental substrate similar to their characteristic natural habitat (Tulli et al. 2012, Vanhooydonck et al. 2015). This study suggests one potential explanation: the inter-population performance differences observed in this study are commensurate with some published inter-specific comparisons (Tulli et al. 2012, Vanhooydonck et al. 2015), meaning that variability among source populations could change the interpretation of these comparisons. Inter-population context-dependence in locomotion morphometrics have been demonstrated between physically isolated populations (*e.g.* island vs mainland; Van Damme et al. 1998), and populations inhabiting dramatically different natural ecological contexts (*e.g.* Des Roches et al. 2014). The differences related here, particularly in morphology and jumping behavior, over such small spatial scales are noteworthy, and demonstrate the significant potential effect of anthropogenic habitat alteration within a species (Donihue and Lambert 2014).

Competing Interests:

I have no competing interests.

Acknowledgements:

Thanks go to P. Pafilis and J. Foufopoulos for logistical aid in-country; K. Culhane, Z. Miller, and A. Mossman for help in the field; B. Kazez and B. Redding for video analysis assistance; and A. Herrel, M. Lambert, O. Schmitz, and D. Skelly for manuscript comments.

Funding:

Funding provided by National Geographic Waitt Foundation and the Yale Institute for Biospheric Studies.

LITERATURE CITED

Bauwens D, Garland Jr. T, Castilla AM, van Damme R. 1995 Evolution of sprint speed in lacertid lizards: morphological, physiological and behavioral covariation. *Evolution* **49**, 848–863.

Bonine KE, Garland Jr. T. 1999 Sprint performance of phrynosomatid lizards, measured on a high-speed treadmill correlates with hindlimb length. *Journal of Zoology* **248**, 255–265. (doi: 10.1111/j.1469-7998.1999.tb01201.x)

Des Roches S, Torresdal J, Morgan TW, Harmon LJ, Rosenblum EB. 2014 Beyond black and white: divergent behavior and performance in three rapidly evolving lizard species at White Sands. *Biological Journal of the Linnaean Society* **111**, 169–182. (doi: 10.1111/bij.12165)

Donihue CM, Lambert MR. 2014 Adaptive evolution in urban ecosystems. *AMBIO* **44**, 194–203. (doi: 10.1007/s13280-014-0547-2)

Irschick DJ, Garland TJ. 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Annual Review of Ecology, Evolution, and Systematics* 32: 367–396.

Irschick DJ, Meyers JJ, Husak JF, Le Galliard J. 2008 How does selection operate on whole-organism functional performance capacities? A review and synthesis. *Evolutionary Ecology Research* **10**, 177–196.

Jones CG, Lawton JH, Shachak M. 1994. Organisms as ecosystem engineers. *Oikos* **69**, 373–386.

Langsrud, Øyvind. 2003 ANOVA for unbalanced data: Use Type II instead of Type III sums of squares. *Statistics and Computing* **13**, 163–167.

Losos JB. 2011 *Lizards in an evolutionary tree. Ecology and adaptive radiation of Anoles*. Berkeley, CA: University of California Press.

Richardson JL, Urban MC, Bolnick DI, Skelly DK. 2014 Microgeographic adaptation and the spatial scale of evolution. *Trends in Ecology and Evolution* **29**, 165–176. (doi: doi:10.1016/j.tree.2014.01.002)

Toro E, Herrel A, Irschick D. 2004 The evolution of jumping performance in Caribbean Anolis lizards: solutions to biomechanical trade-offs. *American Naturalist* **163**, 844–856.

Tulli MJ, Abdala V, Cruz FB. 2012 Effects of different substrates on the sprint performance of lizards. *Journal of Experimental Biology* **215**, 774–784. (doi: 10.1242/jeb.065490)

Valakos E, Pafilis P, Sotiropoulos K, Lymberakis P, Maragou P, Foufopoulos J. 2008 *The Amphibians and Reptiles of Greece*. Frankfurt Germany: Chimaira.

Van Damme R, Aerts P, Vanhooydonck B. 1998 Variation in morphology, gait characteristics and speed of locomotion in two populations of lizards. *Biological Journal of the Linnaean Society* **63**, 409–427. (doi: 10.1111/j.1095-8312.1998.tb01526.x)

Van Damme R, Vanhooydonck B. 2001 Origins of interspecific variation in lizard sprint capacity. *Functional Ecology* **15**, 186–202.

Vanhooydonck B, Measey J, Edwards S, Makhubo B, Tolley KA, Herrel A. 2015 The effects of substratum on locomotor performance in lacertid lizards. *Biological Journal of the Linnaean Society* (doi: 10.1111/bij.12542)

Vanhooydonck B, Van Damme R. 2003 Relationships between locomotor performance, microhabitat use and antipredator behavior in lacertid lizards. *Functional Ecology* **17**, 160–169. (doi: 10.1046/j.1365-2435.2003.00716.x)

Walker JA. 1998 Estimating velocities and accelerations of animal locomotion: a simulation experiment comparing numerical differentiation algorithms. *Journal of Experimental Biology* **201**, 981–995.

Figure 1: The island of Naxos in the Greek Cyclades and representative pictures of the sites with and without walls. I found significant differences in the body size (SVL) and leg morphology of males (bold blue) and females (light red) from wall (top) and no-wall (bottom) sites. Mean and standard error are presented for each measurement along with the p-value of the size-corrected LME model (see Table 1).

Figure 2: Lizards from wall sites had proportionally longer hindlimbs, relative to SVL (a). These differences in hindlimbs corresponded to significantly faster accelerations over the rocky substrate (b), and to increased jumping propensity (c). All comparisons with (*) are significant $p<0.05$.

Table 1: Results of the linear mixed effects models of morphological measurements and performance metrics. A (*) denotes significance at the $p<0.05$ level.

Table 2: The average and standard deviation of the calculated velocity and acceleration of male and female lizards from wall and no-wall populations on Naxos.

Figure 1

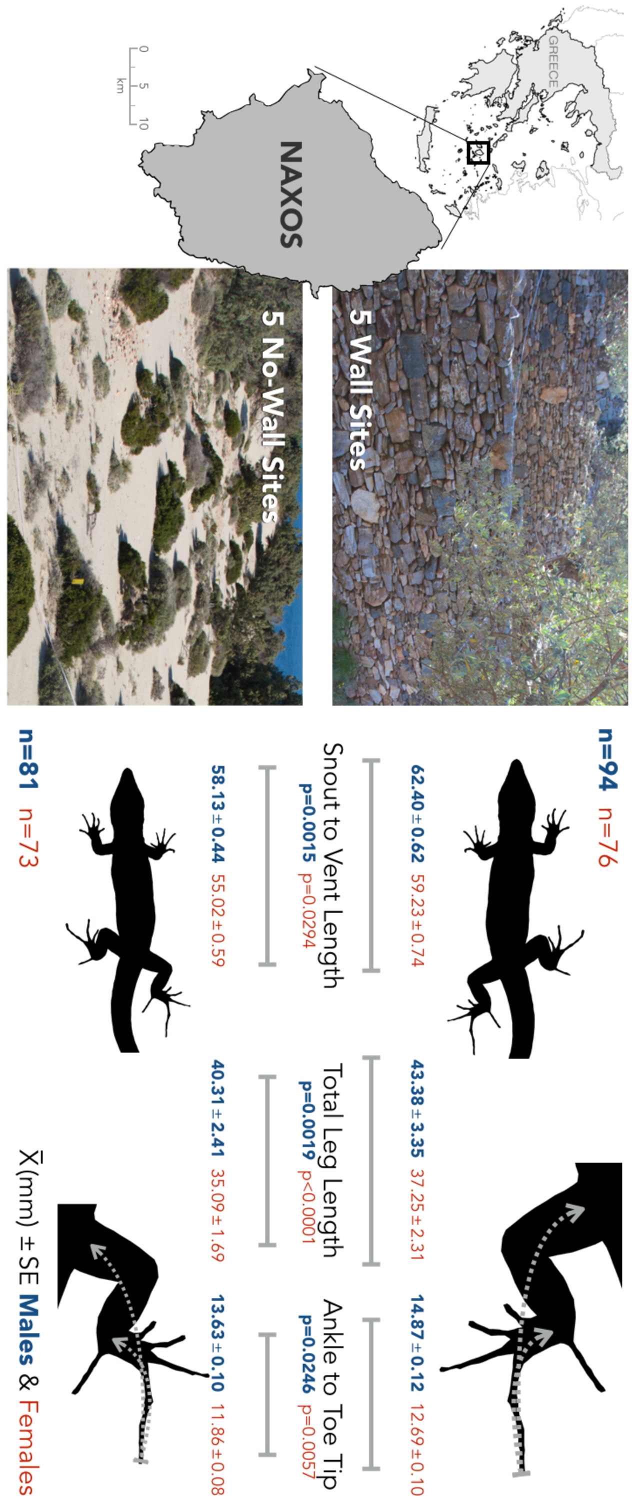
GREECE
NAXOS
0 5 10
km
5 Wall Sites
5 No-Wall Sites
n=94 n=76
n=81 n=73
Snout to Vent Length
62.40±0.62 59.23±0.74
p=0.0015 p=0.0294
58.13±0.44 55.02±0.59
Total Leg Length
43.38±3.35 37.25±2.31
p=0.0019 p<0.0001
40.31±2.41 35.09±1.69
Ankle to Toe Tip
14.87±0.12 12.69±0.10
p=0.0246 p=0.0057
13.63±0.10 11.86±0.08
X̄(mm) ± SE Males & Females

Figure 2

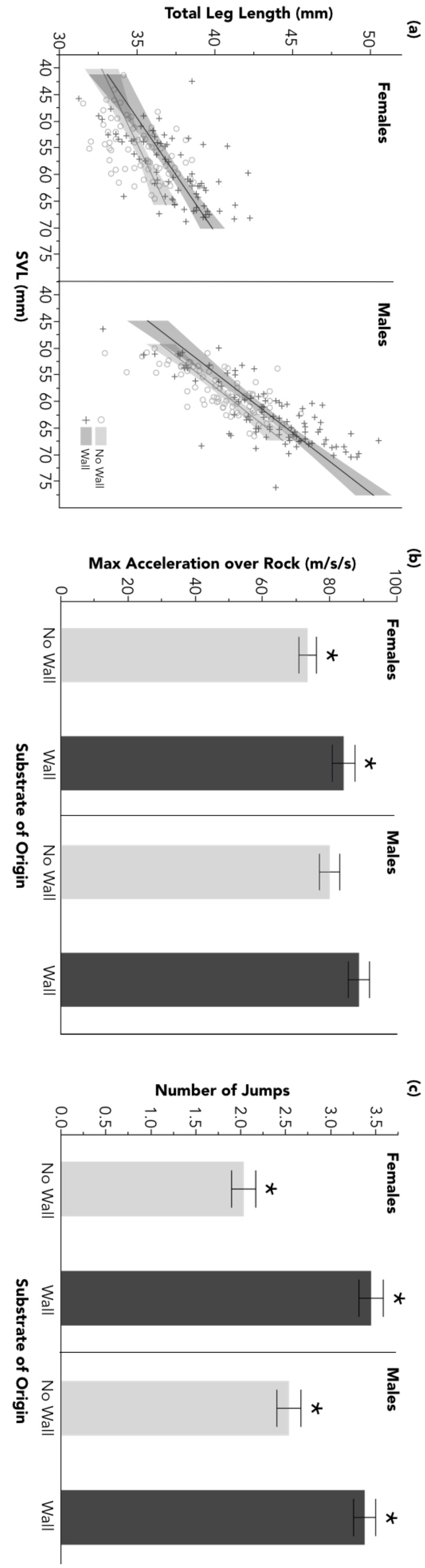
(a)
Total Leg Length (mm)
Females
Males
SVL (mm)
No Wall
Wall
(b)
Max Acceleration over Rock (m/s/s)
Females
Males
No Wall
Wall
No Wall
Wall
Substrate of Origin
(c)
Number of Jumps
Females
Males
No Wall
Wall
No Wall
Wall
Substrate of Origin

Table 1

Model:	~ Wall | Site								~ Wall + SVL | Site							
	Males				Females				Males				Females			
Morphometric:	N	X^2	DF	*p*	N	X^2	DF	*p*	N	X^2	DF	*p*	N	X^2	DF	*p*
SVL	175	10.133	1	0.0015 *	149	4.745	1	0.0294 *								
Shoulder to elbow	175	2.414	1	0.1203	149	3.059	1	0.0803	175	0.002	1	0.9625	149	1.129	1	0.2881
Elbow to wrist	175	4.341	1	0.0372 *	149	3.897	1	0.0484 *	175	0.097	1	0.7558	149	2.036	1	0.1536
Wrist to tip of finger	175	2.710	1	0.0997	149	5.930	1	0.0149 *	175	0.005	1	0.9452	149	1.923	1	0.1655
Longest finger	175	4.742	1	0.0294 *	149	2.507	1	0.1134	175	0.210	1	0.6470	149	1.116	1	0.2909
Total arm length	175	9.888	1	0.0017 *	149	7.511	1	0.0061 *	175	0.000	1	0.9997	149	3.693	1	0.0546
Hip to knee	175	5.588	1	0.0181 *	149	2.485	1	0.1149	175	0.625	1	0.4294	149	1.125	1	0.2888
Knee to ankle	175	4.813	1	0.0283 *	149	7.985	1	0.0047 *	175	0.047	1	0.8289	149	3.330	1	0.0680
Ankle to tip of toe	175	14.765	1	0.0001 *	149	23.869	1	< 0.0001 *	175	5.053	1	0.0246 *	149	7.639	1	0.0057 *
Longest toe	175	27.850	1	< 0.0001 *	149	34.283	1	< 0.0001 *	175	5.605	1	0.0179 *	149	19.683	1	< 0.0001 *
Total leg length	175	27.440	1	< 0.0001 *	149	23.925	1	< 0.0001 *	175	9.688	1	0.0019 *	149	15.170	1	< 0.0001 *

Model:	~ Wall | Site + SprintTemp								~ Wall + SVL | Site + SprintTemp							
	Males				Females				Males				Females			
Performance metric:	N	X^2	DF	*p*	N	X^2	DF	*p*	N	X^2	DF	*p*	N	X^2	DF	*p*
Max velocity rock	171	0.794	1	0.3730	143	0.909	1	0.3404	171	0.772	1	0.3798	143	2.978	1	0.0844
Max acceleration rock	170	1.098	1	0.2946	143	4.872	1	0.0273 *	170	1.464	1	0.2263	143	5.841	1	0.0157 *
Max velocity sand	166	0.722	1	0.3956	142	0.786	1	0.3754	166	0.390	1	0.5325	142	1.110	1	0.2921
Max acceleration sand	165	0.678	1	0.4104	141	0.000	1	0.9857	165	0.583	1	0.4452	141	0.005	1	0.9420
Jumps	172	6.081	1	0.0137 *	145	3.648	1	0.0561	172	6.317	1	0.0120 *	145	4.078	1	0.0435 *

257 **Table 2**

	Males				Females			
	Wall		No Wall		Wall		No Wall	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Velocity Rock (m/s)	1.99	0.45	1.91	0.47	1.83	0.44	1.76	0.31
Acceleration Rock (m/s/s)	88.57	29.87	79.84	26.89	84.05	28.67	73.32	21.78
Velocity Sand (m/s)	1.98	0.60	1.82	0.42	1.73	0.47	1.67	0.34
Acceleration Sand (m/s/s)	87.88	38.68	81.00	30.60	77.82	29.49	77.73	35.55