

These are electronic supplementary appendices to: Revell, L. J., M. A. Johnson, J. A. Schulte, II, J. J. Kolbe, and J. B. Losos. A phylogenetic test for adaptive convergence in rock-dwelling lizards. *Evolution*.

Supplementary Appendix 1. GenBank accession numbers used in phylogenetic tree reconstruction.

Anolis (*A. luteogularis* AF055977; *A. bartschi* AF055960; *A. griseus* AY296176; *A. vermiculatus* AF055961; *A. bahorucoensis* AF055932; *A. coelestinus* AY296164; *A. cuvieri* AF055973; *A. valencienni* AF055939; *A. allogus* AY296152; *A. distichus* AF528725; *A. gundlachi* AY296177; *A. alutaceus* AF055971; *A. angusticeps* AF055967; *A. porcatus* AY296195)

Phrynosomatinae (*Phrynosoma cornutum* AY297487; *Uma inornata* (to be included upon acceptance of manuscript); *Callisaurus draconoides* AY297492; *Cophosaurus texanus* AY297489; *Holbrookia maculata* AY297490; *Petrosaurus thalassinus* AF049858; *Sator angustus* AF049859; *Urosaurus ornatus* AY297493; *Uta stansburiana* AF049863; *Scel. grammicus* AY297509; *Scel. magister* AF528741; *Scel. malachiticus* AY297518; *Scel. undulatus* AY297514; *Scel. occidentalis* AY297515; *Scel. virgatus* AY297516; *Scel. olivaceous* AY297521)

Cordylidae (Melville et al., in review; GenBank numbers not yet available)

Platysaurus capensis, *Pseudocordylus capensis*, *Cordylus cataphractus*, *Cordylus lawrenci*, *Gerrhosaurus laticaudatus*, *Gerrhosaurus nigrolineatus*, *Pseudocordylus*

microlepidotus, *Cordylus macropholis*, *Cordylus mclachlani*, *Cordylus cordylus*)

Tropidurinae *Stenocercus roseiventris* (to be included upon acceptance of manuscript);

Microlophus occipitalis AY625162; *Microlophus peruvianus* AY625158; *Tropidurus*

etheridgei AF528750; *Tropidurus_hispidus* AY625154; *Tropidurus spinulosus*

AF528751; *Tropidurus plica* AF528748; *Tropidurus flaviceps* AF528747).

Supplementary Appendix 2. Assessment of Type I error in statistical methods employed in this study.

We conducted a simulation test to determine the Type I error rates of tests (1) and (2).

For test (1), we simulated 1000 data sets on each phylogeny. We estimated changes along all branches, and calculated F-values for the MANOVA as with the real data. Then, for each of the 1,000 simulated data sets, we performed 1,000 random permutations of the non-rock to rock transitions among terminal branches, each time recalculating F. For each simulation and associated permutations, the frequency of $F(\text{permutation}) > F(\text{simulated})$ was the P-value of the MANOVA. The frequency of simulated datasets with significant F ($P \leq 0.05$) was the estimate of the Type I error rate. This estimated Type I error was 0.052 ($P[\text{true type I error} \leq 0.05] = 0.41$).

Similarly, for test (2) we used the same 1,000 simulated data sets. For each simulated data set, we estimated changes along all branches and calculated F-values for the MANOVA for that simulated data set as above. For each simulated dataset, we then calculated a pooled variance-covariance matrix of independent contrasts and used that pooled matrix to simulate multivariate character evolution by Brownian motion on the trees 100 times (only 100 Brownian motion simulations were used for each simulated data set in the Type I error test for computational reasons). For each simulated data set, the fraction of times $F(\text{produced using simulations}) > F(\text{from the initial simulated data set})$ was the significance of the F-statistic. The Type I error estimate was the fraction of the 1,000 original data sets with significant MANOVAs. This estimated Type I error was

0.047 ($P[\text{true type I error} \leq 0.05] = 0.69$).

We also conducted a sensitivity analysis to determine if the simulation test (statistical test #2) was robust to violation of the assumption that characters evolved by gradual Brownian motion. To do this, we assumed that the characters actually evolved in a speciation model of character change, but then generated the statistical distribution using gradual Brownian motion. To do this, we generated 1,000 simulated data sets as above, except that character evolution occurred by “speciation Brownian motion” rather than gradual Brownian motion. Speciation Brownian motion is similar to gradual Brownian motion; however the amount of change along each branch is not scaled to be proportional to square root of the branch length. Consequently, it assumes that the difference among species accumulates only during speciation event (hence, it has also been called “punctational”); as a result, the distribution of expected changes on each branch is the same, despite differences in branch length. The sensitivity analysis consisted of analyzing each data set by the method described above, which assumes gradual Brownian motion, even though the distributions were actually generated using a speciation model of character change. The result was actually a lower Type I error rate than when the data were simulated under the gradual Brownian motion model (type I error by simulation = 0.039, $P[\text{true type I error} \leq 0.05] = 0.96$).

Supplementary Appendix 3. Analysis on alternative data set with multiple measurements per species for Phrynosomatinae and *Anolis*.

Branches on which transitions to rock-dwelling occurred differ from those not involving such transitions (MANOVA on morphometric variables, $F_{10,79} = 5.72$; permutation test, $P = 0.005$; simulation test, $P < 0.001$; MANOVA on PC scores for first two axes, $F_{2,87} = 9.97$; permutation test, $P = 0.006$; simulation test, $P = 0.004$). Given the significant MANOVA results, we examined variables individually. Analyses of variance (Appendix Table 1) indicate that the transition to rock-dwelling was associated with differences on both PC axes (PC 1 marginally non-significant in the permutation test), a decrease in head depth and an increase in humerus, metacarpal, and femur. Ulna, tibia, and metatarsal length were marginally non-significant (metatarsal significant in the simulation test). Change in digit lengths did not differ among branches of the phylogeny (Appendix Table 1).

Appendix Table 1. Phylogenetic Analyses of Variance for data set containing multiple individuals for *Anolis* and phrynosomatines.

Variable	F-ratio*	Permutation P-value	Simulation P-value
PC 1	7.46	0.054	0.028
PC 2	10.69	0.024	0.012
Head depth	17.13	0.012	0.001
Humerus	8.31	0.025	0.022
Ulna	5.36	0.079	0.069
Metacarpal	12.85	0.010	0.004
Manus III	0.11	0.76	0.80
Manus IV	2.64	0.15	0.19
Femur	11.60	0.016	0.007
Tibia	5.67	0.064	0.066
Metatarsal	6.40	0.06	0.047
Pedal IV	0.58	0.58	0.56

Supplementary Appendix 4. Testing the assumption of Brownian motion.

The phylogenetic methods of this paper, both the calculation of evolutionary changes along specific branches as well as the simulation based analyses, rely on an underlying assumption of evolution by Brownian motion. This is the same assumption that underlies many other types of phylogenetic comparative statistical analyses, such as independent contrasts.

In Appendix 2, we showed that the phylogenetic methods used in this paper exhibit appropriate Type I error. Furthermore, we also showed in an accompanying sensitivity analysis that Type I error was acceptable even when the assumption of Brownian motion was violated (i.e., when a speciation model of character change was substituted for gradual Brownian motion). Nonetheless, it is still of interest to consider whether or not the data used in this study are consistent with Brownian motion.

To do so, we used two methods. First, we used Garland et al.'s (1992) regression approach in which the absolute value of each contrast is regressed against the square-root of the expected variance of the contrast. As the contrasts have been standardized by their expected variance, thus rendering their expected variances equal after standardization, a significant regression suggests non-Brownian motion evolution. Appendix Table 2 shows the r^2 and the significance [$P(\beta)$] of each regression from each of the four lizard clades. No regression is significant, even at the 0.05 level (thus ignoring multiple tests).

Second, we used Blomberg et al.'s (2003) K-ratio statistic. Blomberg et al.'s (2003) statistic is calculated using a phylogenetic generalized least squares approach (PGLS; e.g., Rohlf 2001) and has an expected value of 1.0 under Brownian motion. To

test for deviation from Brownian motion evolution using Blomberg et al.'s (2003) statistic, we conducted the following procedure: (1) we calculated K-ratios for all traits using the PGLS method described in Blomberg et al. (2003); (2) we estimated an evolutionary rate matrix from the independent contrasts; (3) we simulated multivariate trait evolution by Brownian motion on each of the four lizard phylogenies 1000 times using the independent contrasts matrix as the Brownian motion rate matrix; (4) we calculated K-ratios for all traits from all 1000 simulated data sets for each clade; and (5) we estimated the significance of the observed K-ratio for each trait and clade as the fraction of times in which a more extreme value than the empirical value was observed in the simulated data. Because K is approximately log-normally distributed around $\log(1.0)=0.0$ under the null hypothesis, and only the extremity of the deviation from Brownian motion (and not its direction) was of concern, significance was calculated as the fraction of times in which the absolute value of the logarithm of the observed K-ratio exceeded that obtained via simulation. Appendix Table 3 shows the K-ratios and their significance [P(K)] for each trait and clade. Many of the observed K-ratios were less than 1.0. However, although 9 of 44 tests were individually significantly different from 1.0 at the $\alpha=0.05$ level, after multiple test correction by the sequential Bonferroni method, all K were non-significant. Notably, even before multiple test correction, head depth was not significantly non-Brownian in any lizard clade, and no other trait except ulna length is significantly non-Brownian in more than one clade before correction (Appendix Table 3).

In summary, these tests provide little support for the hypothesis that trait evolution has been consistently non-Brownian.

Appendix Table 2. Regression test for Brownian motion evolution. r^2 and significance for each of 11 linear regressions for each of the four lizard clades. Linear regressions were conducted between the absolute value of the independent contrasts for each trait and their expected variance (calculated from the branch lengths in each phylogeny). N is the number of taxa in each clade. Traits are snout-to-vent length (SVL), head depth (HD), and the lengths of the humerus (HUM), the ulna (ULN), the first metacarpal of the fourth toe of the forefoot (METC), the third toe on the forefoot (MN3), the fourth toe on the forefoot (MN4), the femur (FEM), the tibia (TIB), the first metatarsal of the fourth toe of the hindfoot (METT), and the fourth toe on the hindfoot (T4). No regression indicates significantly non-Brownian motion evolution, even before correcting for multiple tests.

Clade	N		SVL	HD	HUM	ULN	METC	MN3	MN4	FEM	TIB	METT	T4	$\overline{r^2}$
<i>Anolis</i>	14	r^2	0.021	0.006	0.046	0.067	0.021	0.032	0.028	0.038	0.048	0.044	0.008	0.033
		P(β)	0.636	0.795	0.483	0.391	0.636	0.363	0.316	0.526	0.472	0.490	0.767	
Cordylidae	10	r^2	0.159	0.076	0.008	0.007	0.082	0.064	0.100	0.133	0.144	0.175	0.106	0.096
		P(β)	0.287	0.472	0.824	0.831	0.455	0.512	0.401	0.335	0.314	0.263	0.393	
Phrynosomatinae	16	r^2	0.017	0.000	0.007	0.013	0.055	0.018	0.031	0.003	0.007	0.004	0.003	0.014
		P(β)	0.647	0.948	0.767	0.684	0.401	0.629	0.533	0.844	0.770	0.816	0.844	
Tropidurinae	9	r^2	0.024	0.000	0.046	0.021	0.005	0.003	0.036	0.000	0.004	0.002	0.009	0.014
		P(β)	0.713	0.964	0.611	0.729	0.862	0.905	0.652	0.995	0.889	0.920	0.820	

Appendix Table 3. K-test for Brownian motion evolution. K and the significance of K from simulation for each of 11 traits for each of the four lizard clades. K-ratios were calculated using the PGLS approach following Blomberg et al. (2003). N is the number of taxa in each clade. Since we are as concerned about positive as negative deviations from the Brownian motion expectation of K=1.0, the mean value of K in each clade, \bar{K} , for $m = 11$ traits was calculated as $\frac{1}{m} \sum_{i=1}^m \exp[-|\log(K_i)|]$, where the negative sign was chosen arbitrarily because most observed values of K are less than 1.0. Traits are as in Appendix Table 2. Although nine of 44 tests indicated significant non-Brownian motion evolution at the 0.05 level, none were significant after sequential Bonferroni correction for multiple tests (conducted separately within each clade).

Clade	N		SVL	HD	HUM	ULN	METC	MN3	MN4	FEM	TIB	METT	T4	\bar{K}
<i>Anolis</i>	14	K	0.877	0.884	1.139	0.641	0.711	0.757	0.860	1.020	0.850	0.695	0.950	0.826
		P(K)	0.584	0.571	0.574	0.032	0.096	0.177	0.505	0.936	0.488	0.082	0.794	
Cordylidae	10	K	0.908	0.713	0.451	0.462	0.518	0.492	0.427	0.605	0.761	0.599	1.005	0.630
		P(K)	0.775	0.333	0.018	0.025	0.060	0.041	0.012	0.143	0.433	0.122	0.994	
Phrynosomatinae	16	K	0.713	0.680	0.749	0.675	0.702	0.687	0.554	0.627	0.543	0.497	0.795	0.656
		P(K)	0.287	0.233	0.383	0.204	0.289	0.231	0.052	0.140	0.053	0.025	0.506	
Tropidurinae	9	K	0.610	0.732	0.388	0.573	0.565	0.700	1.023	0.369	0.571	0.671	0.468	0.601
		P(K)	0.249	0.481	0.013	0.164	0.155	0.419	0.962	0.006	0.155	0.363	0.047	

Supplementary Appendix 5. McPeck's (1995) method and the present study.

McPeck (1995) suggested a modification of the independent contrasts method that calculates ancestor-descendant contrasts on focal branches of a phylogeny and compares them to sister-taxon contrasts calculated throughout the rest of the tree. At first glance, McPeck's method, in which changes along single branches of the phylogeny are isolated, would seem ideal to this study. Unfortunately the method is not appropriate to our data and question for several reasons.

First, McPeck's method is primarily suited to assess whether the rate of evolution is higher on particular branches in a phylogeny (McPeck 1995; Felsenstein 2004; e.g., Klingenberg and Ekau 1996; McPeck 1999; McPeck and Brown 2000; Stoks and McPeck 2006). However, sister-taxon contrasts are non-directional and consequently the absolute values of both types of contrasts must be used in any test for exceptional evolution along the phylogenetic branches of interest (e.g., McPeck 1999). In our study, we are interested in the rate and direction of evolution, but in particular in the direction of evolution of our focal taxa.

Some authors have also used McPeck's method to test a hypothesis of large and directed evolutionary change along specific branches (e.g., McPeck et al. 1996; Harrison and Crespi 1999). In this case the magnitudes (absolute values) of the sister-taxon contrasts are compared to the directional (signed) contrasts calculated along single branches (e.g., Stoks et al. 2003). The magnitudes of sister-taxon contrasts are still used because the sign of such comparisons are arbitrary (Garland et al. 1992; McPeck 1999). The results are then typically analyzed using a parametric statistical test, such as a *t*-test.

Unfortunately, this approach will fail to satisfy distributional assumptions of

parametric statistics under the null hypothesis of undirected constant rate Brownian motion. This is because, under the null hypothesis of Brownian motion, the signed changes along single branches have an expected value of 0, whereas the absolute values of the contrasts have an expected value of $\sqrt{2\sigma^2/\pi}$, in which σ^2 is the Brownian motion rate parameter. This is derived from the expected value of a reflected Gaussian distribution: $E(x) = \int_0^\infty \left\{ 2 \exp(-x^2/2\sigma^2) / \sqrt{2\pi\sigma^2} \right\} x dx$, and can be confirmed empirically. Similarly, the signed changes along single branches have an expected distribution which is Gaussian with a variance equal to σ^2 , whereas the absolute values of the sister-taxon contrasts have an expected distribution which is reflected Gaussian with a variance equal to $\sigma^2(\pi - 2)/\pi$ (also similarly derived). Consequently, this approach is expected to have low power in a one-tailed test for large positive changes along focal branches (due to $\sqrt{2\sigma^2/\pi} \geq 0$), and unknown error rates due to violations of parametric assumptions, such as normality. Note that even in the standard rate test, in which absolute values of both sister-taxon and single branch contrasts are used, a key distributional assumption of parametric statistical tests (normality) will not be satisfied by the data under the null hypothesis.

Second, McPeck's test will only tend to be significant when evolution is exceptional in magnitude and direction (e.g., Stoks et al. 2003), while in this study we are primarily concerned with the hypothesis that evolutionary change along specific branches in the phylogeny is exceptional in direction.

Thus, instead of employing McPeck's method, we calculated directional ancestor-descendant comparisons to test only whether the multivariate direction of evolutionary

change is more similar on focal branches than on other branches in the phylogeny.

Although we know of no existing phylogenetic method for this specific test, a recent study by Wiens et al. (2007) used a highly similar approach to test the hypothesis that branches identified to possess significant changes in diversification rate among salamanders were associated with significant changes in elevation.

Appendix Table 4. Relative head depth and changes in relative head depth

A) Relative head depth

<i>P. capensis</i>	-0.4798488
<i>T. semitaeniatus</i>	-0.3419532
<i>C. mclachlani</i>	-0.2511876
<i>C. lawrenci</i>	-0.2262920
<i>G. nigrolineatus</i>	-0.2185278
<i>P. thalassinus</i>	-0.1950145
<i>A. alutaceus</i>	-0.1453173
<i>A. angusticeps</i>	-0.1244653
<i>Um. inornata</i>	-0.1213971
<i>G. laticaudatus</i>	-0.1083191
<i>Ps. capensis</i>	-0.0943013
<i>Ca. draconoides</i>	-0.0924054
<i>A. bartschi</i>	-0.0488138
<i>Ps. microlepidotus</i>	-0.0451888
<i>A. vermiculatus</i>	-0.0415015
<i>M. peruvianus</i>	-0.0377362
<i>T. hispidus</i>	-0.0230923
<i>S. undulatus</i>	-0.0228137
<i>A. valencienni</i>	-0.0186313
<i>A. porcatus</i>	-0.0146487
<i>Ut. stansburiana</i>	-0.0112568
<i>Co. texanus</i>	-0.0082660
<i>H. maculata</i>	0.00256889
<i>A. distichus</i>	0.00732570
<i>T. plica</i>	0.01755921
<i>Ur. ornatus</i>	0.02097794
<i>S. grammicus</i>	0.02519510
<i>C. cordylus</i>	0.02868148
<i>A. griseus</i>	0.02882918
<i>A. coelestinus</i>	0.03670811
<i>A. luteogularis</i>	0.04456753
<i>T. spinulosus</i>	0.04695933
<i>A. bahorucoensis</i>	0.05310853
<i>C. cataphractus</i>	0.05830724
<i>S. olivaceous</i>	0.07669904
<i>T. etheridgei</i>	0.08872506
<i>S. malachiticus</i>	0.09325548
<i>S. virgatus</i>	0.09354316
<i>Sa. Angustus</i>	0.10682933
<i>S. magister</i>	0.12393345
<i>S. occidentalis</i>	0.13006450
<i>A. allogus</i>	0.14627334

<i>T. flaviceps</i>	0.14685566
<i>C. macropholis</i>	0.15113411
<i>M. occipitalis</i>	0.18450790
<i>A. gundlachi</i>	0.18990802
<i>S. roseiventris</i>	0.21973354
<i>Ph. cornutum</i>	0.26282933
<i>A. cuvieri</i>	0.28589834

B) Changes in relative head depth. Transitions to rock-dwelling species are identified by the terminal species.

-0.05205785 *T. semitaeniatus*

-0.03009921

-0.02477931 *P. capensis*

-0.02234998 *P. thalassinus*

-0.01868882

-0.01611037

-0.01527345

-0.01332106

-0.01266743

-0.01132018

-0.01069888

-0.01011552

-0.01005816

-0.00956849

-0.00923488

-0.00893198

-0.00845666

-0.00667099

-0.00659451

-0.00578174

-0.00555134

-0.00551266

-0.00538130

-0.00439201

-0.00413221

-0.00403552

-0.00343875

-0.00338392 *A. bartschi*

-0.00337642

-0.00325233

-0.00263319 *Ps. capensis*

-0.00245461

-0.00242904

-0.00227285

-0.00222635
-0.00189348
-0.00130226
-0.00111142
-0.00091707
-0.00083608
-0.00060752
-0.00011475
0.00024802
0.00031940
0.00060525
0.00065946
0.00074681
0.00078025
0.00090740
0.00125112
0.00146004
0.00155186
0.00178469
0.00249069
0.00276003
0.00300723
0.00324264
0.00357462
0.00359093
0.00368705
0.00378594
0.00417970
0.00424311
0.00425437
0.00438459
0.00448570
0.00457657
0.00476274
0.00511369
0.00530878
0.00574779
0.00602681
0.00617504
0.00665489
0.00679899
0.00797283
0.01049317
0.01179984
0.01197239
0.01296034

0.01421727
0.01546385
0.01675965
0.01755212
0.01859466
0.01980965
0.02171727
0.02255071
0.02346912
0.02953542

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