A comparative analysis of clinging ability among pad-bearing lizards

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We examined clinging ability, subdigital pad area and body mass in 14 pad-bearing lizard species from three families to test three predictions: (1) clinging ability and pad area should be tightly correlated among species; (2) pad area and clinging ability should scale similarly to body mass among 14 species; and (3) functional similarity in clinging capabilities should exist among species despite differences in body mass. Our results confirm two predictions; clinging ability is tightly correlated with pad area, even when the effects of body size are removed, and the lizards examined are approximately functionally similar in their clinging capabilities. Nevertheless, despite the tight correlation between pad area and clinging ability, pad area scales with body mass by a lower slope than clinging ability. Overall, these results indicate that although pad area is a strong determinant of clinging ability, other factors enable these lizards to maintain functional similarity.

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ADDITIONAL KEY WORDS:— clinging ability – pad area – body mass – phylogenetic analyses – Gekkonidae – Scincidae – *Anolis.*

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INTRODUCTION

Understanding how phenotypic traits function is essential to testing adaptationist hypotheses (Bock & von Wahlert, 1965; Arnold, 1983, 1986; Wainwright, 1994). Consequently, substantial attention has been devoted to linking interspecific variation in functional (performance) capability with variation in morphology and physiological characteristics (Bennett & Huey 1990; Losos, 1990a, b; Emerson, 1991; Miles, 1994; Garland, 1994; Garland & Losos, 1994). However, comparative studies of performance and morphology should also consider the historical context in which these traits evolved (Felsenstein, 1985; Huey, 1987; Baum & Larson, 1991; Harvey & Pagel, 1991; Losos & Miles, 1994; Arnold, 1994). By gathering both performance and morphological data and analysing them in a phylogenetic context, hypotheses about the expected evolutionary relationship between form and function can be tested. Subdigital pads in lizards, which assist in allowing them to climb vertical and overhanging surfaces, provide an excellent opportunity to test hypotheses in this context.

The ability of lizards to cling to substrates by means of subdigital pads has long intrigued biologists. These pads, which have arisen independently at least three times within lizards in the families Gekkonidae, Scincidae and Polychridae, consist of laterally expanded scales (lamellae) covered with modified scale derivatives either in the form of stalks termed setae (found in geckos, anoles and one skink, *Prasinohaema virens*) or elongated 'folds' or 'ridges' (found in skinks) (Ruibal & Ernst, 1979; Williams & Peterson, 1982; Peterson, 1983; Austin, unpubl.). A number of studies have examined the mechanistic basis of clinging ability (Hora, 1923; Hiller, 1975; Losos, 1990c), as well as the functional anatomy of the toepad structure that provides the clinging force (Russell, 1975, 1981, 1986; Ruibal & Ernst, 1979; Williams & Peterson, 1982; Peterson, 1983; Bauer & Russell, 1988; Bels & Theys, 1989). Despite this work, however, few attempts have been made to examine which morphological characteristics influence clinging ability in pad-bearing lizards (but see Mahendra, 1941; Hiller, 1975; Losos, 1990a). Nevertheless, evolution of the subdigital pad has been invoked as an important adaptation allowing pad-bearing lizards to exploit arboreal habitats (Russell, 1979; Williams & Peterson, 1982, Peterson, 1983). However, without understanding how variation in pad characteristics affects clinging ability in pad-bearing lizards, hypotheses about the adaptive significance of the subdigital pad will be difficult to test.

Here we test three predictions about the relationship between body mass, pad area and clinging ability among 14 pad-bearing representatives of the three lizard lineages that have independently evolved subdigital pads. First, we predict that pad area should be positively correlated with clinging ability among species, either when absolute (non size-adjusted) or relative (size-adjusted) pad area and clinging ability are examined. This prediction is based on the assumption that increases in pad area should lead to a proportional increase in force transmitted to the substrate on which the lizard is clinging. In addition, this prediction assumes that factors other than pad area are not strong influences on clinging ability (unless they are also correlated with pad area). If, for example, phenotypic differences among species (e.g. in pad structure) strongly influence clinging ability, then these factors may override the effect of pad area.

Second, we predict that pad area and clinging ability should scale similarly to body mass. This second prediction is based on the assumption that pad area is the primary

determinant of clinging ability, and that both should therefore scale to body mass in the same manner. Third, we predict that these 14 species should be functionally similar in their clinging capabilities despite differences in body mass. By functionally similar we mean that lizards differing in body size will exhibit the same ratio of clinging ability to body mass (i.e. that clinging ability scales to body mass by a slope of 1.0). This prediction of functional similarity is based on the assumption that selection will favor similar clinging capabilities for large and small lizards that are using approximately the same habitats (i.e. arboreal environments). Otherwise, if clinging ability scales to body mass by a slope less or greater than 1.0, then large and small lizards would be relatively worse clingers (for their body size) than their smaller or larger congeners, respectively. We view this latter possibility as unlikely, and therefore predict that selection will favor functional similarity in clinging ability among lizards of different body sizes.

Because we earlier predicted that pad area and clinging ability should scale similarly with body mass, this third prediction also entails an expectation that pad area will scale with body mass by a slope of 1.0. Therefore, because isometric growth would result in pad area scaling to body mass by a slope of 0.67 (see Bauer & Good, 1986), this prediction implies that pad area will exhibit positive allometry with body mass. To test the above predictions, we examined the clinging ability, pad area and body mass of 14 lizard species that differ substantially in all three characteristics.

MATERIAL AND METHODS

Species examined

We quantified clinging ability in 14 lizard species: six geckos (*Hemidactylus frenatus, H. turcicus, Gehyra oceanica, G. mutilata, Gekko gecko* and *Lepidodactylus lugubris*), four anoles (*Anolis carolinensis, A. sagrei, A. grahami* and *A. leachi*) and four skinks (*Prasinohaema virens, p. flavipes, P. prehensicauda* and *Lipinia leptosoma*). Two of the geckos (*G. gecko* and *H. turcicus*) and one of the anoles (*A. sagrei*) were ordered from animal suppliers. Three of the geckos (*H. frenatus, G. mutilata* and *L. lugubris*) and one of the anoles (*A. carolinensis*) were collected from introduced populations on Oahu, Hawaii whereas *G. oceanica* was collected from the Cook Islands. Finally, the skinks were collected from either New Guinea (*Prasinohaema* spp.) or the Belau Islands (*L. leptosoma*).

Substantial variation in body mass exists among these species. For instance, *G. gecko* weighs approximately 31 times more than *L. lugubris* (Table 1). Similarly, substantial variation in pad area and in the ability to climb vertical and overhanging surfaces exist among these 14 species. For example, all six geckos possess large pads and readily climb vertical and overhanging surfaces (K. Petren, pers. obs.; R. Fisher, pers. obs.). In contrast, the anoles and skinks possess smaller pads and climb overhanging surfaces with difficulty or not at all (D. Irschick, pers. obs.; C. Austin, pers. obs.). All the geckos included in this study are commonly observed on house walls, but few quantitative data are available regarding habitat use in more natural settings. The anoles differ ecologically: *Anolis leachi, A. carolinensis* and *A. grahami* are more aboreal than *A. sagrei* (Schoener, 1968; Losos 1990a). Few ecological data are available for the skinks, but all use arboreal habitats (e.g. trees) in either lowland or montane tropical rainforest (Brown & Fehlman, 1958; Austin & Jessing, 1994; Austin, 1995).

Performance trials

Our measure of clinging ability was the vertical force required to pull a lizard down a standard, nearly vertical (85° to the horizonal) surface. Because the substrate on which lizards were placed was smooth (see below), our measure of clinging ability is not influenced by claws on the lizards. More tests need to be carried out to examine how claws influence clinging ability in pad-bearing lizards. Force was measured using a vertically oriented force platform. The surface to which the lizards adhered was an acetate overhead transparency sheet attached to and overlying a stiff plexiglass plate 6.4 mm thick and 13 by 15 cm long and wide respectively. An acetate transparency was used because lizards were unable to cling to numerous other cleaned surfaces. The acetate was cleaned between performance trials with 95% ethanol to prevent particles from collecting on the acetate. The Plexiglass plate was attached to two square, hollow, horizontal, metal beams (6.4 mm square) which were in turn attached to a heavy, stiff metal plate. The beams had windows cut in them so that they flexed vertically at specific locations. The flexing portion of the beam was 0.62 mm thick. On the flexing portion, strain gauges were attached to measure the deformations. Strain gauge signals were amplified using a strain gauge amplifier which produced a voltage signal that was calibrated to force (newtons) by hanging weights from the force platform. Calibration curves were linear and had a squared correlation always greater than 0.99.

During trials with lizards, the voltage signal was digitized (12-bit) and viewed as a function of time using a dynamic signal analyser sampling at 25 Hz. The unloaded force platform system had a narrow resonance frequency at 68 Hz, and when loaded with 100 grams the system had a narrow resonance frequency at 53 Hz. At frequencies below the resonance frequency, the system is a stiffness-controlled oscillator in which displacement tracks force independent of frequency. Because typical force versus time traces were several seconds long, the force platform adequately tracked vertical force generated as a lizard was pulled down the force platform. Peak force before detachment was compared among lizards. Errors due to calibration and digitization were negligible compared to variation among lizards because force was resolvable to less than ± 0.01 N.

Performance trials took place during two time periods at the University of California at Davis. The clinging ability of skinks was measured during March of 1991 and that of geckos and anoles during December of 1994. Housing and experimental conditions were identical in both trials. Lizards were maintained in plastic shoeboxes or ten-gallon aquaria at 27°C during these experiments. Lizards were fed vitamin-dusted crickets *ad libitum* and misted with water regularly prior to trials. However, because trials took place over a short time period (2 days per lizard), lizards were not fed during trials. The four skinks, two of the anoles (*A. sagrei* and *A. carolinensis*) and three of the geckos (*G. oceanica, H. turcicus* and *G. gecko*) had been maintained in captivity for at least several months prior to the experiments. The remaining species were captured immediately prior to trials. Only healthy adult lizards in good condition were used in performance trials (e.g. lizards with shrunken tail bases were excluded).

Clinging ability was measured four times, once in the morning and once in the afternoon on two consecutive days. Lizards were placed in plastic cups an hour before each trial and allowed to acclimate to room temperature. After each trial was completed, the lizards were returned to their cages. The temperature of the room in

which the clinging experiments were performed was 22°C. Trials were conducted as follows. Each lizard was gently picked up and placed with its front feet contacting the acetate sheet. The lizard was then gently and slowly pulled in a downward direction until the lizard refused to cling further and removed its feet from the surface. This method was repeated until either an acceptable trial was obtained or the lizard became fatigued. An acceptable trial consisted of the lizard placing both front feet on the acetate and completely extending its forearms. We considered extension of the forearms to be important because we observed that when lizards did not extend their arms, they often did not place all toepads flush against the acetate paper. Trials in which the lizard extended its forearms completely were considered 'good,' whereas trials in which the lizard did not extend its forearms fully were considered 'poor'. In the statistical analyses, we used the best of the good trials as the measure of maximal clinging ability for each lizard. Lizards with no good trials were excluded from the analyses.

Subsequent to performance trials, body mass for each lizard was measured with a Mettler top-loading balance accurate to 0.005 g. Due to the difficulty of measuring pad area on large numbers of live lizards, lizards were euthanized and preserved with 10% formalin after performance trials and transported to Washington University in St. Louis. However, measurements of pad area were obtained from the four skinks and *G. oceanica* (23 out of 106 lizards examined) while still alive because these lizards were needed for other studies. Pad area was quantified as the combined area of all toes which we considered to contain adhesive structures (e.g. setae) on both front feet. Pad area was measured by placing each foot flush against a flat glass plate. Images were captured with a video camera connected to a video digitizing system (Morpho-Sys; Meacham & Duncan, 1990). Because adhesive structures exist on the palm as well as on the toes of the skinks (Austin, unpubl.), we measured the area of both the palm and the toes for these species and used the combined area as the estimate of the area containing adhesive structures.

Statistical analyses

Considerable controversy exists over which regression technique (model I, major axis or reduced major-axis) is appropriate for determining scaling relationships (see LaBarbera [1989] for a recent review). The most commonly used regression technique in scaling studies, model I (ordinary least squares) regression, may underestimate the slope if the independent variable is measured with error. However, one can correct this error by dividing the slope by the correlation (h) between successive determinations of the independent variable (Fuller, 1987; see Moore & Ellers, 1993 for an empirical example). We estimated the error in our independent variable (body mass) by taking a second measurement of body mass on 18 lizards (two per species) from nine lizard species (*G. oceanica* and the skinks were not used). The Pearson correlation coefficient was 0.997 between measures of body mass. We then divided the calculated slopes by this correlation value to correct for error in the independent variable. All statistical tests were performed using SYSTAT (Wilkinson, 1990). All variables were $\log_{10}$ transformed prior to statistical analyses.

Non-phylogenetic methods for calculating slopes and correlation coefficients may be invalid if sister taxa tend to be similar to one another (i.e. a phylogenetic 'effect' exists; Felsenstein, 1985; Harvey & Pagel, 1991; Martins & Garland, 1991; Garland,

Harvey & Ives, 1992). Therefore, we used the independent contrasts method (see Felsenstein [1985] and Garland *et al.*, [1992] for descriptions of this technique) to estimate scaling relationships and to perform regressions. The independent contrasts method require knowledge of both the topology and branch lengths of the phylogeny for taxa considered in a study. However, phylogenetic relationships among the 14 lizard species examined are only partially understood (Fig. 1) and branch length information is not available for these species. Below we outline the available phylogenetic data for the species examined.

Based on a cladistic analysis of osteological characters, geckos and skinks are considered to be more closely related to one another than either is to anoles (Estes, de Queiroz & Gauthier, 1988). Morphological evidence indicates that the skink genus *Prasinohaema* is a monophyletic group with *P. flavipes* and *P. prehensicauda* sister taxa relative to *P. virens* and *Lipinia* the outgroup to *Prasinohaema* (Greer, 1974). Based on overall foot structure, Russell (1972) placed the gecko genera *Gekko, Gehyra* and *Lepidodactylus* in the *Gekko* group and *Hemidactylus* in the *Hemidactylus* group. Here, we use Russell's taxonomic arrangement and considered *Gehyra, Lepidodactylus* and *Gekko* to form a monophyletic group relative to *Hemidactylus*. However, we consider the relationships among *Gehyra, Lepidodactylus* and *Gekko* unresolved. Species within each genus (*Hemidactylus* and *Gehyra*) are considered more closely related to one another than to any other species in the study. Etheridge (1960) divided the genus *Anolis* into two groups based on the presence or absence of transverse processes in distal caudal vertebrae (beta [transverse process] and alpha [no transverse process]). On biogeographic considerations, as well as on the assumption that this character was not likely to be homoplastic, Williams (1969) treated these two divisions as defining separate monophyletic groups. More recent analyses (Guyer & Savage, 1986, 1992; Cannatella & de Queiroz, 1989) indicate that alpha anoles are likely paraphyletic, but that beta anoles may be monophyletic. *Anolis sagrei* and *A. grahami* are both beta anoles, whereas *A. leachi* and *A. carolinensis* are alpha anoles. Therefore, we consider *A. sagrei* and *A. grahami* to be sister taxa among the species in this study, but considered the relationships of *A. leachi* and *A. carolinensis* to be uncertain.

Because our tree has two unresolved tritomies, nine possible fully-resolved trees (three possible resolutions in each of the gecko and anole clades) exist that are consistent with the relationships among the 14 species depicted in Figure 1. We performed phylogenetic analyses on each of the nine possible trees. Since branch length information is not available for these species, we examined the sensitivity of the results using two branch length models. First, we assumed that morphological change occurred primarily at speciation (speciational model) and set all branch lengths equal to one another (see Martins & Garland, 1991; Garland *et al.*, 1993). Second, because geckos, anoles and skinks have each likely been evolving independently from one another much longer than species within these families, we set the branch lengths between families to be ten times longer than branches within families (extended branches model). Finally, the phylogenetic analysis used here assumes that the taxa examined are in evolutionary equilibrium (i.e. that the traits examined are no longer changing rapidly due to selection). Due to the relatively old age of the gecko, skink and anole clades, we consider this assumption reasonable.

Mean values for body mass, pad area and clinging ability for each species were used in statistical analyses. For each analysis (e.g. pad area vs. body mass), we calculated contrasts for both variables on each of the nine possible trees, each with two possible branch lengths (18 trees overall), and calculated slopes by regression
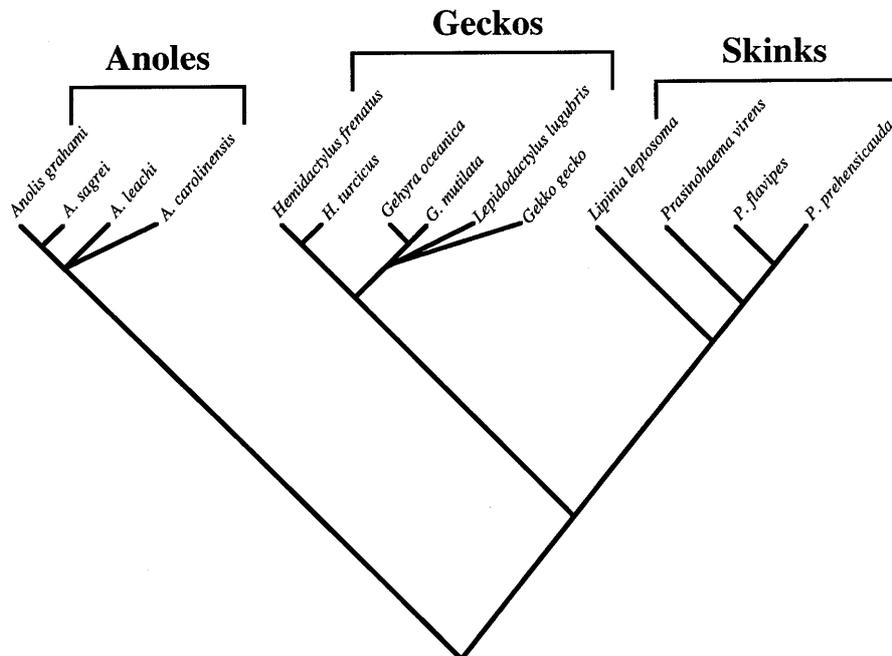
Figure 1. Phylogenetic relationships among the 14 lizard species examined in this study. Branch lengths do not represent time since separation from a common ancestor. See text for details.

through the origin (see Garland *et al.*, 1992 for equations used). We tested the predictions that pad area and clinging ability scale similarly to body mass, and that both variables scale to body mass[1.0] by using Clarke's T-statistic (Clarke, 1980; McArdle, 1988). This test is highly conservative and therefore biases against finding a significant difference between actual and predicted values or between two sample values. For each analysis, the most extreme (high and low) slopes for the pad area-body mass and clinging ability-body mass relationships were compared. Similarly, these extreme values were also compared against the expected value of 1.0.

To examine whether pad area is positively correlated with clinging ability, we also performed regressions (ordinary least squares) across all 18 possible trees with pad area and clinging ability as independent and dependent variables respectively. First, we regressed non size-adjusted contrasts of clinging ability on pad area. Second, we removed the effects of size from both pad area and clinging ability by calculating residuals from regressions of contrasts of each variable on body mass contrasts. We then examined whether these body mass and phylogeny-free contrasts were positively correlated by performing regressions on the residuals.

RESULTS

The 14 lizard species vary substantially in pad area and clinging ability (Table 1). The most extreme difference is a 126-fold difference in absolute (non size-adjusted) clinging ability and 25-fold difference in absolute pad area between *Gekko gecko* and *Lipinia leptosoma*. In all analyses, the contrast between the skink and gecko clades

TABLE 1. Mean (± 1 SE) values for body mass (gms), pad area (mm^2) and clinging ability (newtons) for 14 lizard species. N represents the sample size for each species. See text for which species are geckos, anoles and skinks

Species	*n*	Body mass	Pad area	Clinging ability
Hemidactylus frenatus	15	3.0±0.21	25.3±1.34	1.0±0.08
H. turcicus	5	2.1±0.28	21.5±1.69	0.8±0.24
Gehyra oceanica	11	7.9±0.50	69.1±3.22	4.7±0.31
G. mutilata	9	1.7±0.09	18.1±1.17	0.8±0.04
Lepidodactylus lugubris	8	1.4±0.05	12.1±0.58	0.9±0.09
Gekko gecko	6	43.4±1.48	227.1±10.81	20.04±1.33
Anolis carolinensis	15	5.5±0.52	30.1±2.20	1.5±0.13
A. sagrei	9	4.4±0.48	21.4±1.69	1.3±0.19
A. leachi	7	18.1±2.05	60.8±5.64	4.9±0.79
A. grahami	9	6.9±0.34	35.9±1.61	2.5±0.26
Prasinohaema virens	3	3.1±0.13	18.9±1.77	0.4±0.05
P. prehensicauda	3	7.1±0.90	21.1±1.24	0.2±0.01
P. flavipes	4	23.9±1.73	52.5±1.89	0.8±0.65
Lipinia leptosoma	2	1.3*	9.1±0.70	0.2±0.01

*= No standard error bars because no variation in body mass exists.

tended to fall below the regression line in scaling relationships (Fig. 2A, B) as well as in plots between clinging ability and pad area (either absolute or relative values, Fig. 3A, B). However, the contrast between the anole clade and the ancestor of all other taxa tended to fall much closer to the regression line both in scaling relationships (Fig. 2A, B) and in plots between clinging ability and pad area (Fig. 3A, B). Overall, despite some deviations, contrasts between the larger clades are not obvious outliers relative to contrasts within these clades.

In all analyses, body mass and clinging ability are not correlated when the effects of pad area are removed (Phylogenetic partial correlation analysis: $P > 0.05$ for all comparisons). Absolute clinging ability is strongly correlated with absolute pad area ($P < 0.01$ for all comparisons, Table 2, Fig. 3A). Similarly, relative clinging ability is positively correlated with relative pad area ($P < 0.01$ for all comparisons, Table 2, Fig. 3B). Differences in topology or branch lengths changed r^2 values only slightly and no obvious tendency for either branch length model to generate high or low r^2 values emerged. Further, slopes changed only slightly among different topologies and branch lengths (Table 2). Pad area scales at a lower slope than clinging ability with body mass (*t*-Tests, $P < 0.01$ for all comparisons).

All slopes between pad area and body mass are significantly lower than the predicted value of 1.0 ($P < 0.01$ for all comparisons, Table 2, Fig. 2A). Indeed, only the highest slope value in the pad area-body mass comparison (0.78) was significantly greater than the expected slope based on isometry (expected b = 0.67; $t = 2.53$, 9 df, $P < 0.05$). In contrast, all slopes between clinging ability and body mass are not significantly different from 1.0 ($P > 0.20$ for all comparisons, Table 2, Fig. 2B).

DISCUSSION

We examined body mass, clinging ability and pad area in 14 pad-bearing lizards to test three predictions: (1) that pad area and clinging ability should be strongly correlated among species; (2) that pad area and clinging ability should scale similarly

to body mass; and (3) that the lizard species examined should be functionally similar in their clinging capabilities despite differences in body mass. Our results confirm the first prediction. However, despite the strong correlation between pad area and clinging ability, prediction 2 was not upheld; pad area scales with a lower slope than clinging ability to body mass. Finally, prediction 3 was upheld; the lizards examined are approximately functionally similar in their clinging capabilities.

Correlation between pad area and clinging ability

In all analyses, pad area (both absolute and relative) explained a large fraction of variation in clinging ability among species (> 92% between absolute values, > 49% between relative values). Furthermore, once the effects of pad area are removed, body mass is not correlated with clinging ability, which indicates that pad area,
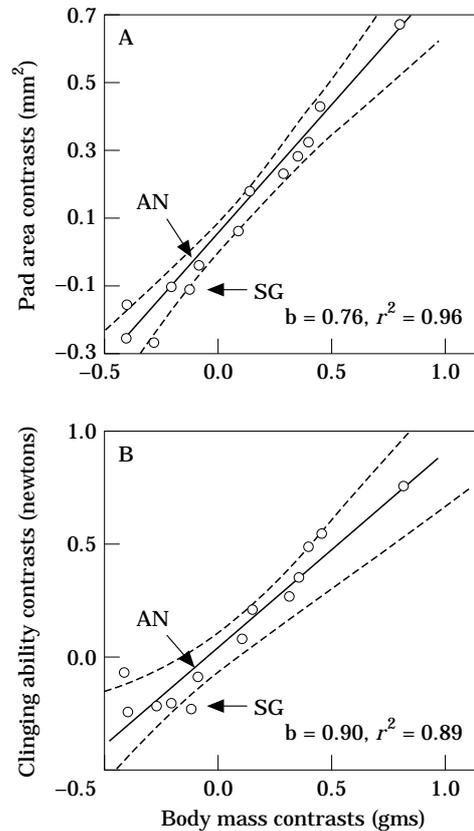

Figure 2. A, pad area contrasts (y-axis) vs. body mass contrasts (x-axis) for 14 lizard species. Each point represents an independent contrast for each variable. This plot, as well as those in (B) and Fig. 3A, B are derived from the same phylogenetic analysis. However, because results change only slightly among different phylogenetic analyses (Table 2), these plots can be considered representative of all analyses. All regression lines in plots are from ordinary least squares regressions. Dotted lines are 95% confidence bands. AN = contrast between the anole clade and the ancestor of all other taxa. SG = contrast between the skink and gecko clades. B, clinging ability contrasts (y-axis) vs. body mass contrasts (x-axis) for 14 lizard species.

rather than body size *per se*, is causally related to clinging ability. Clearly, when the effects of body size are not removed, pad area is the primary causal factor behind variation in clinging ability (excluding the unlikely possibility that another, unknown factor closely correlated with pad area is the causal agent behind variation in clinging ability). Nevertheless, once the effects of body size are removed, approximately 50% of the variation in clinging ability remains unexplained, indicating that factors in addition to pad area are influencing clinging ability.

Several possible factors may account for this unexplained variation in relative clinging ability. First, geckos, skinks and anoles are known to differ in the microscopic adhesive structures on their pads (Ruibal & Ernst, 1979; Williams & Peterson, 1982; Austin, unpubl.). If these structural designs differ in their adhesive qualities (i.e. one pad design is superior than another in its clinging properties), then the effects of pad area might be overshadowed by these functional differences. The effect of such functional differences would be to diminish the correlation between relative pad area and relative clinging ability. However, the small sample size for each pad design
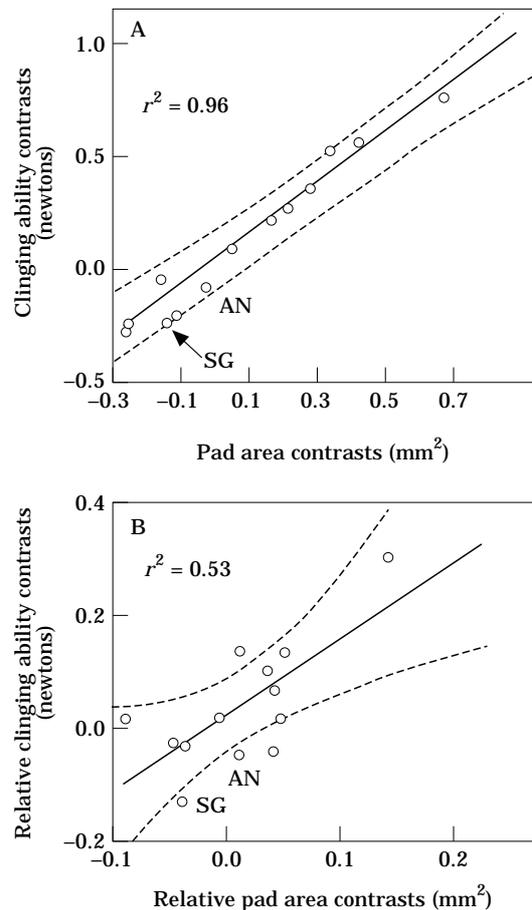

Figure 3. A, non size-adjusted clinging ability contrasts (y-axis) vs. non size-adjusted pad area contrasts (x-axis) for 14 lizard species. See Fig. 2A, B for details, B, size-adjusted clinging ability contrasts (y-axis) vs. size-adjusted pad area contrasts (x-axis) for 14 lizard species.

TABLE 2. Coefficients of determination (r^2 values) and slopes as derived from phylogenetic analyses of relationships among pad area (mm^2), clinging ability (newtons) and body mass (gms) among 14 lizard species. The numbers in the 'Speciational' and 'Extended branches' columns are ranges of either r^2 values or slopes. See text for details

Relationship	Speciational	Extended branches	P_H[#]	P_L[#]
Relative clinging ability vs. relative pad area*	0.52 – 0.59	0.49 – 0.58	<0.01 (12)[‡]	<0.01 (12)
Clinging ability vs. pad area*	0.92	0.92 – 0.96	<0.01 (12)	<0.01 (12)
Pad area vs. body mass[†]	0.75 – 0.78	0.75 – 0.77	<0.01 (9)	<0.01 (9)
Clinging ability vs. body mass[†]	0.90 – 0.95	0.88 – 0.90	>0.50 (9)	>0.20 (9)

*=Coefficients of determination (r^2 values).
[†]=Slopes.
[‡]=Degrees of freedom (See Clarke [1980], McArdle [1988] for calculation of degrees of freedom for t-tests).
[#]=For regressions, P values are one-tailed significance tests. For slopes, P values were calculated by comparing t-statistics against t-tables (Rohlf & Sokal, 1981). P_H are probability values for extreme high values whereas P_L are probability values for extreme low values.

examined here (six representatives of the gecko pad design, four of the anole pad design and three different pad designs within the four skinks examined [Williams & Peterson, 1982; Austin, unpubl.]) make testing of such functional differences difficult. Clearly, more samples of each pad design are needed to test the hypothesis that differences in pad structure affect clinging ability (see e.g. Garland *et al.*, 1993). In a similar vein, differences among lizard taxa in foot anatomy may also influence clinging ability (see e.g. Russell [1975, 1976]). Of course, differentiating between pad design and foot anatomy as causal factors of clinging ability will prove to be difficult, as these characteristics may be correlated among higher taxonomic groups (i.e. between geckos, anoles and skinks).

One of the primary assumptions in performance studies is that measures of performance are ecologically-relevant to the organisms under study (Arnold, 1983; Hertz, Huey & Garland, 1988; Garland & Losos, 1994). Under what ecological circumstances might clinging ability be critical to fitness? Unfortunately, as in most studies of performance (see Hertz *et al.*, 1988; Garland & Losos, 1994), little data are available concerning when lizards use their maximal performance capabilities in nature. Still, many pad-bearing lizards spend a large fraction of their time moving on vertical or overhanging surfaces and often capture prey or escape predators when moving on such surfaces (D. Irschick, unpubl.). Intuitively, we would expect maximal capabilities such as clinging to be relevant in such contexts. Nonetheless, no studies have demonstrated any causal relationship between pad area, clinging ability and fitness although Hecht (1951) has suggested that directional selection for lamellae number (the number of scales under each pad) occurs in the gecko *Aristelliger praesignis*. Whether lamellae number is correlated with either pad area or clinging ability in lizards remains to be demonstrated, however.

Scaling of pad area and clinging ability to body mass

Because lizards must support their weight while climbing vertical substrates, one might expect that selection would favour functional similarity in clinging ability

across lizards of different body sizes. Alternatively, evolution of increased body size would be either a benefit (if clinging ability scales to body mass by an exponent > 1.0) or a detriment (exponent < 1.0) for the ability of a lizard to support its weight while climbing. Among the 14 species examined here, clinging ability scales very close to, but slightly lower than, the slope expected under functional similarity. In contrast to our *a priori* predictions, however, pad area scales to body mass by a lower slope than clinging ability. Therefore, as body size increases, clinging ability increases at a faster rate than pad area. This lack of correspondence between the scaling of pad area and clinging ability to body mass is puzzling because only a small fraction of the variation in absolute clinging ability (between 4% and 8%) is unexplained by pad area. Nonetheless, factors other than pad area must enable these lizards to maintain functional similarity despite variation in body size.

In another comparative study of scaling between pad area and body mass, Bauer and Good (1986) determined that the slope for this relationship (among species in the lizard genus *Gekko*) was lower (0.59), not higher as in our study (0.75–0.78), than the expected slope of 0.67 based on isometry. Because of the scale of comparison differs greatly between their study and ours (i.e. species within a genus vs. an among and within family comparison), one might expect differences based on this factor alone. Nevertheless, why the two estimates differ so substantially is not obvious. Bauer and Good (1986) suggested that the negative allometry observed in their study might be evidence of 'overdesign' in small geckos, but the high value observed here does not support that hypothesis. Clearly, more work needs to be done to examine scaling relationships at different hierarchical levels within pad-bearing lizards.

Phylogenetic analyses

Recent workers have emphasized that phylogenetic information is necessary to accurately estimate scaling relationships (Martins & Garland, 1991; Harvey & Pagel, 1991; Pagel, 1993). The data presented here provide a clear example of the manner in which a non-phylogenetic regression can provide an inaccurate result. A non-phylogenetic regression of clinging ability vs. body mass reveals a slope of 0.80 (SE = 0.25, Fig. 4), a value substantially lower than slopes derived from phylogenetic analyses (Table 2). Inspection of Figure 4 reveals that the lower non-phylogenetic slope results primarily from the inclusion of the four skinks, all of which are poor clingers relative to body mass. Because the poor clinging ability of these skinks probably evolved once in a common ancestor, inclusion of all four species as independent data points likely overestimates the importance of this single evolutionary event and thus results in the regression line being pulled downwards (Fig. 4). By contrast, the independent contrasts method more accurately represents the evolution of poor clinging ability in skinks as having occurred once. Because this evolutionary event is counted once, it has a much smaller effect on the regression line, thus resulting in a higher slope.

The advent of phylogenetic approaches has also led to discussion about how one should incorporate phylogenetic uncertainty (i.e. polytomies) into comparative studies (Grafen, 1989, 1992; Maddison, 1989; Harvey & Pagel, 1991; Losos, 1994; Martins, 1996). Central to this debate is the possibility that correlations or slopes can change radically when different phylogenies are used. The data here indicate that the effects of variation in topology, and to a lesser extent branch lengths, on correlation
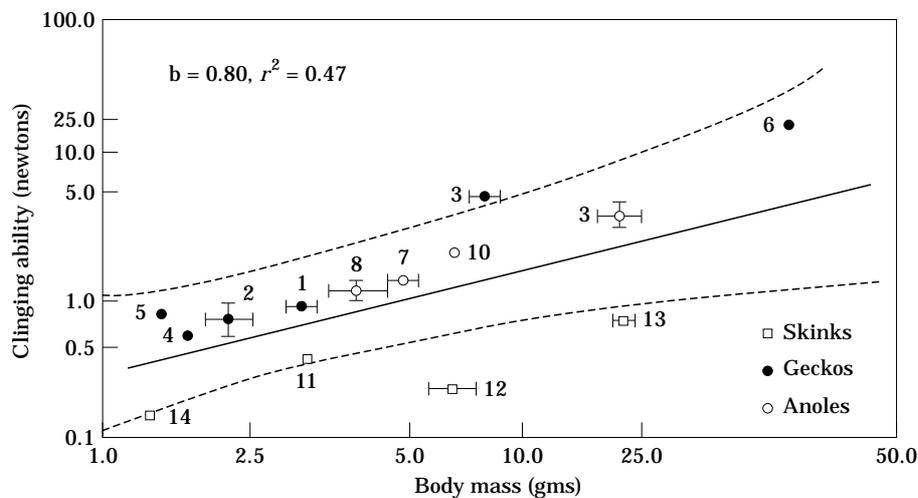
Figure 4. Clinging ability (y-axis) vs. body mass (x-axis) for 14 lizard species. Each point represents a mean value (± 1 SE) for a species. Where there are no error bars, standard errors are too small to be seen on the plot. Different taxonomic groups are represented by symbols. 1 = *Hemidactylus frenatus*, 2 = *H. turcicus*, 3 = *Gehyra oceanica*, 4 = *G. mutilata*, 5 = *Lepidodactylus lugubris*, 6 = *Gekko gecko*, 7 = *Anolis carolinensis*, 8 = *A. sagrei*, 9 = *A. leachi*, 10 = *A. grahami*, 11 = *Prasinohaema virens*, 12 = *P. prehensicauda*, 13 = *P. flavipes*, 14 = *Lipinia leptosoma*.

and slope values are small. However, all phylogenetic analyses provided substantially different results from a non-phylogenetic analysis. In addition to the above example, the slope between pad area and body mass was substantially lower (b = 0.69, SE = 0.23) and the correlation between relative pad area and relative clinging ability was inflated ($r = 0.85$) in non-phylogenetic analyses, indicating that testing multiple phylogenetic hypotheses provides a much more powerful approach than ignoring phylogeny altogether.

By gathering functional and morphological data within a phylogenetic framework, one can rigorously test *a priori* predictions about relationships between form and function. Here, we tested several predictions about relationships between body mass, pad area and clinging ability. Naturally, the results outlined here should be viewed with caution. The 14 species examined represent only a small fraction of the diversity of the gecko, anole and arboreal skink clades. Larger samples from each of these clades are needed to further test the predictions put forth here.

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