Do morphological adaptations for gliding influence clinging and jumping?

Morphological adaptations for gliding

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Abstract

The ability to glide has evolved in multiple taxa and is usually associated with highly specialized morphological adaptations. Traits that evolve for one reason, can, however, influence other traits and abilities. In this study, we examined the influence of adaptations for gliding on the jumping and clinging abilities of frogs by comparing specialized flying frogs (*Rhacophorus*) and unspecialized nonflying frogs (*Polypedates*) from the family Rhacophoridae. Flying frogs had more webbing, longer legs, and greater clinging abilities than nonflying frogs. Clinging abilities, jumping distance, and gliding distance all correlated positively with body size for both flying and non-flying frogs. We did not, however, find any significant differences between the two groups in horizontal jumping distance or glide distance from a low starting point of 1.56 m. The morphological adaptations that evolved for gliding have not significantly influenced the flying frogs' short-distance jumping abilities, but may have influenced their clinging abilities. Alternatively, there may have been direct selection for clinging ability because of flying frogs' increased need, compared with other frogs, to land on vertical surfaces without slipping off.

Key words: Adhesion; Borneo; Evolution; Jumping ability; Rhacophoridae; Size

Introduction

The ability to glide has evolved independently numerous times (Emmons and Gentry 1983; Dudley et al. 2007; Heinicke et al. 2012; Socha et al. 2015). In most cases, gliding ability is associated with adaptive morphological features such as long leg bones (Runestad and Ruff 2005), skin flaps and patagia (Thorington and Heaney 1981), and increased surface area of inter-digital webbing (Emerson and Koehl 1990, Emerson et al. 1990; Young et a. 2002); however, the degree of specialization varies greatly, and some gliding animals are more adept than others. Nevertheless, even a modest gliding ability appears to be adaptive for protecting animals from injury when falling, providing an escape mechanism, assisting in the pursuit of prey, or all three (Savile 1962; Holms and Austad 1994; Byrnes and Spence 2011). These abilities may also expand the range of accessible habitat (Stewart 1985; Dudley et al. 2007).

To better understand the roles of adaptations for specific functions, it can be useful to compare related species that differ in their degree of specialization, and the frogs of the family Rhacophoridae provide an excellent opportunity to do this. This family contains several non-specialized frogs in the genus *Polypedates*, which have small amounts of webbing that are likely capable of generating some lift and controlled descent, but they lack extensive webbing or folds. Rhacophoridae is, however, most famous for its highly specialized "flying frogs" in the genus *Rhacophorus* (Emerson and Koehl 1990; Dudley et al. 2007). These frogs are well known for their proficiency as gliders, and they exhibit adaptations such as enlarged hands and feet, extensive webbing between all fingers and toes, and accessory skin flaps on the lateral margins of the arms and legs (Inger 1966; Emerson and Koehl 1990; Emerson et al. 1990).

Wind tunnel experiments on models have been used to study the effects of the morphological differences between *Rhacophorus* and *Polypedates*, finding that the adaptations

of flying frogs serve four major roles: (1) increasing the horizontal distance travelled, (2) reducing the minimum speed required to glide, (3) increasing drag, which increases time aloft and lowers the force of impact, and (4) improving maneuverability by reducing the turning radius (Emerson and Koehl 1990; Emerson et al. 1990). Specialized morphology such as increased webbing may also affect other mobility performance variables such as horizontal jumping and gripping ability, but the influence of "flying" morphology on these variables has not been examined.

One of the major determinants of frog jumping ability is rear leg length relative to body size (Rand 1952; Gray 1968; Zug 1972; but see Emerson 1978), and many gliders exhibit elongate limbs (Runestad and Ruff 2005) which may increase their jumping abilities. There are also reasons why gliding frogs may be unable to jump as far as non-gliders, for example, the additional mass and drag from webbing and skin folds could adversely affect horizontal jumping performance in situations where individuals are not jumping from points high enough to require gliding, but the influence of adaptations for gliding on jumping ability has not been studied.

Adaptations for gliding could also affect clinging ability. This could occur either as a byproduct of the adaptations, or as a direct consequence of selection for better clinging. All flying frogs are arboreal and possess adhesive toe pads, which use wet adhesion to adhere to smooth, vertical, or overhanging surfaces, such as leaves (Emerson and Deihl 1980; Green 1981; Hanna and Barnes 1991; Barnes et al. 2006; Barnes et al. 2011; Endlein et al. 2013). Wet adhesion operates by exploiting water's viscosity and surface tension, and its adhesive force is proportional to the wet surface area (Emerson and Diehl 1980; Emerson 1991; Barnes et al 2006). As a result, the exaggerated characteristics of flying frogs such as skin flaps and extensive webbing could affect their clinging abilities by providing a greater surface area for wet adhesion.

Alternatively, because flying frogs often need to land on vertical surfaces without sliding off, there may be selection for features like large toe pads.

We conducted a series of experiments to examine the relationships between flying frog morphology and both jumping performance and shear forces. We predicted that flying frogs would jump relatively farther than their nonflying counterparts, and we expected flying frogs to have greater relative clinging abilities than nonflying frogs.

Materials and Methods

Study sites and species

We collected frogs and conducted trials in December 2013 at the Danum Valley Field Centre, Sabah Malaysia (4.9167˚ N, 117.6667˚ E, datum = WGS84). We captured frogs by hand at night, tested them the following morning, and released them at their capture locations within 24 hours of capture. To minimize confounding variables, we conducted all tests indoors at temperatures between 27.4–30.4 ºC.

We used six species of arboreal frogs from the family Rhacophoridae. Three species (44 individuals) were in the genus *Rhacophorus*, and three species (25 individuals) were non-gliding frogs in the genus *Polypedates* (Table 1). The *Rhacophorus* spp. all exhibited the morphological features of a flying frog, including enlarged hands and feet, extensive inter-digital webbing, and accessory skin flaps, whereas the *Polypedates* spp. did not exhibit flying frog morphology, with minimal inter-digital webbing and no accessory skin flaps. Despite these morphological differences, all species were similar in general body shape and habits, and a previous examination of adaptations for gliding similarly compared several species from the genus

Rhacophorus to several species of *Polypedates* (Emerson and Koehl 1990). All data are available in S1 Data.

Table 1. The number of individuals of each species examined.

Numbers of individual frogs of various species available after removing outliers (performance data points that were two standard deviations or more below the mean). All performance metrics = individuals with no missing data for performance responses.

	Total	SUL, mass, and leg length	Pad area and web area	Glide distance	Jump distance	Shear force	All performance metrics
Rhacophorus dulitensis	2	2	2	1	2	2	1
Rhacophorus nigropalmatus	3	3	2	2	2	2	1
Rhacophorus pardalis	39	39	37	38	36	37	35
Polypedates leucomystax	8	8	8	5	7	7	5
Polypedates macrotis	6	6	6	3	4	6	3
Polypedates otilophus	11	11	11	11	10	11	10

Morphology

For each individual, we used dial calipers to measure the snout to urostyle length (SUL) and the proximal (body to outside of knee) and distal (outside of knee to the joint of the ankle) lengths of the right hind limb. We used spring balances (Models PESO 10010, 10050, 10100, Pesola, Switzerland) and placed frogs in pre-weighed plastic bags to measure mass to the nearest 0.5 g. We also measured the webbing area and toe pad area of the front and rear limbs on the right side, with the limbs extended and the webbing maximally splayed. To make those measurements, we placed frogs on 1-mm graph paper on glass and illuminated them, then photographed them and analysed them in ImageJ (Schneider et al. 2012). Briefly, we used a scale bar derived from the squares on the graph paper (using the 'set scale' feature), drew around

the webbing area, or each toe pad (using the 'polygon selections' tool), and summed the areas provided by ImageJ using 'analyse' and 'measure' features. Webbing area and toe pad area are provided in the data set (S1 Data). For statistical analyses, the front and rear limb measurements were combined for leg length, pad area, and webbing area.

Horizonal jump distances

To measure horizontal jumping ability, we placed frogs on the ground and encouraged them to jump by gently tapping their rear legs and the posterior portion of their bodies with the blunt end of a pencil. After a frog jumped, we measured the horizontal distance from its starting position to the position where it landed (measured at the posterior of its urostyle). We encouraged each frog to jump three times in succession and used the maximum distance from the three tests in the analyses (some frogs only jumped twice; we did not include frogs that jumped only once). We used the maximum rather than the average both because fatigue can affect the results of multiple jumps and reduce the mean, and because we were interested in quantifying the frogs' maximum performance potential (Emerson 1978; Watkins 1997). Previous work has shown that increasing the number of replicates per individual results in a more accurate estimate of maximum performance (Adolph and Pickering 2008); however, we were constrained to only three replicates per individual because frogs became fatigued. Therefore, we limited our replicates to avoid undue stress on the animals and allow us to release them within 24 hours of capturing them. Three replicates were sufficient to provide a good estimate of the frogs' performance (modelling in S2).

'Glide' distance from a low starting point

To measure gliding ability from a low starting point, we placed frogs on a podium (1.56 m high) and encouraged them to jump off by gently tapping their rear legs and the posterior portion of their bodies with the blunt end of a pencil. After a frog jumped, we measured the horizontal distance from the frog's starting position (measured from the base of the podium, which was aligned with the frog's snout) to the position where it landed (measured at its posterior). In preliminary trials, the frogs were reluctant to jump unless they had an elevated target to aim for. Therefore, we placed a semi-circle of chairs outside of the maximum jump distance (approximately 3 m) to provide them with vertical targets. We encouraged each frog to jump three times and used the maximum distance of the three tests in the analysis (some frogs only jumped twice; we did not include frogs that only jumped once).

The purpose of this experiment was to test whether or not the morphological features that are associated with gliding would allow flying frogs to travel farther when jumping from a sub-optimal, 'non-gliding' height. In other words, it is well known that flying frogs can travel farther than nonflying frogs when jumping from very high up, but the adaptations for gliding may not offer an advantage when the starting point is not very high. Indeed, the added mass and drag from those traits could even have a negative effect when frogs are jumping from a low starting point. Therefore, we wanted to compare the performance of flying and nonflying frogs when they jumped from a low starting point, and we deliberately chose a platform that we suspected would be sub-optimal for them (in the sense that they couldn't glide from this height) but was still ecologically meaningful. All of the species used in this study are often found less than two meters off the ground, and all individuals in this study were collected from less than two meters. Additionally, at the study site, it is common to see vipers and other snakes foraging 1–2 meters

from the ground. Therefore, jumping ability from this height would be important for escaping predators.

Shear force

We estimated the frogs' clinging abilities by measuring the force required to pull the frogs along a tile surface. To conduct this test, we placed frogs in a fabric harness, which wrapped around the inguinal area like a belt. We then attached the harness to a spring balance, pulled it at a 20˚ angle along a dry tile surface, and recorded the force (measured in grams) necessary to pull the frog backwards (hereafter referred to as the shear force) (Niewiarowski et al. 2008). For each frog, the shear force was only measured once.

Data analysis

Although flying and nonflying frogs do differ morphologically, the species we used overlapped substantially in some or all of the characteristics we measured. We tested for differences between the morphology of our flying (*Rhacophorus*) and nonflying (*Polypedates*) frog species using a permutational MANOVA with 5000 permutations. We structured the analysis with mass, pad area, web area, and leg length as the response variables, group (flying or nonflying) as a fixed factor, SUL as a covariate, and species as the strata (i.e., nesting factor), and we used Euclidian distances. We chose SUL as the covariate rather than mass, because anuran mass can be strongly influenced by food and water intake; therefore, we expected SUL to correlate more closely with the other body metrics. Initially, we included an interaction term between group and SUL, but this was not significant so we removed it. We used the function

"adonis" in the package "vegan" (v2.3-4) (Oksanen et al. 2016) in R (v3.2.3; R Development Core Team, Vienna, Austria) for this analysis. We used the mean square errors and the function "pf" in the package "stats" (v3.2.3; R Development Core Team) to obtain F statistics and P values. We $\log_{10}$ transformed all of the data prior to statistical testing.

Following the perMANOVA, we used post-hoc linear mixed models (lmm) to compare our flying and nonflying frogs for each of the individual response variables used in the MANOVA (Hochberg and Tamhane 1987). For each model, SUL was included as a covariate and species was nested under group. We used the function "lmer" in the R package "lme4" (v1.1-11) (Bates et al. 2015) to construct the models, and we calculated P values using the function "Anova" in the package "car" (v2.1-1) (Fox and Weisberg 2011). Finally, for each lmm we used the function "effect" in the package "effects" (v3.0-7) (Fox 2013) to visualize the main effect and 95% confidence intervals for flying and nonflying frogs for the body metric being examined by the lmm.

We used the same analytical methods to compare the performance abilities of flying and non-flying frogs (i.e., the response variables were horizontal jump distance, glide distance, and shear force). For these analyses, we used mass as the covariate rather than SUL. This decision was made *a priori* and was based on the fact that mass should have a greater impact on jumping abilities than does SUL (i.e., mass is affected by things like recent food intake, whereas SUL is not). Initially, we included an interaction term between group and mass, but this was not significant so we removed it. Additionally, for performance measures, we removed any individuals with a response that was two standard deviations or more below the mean (this was done on the log transformed data, separately for flying and nonflying frogs, and separately for each performance response). We did this because we were measuring maximum performance,

and individuals with a measured maximum well below the mean likely did not exhibit their true maximum performance. Therefore, we were concerned that these data points were unreliable and would bias the data (this is also the reason that we did not include individuals who jumped only once).

Several individuals were missing data for one or more of the performance responses or body metrics and had to be removed from the perMANOVAs. Most of the individuals were, however, only missing data for one of the metrics. In some cases, particularly for shear force, using only individuals with complete data resulted in very small sample sizes for some species (Table 1). Therefore, we decided to use all of the available data for the post-hoc lmms rather than limiting the analysis to the same data points that were used in the perMANOVAs. This decision was made *a priori,* and we believe that this is justified because the purpose of the perMANOVAs was to tell us about the population of data from which the samples were collected, and the additional data points used in the lmms were from the same population. Therefore, their inclusion should have simply increased the power of the lmms by increasing the sample size.

Finally, we used linear regression to examine the relative effects of mass on glide distance, jump distance, and shear force (the data were $\log_{10}$ transformed prior to the regression analysis). We aimed to determine whether jumping performance scaled with body size. If performance, relative to body size, did not change with increasing body size, we expected the $\log_{10}$ transformed data to have a slope of one; whereas if relative performance decreased with increasing body size (i.e., larger frogs performed relatively worse than smaller frogs), i we expected the slopes to be less than one. Our prediction was that, in all cases, absolute performance would increase with increasing body size (resulting in positive slopes), but relative performance would decrease with increasing body size (resulting in slopes less than 1 but greater

than zero). Further, if performance scaled isometrically with body size, then for glide distance and jump distance, we expected an isometric slope of 0.33 because we expected distance to scale linearly, while mass scaled exponentially (as a cubed relationship). For shear force, however, we expected an isometric slope of 0.67 because we expected shear force to be determined by pad area (which should have scaled as a squared relationship), while mass scaled as a cubed relationship (Smith et al. 2006).

To test whether slopes were significantly different from one, we determined whether the 95% confidence intervals of the calculated regression lines included a slope of one. Similarly, to assess isometry, we tested whether the expected slopes for isometric relationships fell within the 95% confidence intervals of our regressions.

We did not explicitly include a phylogenetic component in any of our analyses because all flying frogs were in the genus *Rhacophorus,* and all nonflying frogs were in the genus *Polypedates*. Thus, all members within each group were more closely related to each other than to any member of the other group (Garland et al. 2005), making phylogentic analyses uninformative.

Results

The permutational MANOVA on morphological metrics showed that there were significant differences between our flying and nonflying frogs ($F_{1,65}$ = 108.37, P = 0.0005) and among species ($F_{1,65}$ = 3.8, P = 0.0060). There was also a significant relationship between snout-urostyle length (SUL) and other body metrics ($F_{4,65}$ = 773.41, P = 0.0002). The post-hoc linear mixed models showed that when flying frogs and nonflying frogs were compared (with SUL as a covariate), there was no significant difference in mass (P = 0.717) or pad area (P = 0.1358), but

flying frogs had significantly greater rear leg lengths ($P < 0.0001$) and webbing area ($P < 0.0001$). SUL was a significant covariate for all comparisons ($P < 0.0001$; Figs 1 and 2).

Fig 1. Leg length, pad area, and shear forces differed significantly between flying and nonflying frogs.

Main effects and 95% confidence intervals from linear mixed models comparing flying (F; *Rhacophorus*) and nonflying (NF; *Polypedates*) frogs for each parameter of the models (* = statistically significant at $\alpha = 0.05$).

Fig 2. Regressions of body metrics for flying and nonflying frogs.

Snout to urostyle length (SUL) versus (a) mass, (b) toe pad area (front right foot + rear right foot), (c) web area (front right foot + rear right foot), and (d) rear leg length (proximal + distal length) for flying (*Rhacophorus*) and nonflying (*Polypedates*) frogs.

The permutational MANOVA on performance metrics showed that there were significant differences between flying and nonflying frogs ($F_{1,54} = 21.19$, $P = 0.0100$) but not among species within genera ($F_{4,54} = 1.15$, $P = 0.3265$). There was also a significant relationship of performance metrics with mass ($F_{1,54} = 40.03$, $P = 0.0002$). Post-hoc linear mixed models showed that flying frogs had significantly greater shear forces than nonflying frogs ($P = 0.0319$) and that mass was a significant covariate for all comparisons (gliding: $P = 0.0002$, jumping: $P = 0.0432$, shear force: $P < 0.0001$), but there were no significant differences between flying and nonflying frogs for gliding ($P = 0.9081$) or jumping ($P = 0.7607$) distance.

The slopes of the regression lines of performance against mass were as follows: flying frog glide distance = 0.261, nonflying frog glide distance = 0.077, flying frog jump distance = 0.173, nonflying frog jump distance = 0.067, flying frog shear force = 0.648, and nonflying frog shear force = 0.605 (Fig 3). For all regressions ($\log_{10}$ transformed), a line with a slope of one did not fall within the 95% confidence intervals. Thus, there was a negative relationship between size and relative (proportional) performance for all tests (i.e., performance per unit of mass was lower in larger frogs than in smaller frogs). For shear force, the expected isometric relationship (slope = 0.67) was contained within the 95% confidence intervals of both groups of frogs. However, for glide distance and jump distance, only the expected isometric slope for flying frog glide distance was contained within the 95% confidence intervals of the regressions. Further, it was near the edge of the confidence intervals and, after accounting for multiple comparisons, may not represent a true result.

Because our data contained only a few large flying frogs, there was concern that those individuals might bias the data. To address this, we performed a sensitivity analysis by systematically removing them and checking the slopes of the new regressions. They did not deviate strongly from the regressions with all individuals, suggesting that our data were robust (Appendix S2).

Fig 3. Regressions of performance metrics for flying and nonflying frogs.

Mass versus (a) maximum glide distance, (b) maximum jump distance and (c) shear force (i.e., the force required to pull a frog backwards on a tile) for flying (*Rhacophorus*) and nonflying (*Polypedates*) frogs. Shaded areas are 95% confidence intervals. Grey lines showed the expected slopes under isometry.

Discussion

Morphology

After accounting for body size, flying frogs had significantly greater interdigital webbing and longer rear legs than nonflying frogs. It is well known that flying frogs' extensive interdigital webbing, along with other adaptations, acts as airfoils that give them their name-sake gliding abilities (Emerson and Koehl 1990). Similarly, long legs have also been documented in other gliding species (Runestad and Ruff 2005). Pad area was not significantly different between flying and nonflying frogs.

Jumping and gliding

We did not find any significant differences between flying and nonflying frogs with regards to either horizontal jumping ability or jumping ability from a low starting point). Emerson and Koehl (1990) found that nonflying frogs were actually capable of traveling farther than flying frogs if they kept their limbs outstretched; however, when both species held their limbs bent beside the body (in the position described by Stewart (1985)), flying frogs glided farther, and both species increased their maneuverability. During our trials, all frogs moved their limbs to the side of their body and assumed the typical gliding posture. Thus, we expected the flyers to travel farther than the nonflying frogs.

The fact that we did not find a significant difference may be related to minimum glide speed (i.e., the lowest speed at which an animal is capable of gliding). Multiple studies on both flying frogs and other gliding animals have noted that after jumping, animals go through an

initial falling phase (sometimes called a ballistic dive) before they begin to glide (Abdulali and Sekar 1988; Emerson and Koehl 1990; McGuire 2003; McGuire and Dudley 2005; Socha et al. 2005; Bahlman et al. 2013). Presumably this falling phase accelerates them to the minimum glide speed, but because our frogs jumped from such a low starting point (either 1.56 m or horizontal jumps on the ground), they may not have been able to build up enough speed to exit the falling phase and enter the gliding phase. Indeed, Young et al. (2002) found that flying geckos (*Ptychozoon kuli*) were not able to glide when dropped from a low point (3 m) but could glide when dropped from 8 m, and they calculated that the lizards needed to be moving at 650 cm/sec to be able to glide. Thus, the adaptations for gliding may not offer any advantages (in terms of distance travelled) when jumping from low starting points. It would be interesting for future studies to examine this further by using a range of starting points to examine longer trajectories and identify the point at which the glide deviates from the ballistic trajectory.

Although it makes sense that flying frogs can only take advantage of their adaptations at certain heights, it is still somewhat surprising that they were unable to jump farther than nonflying frogs, because we found that they had relatively longer rear legs, and greater rear leg length is often associated with greater jumping abilities (Rand 1952; Gray 1968; Zug 1972; Emerson 1991). Some studies have, however, found that leg length alone is not always an accurate predictor of jumping ability, and both the amount of extensor musculature and the ability to store energy in tendons and ligaments can affect jumping ability (Bennet-Clark 1977; James et al. 2007; Roberts et al. 2011; Astley and Roberts 2012). Thus, leg length in flying frogs may not confer an advantage in terms of jump distance, but instead may have more to do with steering during flight than jumping distance. Also, flying frogs' skin folds increase drag (Emerson and Koehl 1990), and could, conceivably, reduce jumping distance.

We also found that the absolute distance for both horizontal jumps and jumps from 1.56 m increased with frog body size. According to the theoretical model put forth by Hill (1950), frogs of similar morphology should jump the same absolute distance, regardless of their body size. In contrast, experimental results have generally shown that larger frogs jump farther than smaller frogs (Rand and Rand 1966; Zug 1972; Dobrowolska 1973; Emerson 1978; Licht 1986; Gomes et al. 2009), and our results are consistent with those studies. Also, consistent with previous studies, our regression analyses revealed that, relative to body size, jump distance decreased with mass for both tests (Emerson 1978; Zug 1978; Gomes 2009). A decrease in relative jump distance with mass is expected because jumping and gliding distance are expected to increase linearly, while mass, and the force required to move that mass, is expected to have a cubed relationship with length measures (in the non-transformed data). Nevertheless, with the possible exception of glide distance in flying frogs, we did not find the expected isometric slopes of 0.33. Instead, our frogs exhibited negative allometry, with slopes that were significantly lower than 0.33. In other words, as body size increased, absolute performance increased as an exponent of mass, rather than the expected linear relationship (the exponents were the slopes of the log plots described previously), resulting in lower relative performance than we has expected.

Shear force

Our results suggest that flying frogs can impart greater shear forces than nonflying frogs. This makes sense because flying frogs appear to aim for vertical surfaces such as overhanging leaves and branches when jumping and gliding (Stewart 1985; LS pers. obs), and the ability to minimize sliding of the toe pads would be extremely advantageous for landing safely (Bijma et al. 2016). The proximate cause of the difference is, however, not entirely clear. Frogs attach to

smooth surfaces *via* wet adhesion, which is area-dependent (Emerson and Diehl 1980; Green 1981; Hanna and Barnes 1991), but we did not find that flying frogs had significantly larger pads than non-flying frogs, and we measured friction rather than adhesion. It is tempting to postulate that exaggerated morphological features like flying frogs' increased webbing might have been responsible for the increase in adhesive abilities; however, almost none of the webbing contacts the substrate when flying frogs are sliding (Endlein et al. 2013), so this feature probably does not provide increased shear forces. Another possibility is that the increased shear forces may originate from differences in toe pad morphology rather than size. Several studies have found that pad morphology strongly affects gripping strength, with highly arboreal species possessing more specialized pads (Welsch et al. 1974; McAllister and Channing 1983; Green and Simon 1986; Hertwig and Sinsch 1995; Smith et al. 2006). Because we did not examine pad morphology in this study, we cannot rule out the possibility that flying frogs have evolved specialized pads; however, this would be an interesting topic for future studies to investigate.

Shear force increased with mass in our study, which is noteworthy because most studies have found that the clinging abilities of tree frogs are negatively correlated with mass (Emerson and Diehl 1980; Emerson 1991; Barnes et al. 2006). This apparent contradiction is, however, simply a difference in measurement methods. Most previous studies have placed frogs on a rotating plate and recorded adhesive ability as the angle at which the frog detaches, which is actually a measure of adhesive ability relative to mass (Emerson and Diehl 1980; Emerson 1991). In contrast, our method measured the absolute adhesive abilities of the frogs, and because larger frogs have greater surface areas for wet adhesion, we would expect them to maintain greater absolute shear forces. In contrast, our regression analyses (which looked at relative adhesive abilities) showed that larger frogs maintained relatively lower shear forces than smaller

frogs, which is consistent with previous studies. Also, unlike our other performance measurements, our regressions showed the expected isometric relationships.

Conclusions

Our results suggest that flying frogs and nonflying frogs do not differ in either their jumping abilities or their abilities to glide when jumping from a low starting point. This suggests that the adaptations for gliding are neither advantageous nor disadvantageous for horizontal travel unless they begin from a sufficiently high starting point. Our data do, however, suggest that flying frogs have significantly greater adhesion than nonflying frogs, which is a trait that would be greatly advantageous for landing on vertical surfaces without slipping off.

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Appendices

S1 Data. Frog data set.

S2 File. Discussion of repeated measurements tests. Description of the script used for testing the effects of repeated performance measurements, as well as a figure and discussion of the results.

S3 Script. R script for comparing the effects of repeated measurements.

Figure Captions

Fig 1. Leg length, pad area, and shear forces differed significantly between flying and nonflying frogs.

Main effects and 95% confidence intervals from linear mixed models comparing flying (F; *Rhacophorus*) and nonflying (NF; *Polypedates*) frogs for each parameter of the models (* = statistically significant at $\alpha = 0.05$).

Fig 2. Regressions of body metrics for flying and nonflying frogs.

Snout to urostyle length (SUL) versus (a) mass, (b) toe pad area (front right foot + rear right foot), (c) web area (front right foot + rear right foot), and (d) rear leg length (proximal + distal length) for flying (*Rhacophorus*) and nonflying (*Polypedates*) frogs.

Fig 3. Regressions of performance metrics for flying and nonflying frogs.

Mass versus (A) maximum glide distance, (B) maximum jump distance and (C) shear force (i.e.,

the force required to pull a frog backwards on a tile) for flying (*Rhacophorus*) and nonflying (*Polypedates*) frogs. Shaded areas are 95% confidence intervals. Grey lines show the expected slopes under our predictions for isometry.

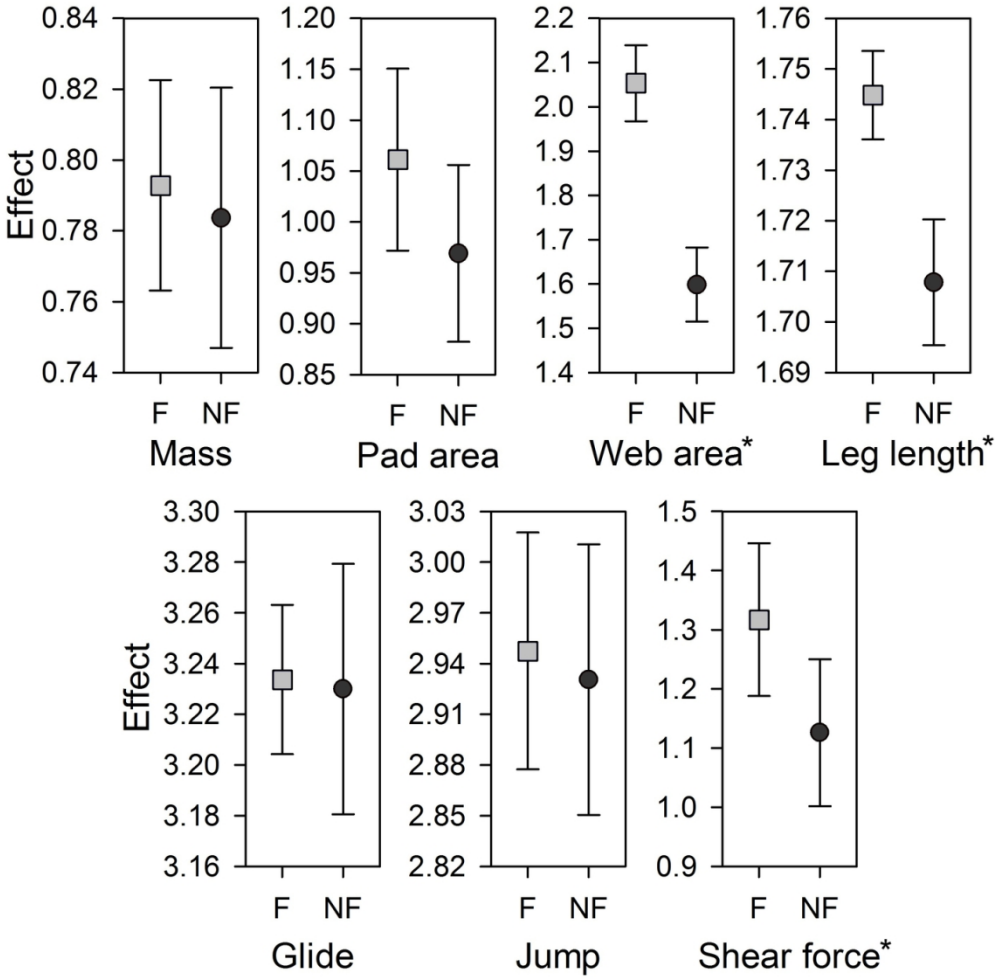

Fig 1. Leg length, pad area, and shear forces differed significantly between flying and nonflying frogs. Main effects and 95% confidence intervals from linear mixed models comparing flying (F; Rhacophorus) and nonflying (NF; Polypedates) frogs for each parameter of the models (* = statistically significant at α = 0.05).

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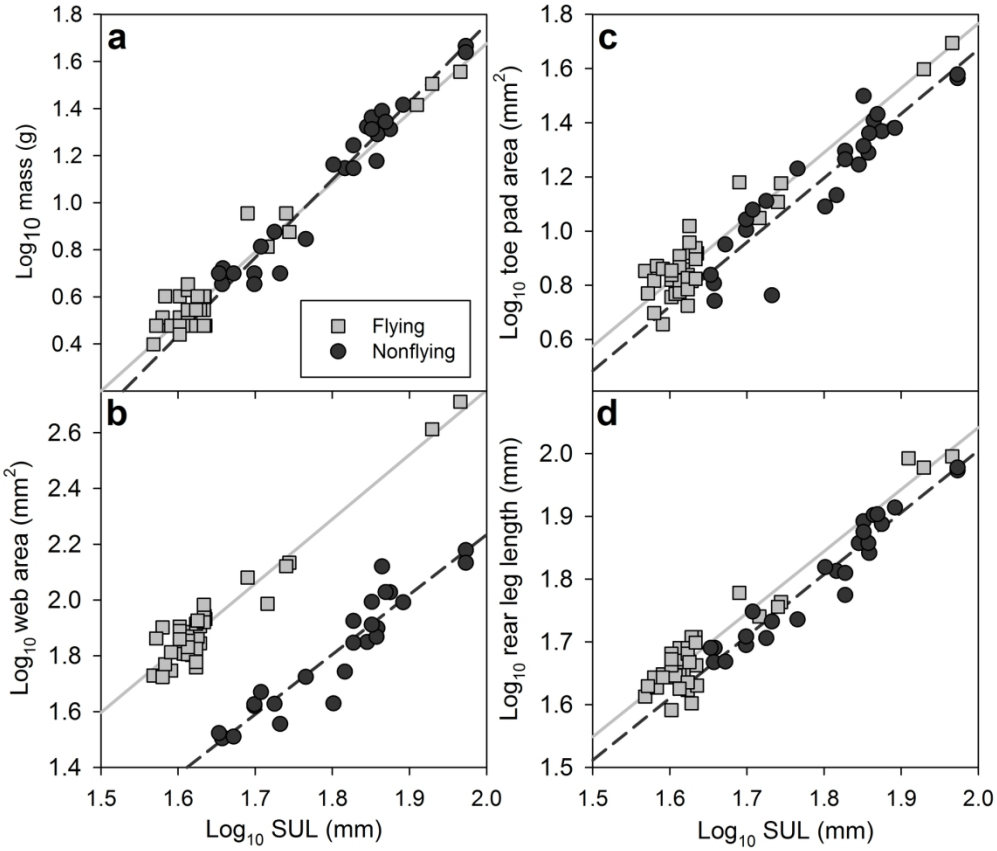

Fig 2. Regressions of body metrics for flying and nonflying frogs.
Snout to urostyle length (SUL) versus (a) mass, (b) toe pad area (front right foot + rear right foot), (c) web area (front right foot + rear right foot), and (d) rear leg length (proximal + distal length) for flying (Rhacophorus) and nonflying (Polypedates) frogs.

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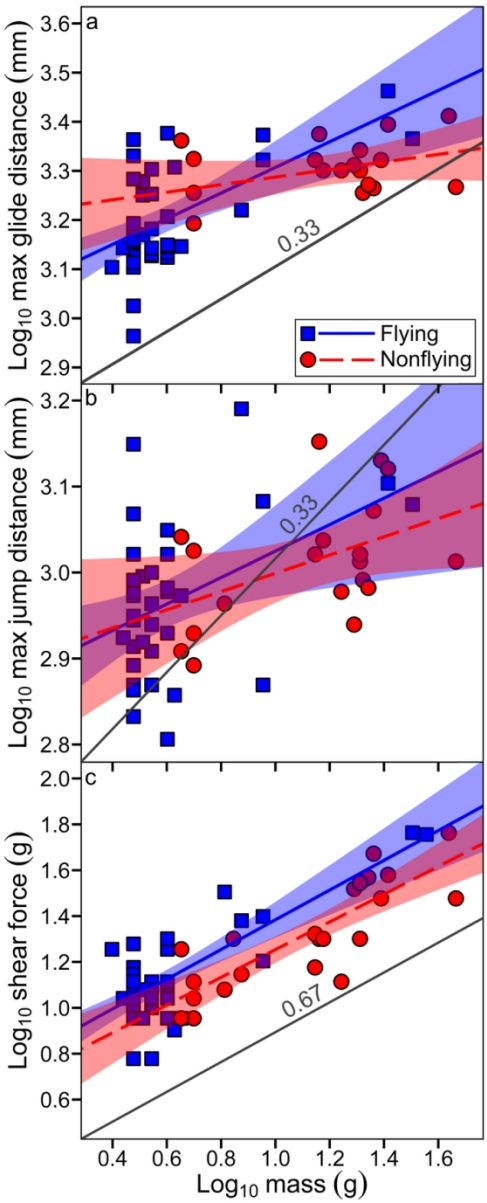

Fig 3. Regressions of performance metrics for flying and nonflying frogs.
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29x73mm (600 x 600 DPI)