

# Structural correlates of increased adhesive efficiency with adult size in the toe pads of hylid tree frogs

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**Abstract** Tree frogs are able to climb smooth, vertical substrates using specialised toe pads which adhere via an area-based wet adhesive mechanism. Although the link between pads and arboreality in frogs is well-established, few studies have investigated the influence of morphology on adhesion. Trinidadian tree frogs from the genus *Hyla* are geometrically similar. There is a tendency towards comparatively reduced mass in larger species, but toe pad area increases as expected with isometry. As adhesion is area-dependent, forces are affected directly by the increase in mass relative to pad area, and there is a decrease in the ability of larger species to adhere to smooth rotation platforms. However, there is an increase in force per unit area that suggests larger species have more efficient toe pads. Toe pad structure is very similar though there are variations in the details of a number of features. Crucially, although differences in morphology appeared small they had demonstrable effects on adhesive efficiency of the pads. Epithelial cell area correlates positively with frog length and adhesive efficiency, related features of cell density and intercellular channel length correlate negatively. These findings are discussed in relation to the different forces involved in the tree frogs' wet adhesive system.

**Keywords** Tree frogs · Allometry · Toe pad morphology · Wet adhesion · Scanning electron microscopy

## Abbreviations

$F$	force normal to the surface
$F_c$	capillarity
$F_{sa}$	stefan adhesion
$g$	the earth's gravitational acceleration
$h$	meniscal height
IRM	interference reflection microscopy
$m$	mass
$r$	pad radius
SEM	scanning electron microscopy
SVL	snout–vent length
$v$	speed of separation
$\alpha_{\text{detachment}}$	angle at which frogs fall from rotation platform
$\gamma$	surface tension
$\eta$	viscosity

## Introduction

The possession of specialised sub-digital pads (toe pads) has become a taxonomic characteristic in many frog families in which the feature is prevalent, particularly amongst frogs in the families Hylidae, Microhylidae, Centrolenidae, Rhacophoridae and Hyperoliidae (Duellman and Trueb 1994). Many are considered 'tree frogs' due to the high incidence of arboreal lifestyles within these families, though in actuality this term encompasses a range of species with great variability in their levels of arboreality. Toe pads are also found in 'torrent' and 'stream' frogs from the families Leptodactylidae and Dendrobatidae, which live in and around fast-flowing mountain streams. In view of the range of species in which these toe pads are found, it is perhaps surprising that their morphology is remarkably similar

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(Hertwig and Sinsch 1995). The high degree of convergence between toe pads of frogs of different families suggests that these structures have arisen in response to a similar functional requirement. Most frogs with toe pads inhabit environments in which the likelihood of dislodgement from a substrate is increased due to increased exposure to extreme external factors. Furthermore, the ground that is available for attachment is often 'slippery'. Where the result of dislodgement, or excessive slipping is likely to be a significant disadvantage to survival it is to be expected that frogs with structures such as adhesive toe pads will have an advantage.

Adhesive toe pads are distinguished from the non-specialised ventral surface of the toe by the presence of specialised epidermal cells covering an expanded disc at the distal end. The epidermis on the pad can be as much as three times the thickness of the dorsal epithelium (Ba-Omar et al. 2000). Typically, it consists of six to eight cell layers (Ernst 1973a), which differentiate gradually into columnar cells with hexagonal apices, separated from one another by intercellular channels. These cells are uniquely different to any other epidermal cells (Duellman and Trueb 1994). Tonofilament bundles fill densely packed villus-like processes that provide a nanoscale roughness to the flat-topped epithelial cells. The fibres continue into the cells, running parallel to the cells' longitudinal axes, and form a filamentous cytoskeleton which probably confers a degree of rigidity to the cells (Green 1979). Intercellular channels are thought to allow individual cells to find their closest contact with the substrate upon which the pads are placed (Green and Carson 1988). They also act to disperse the watery mucus which is produced in glands that open out onto the surface of the pad, so that it forms a complete but thin layer over the whole of the pad surface (Ernst 1973b; Barnes 1999).

A number of comparative studies have explored the relation of the structural development of the digital area to the levels of arboreality seen between species (Welsch et al. 1974; Green 1981; McAllister and Channing 1983; Green and Simon 1986; Hertwig and Sinsch 1995). These studies concur that 'structural complexity', in terms of the extent of specialisation of cell types seen on the toe, increases according to arboreality. Green and Simon (1986) describe a progressive specialisation of the cells of the toe pads in parallel with increasingly arboreal lifestyles of different species of the Microhylid genus *Cophixalus*. Semi-fossorial species (active under leaf-litter), possess only non-specialised squamous epithelium on toe pads; terrestrial species (active in the open on the ground)

have pads with intermediary cuboidal epithelium; semi-arboreal species have cuboidal cells on accessory adhesive areas and toe pads covered with hexagonal columnar cells; while in highly arboreal species both toe pads and accessory areas are covered by hexagonal columnar cells.

The means by which the structure of the toe pad enhances a frogs' ability to maintain a hold on substrates within their environment has been a source of curiosity since the early 20th century, with many different mechanisms being proposed (Hora 1923; Noble and Jaekle 1928). However, more recent studies of adhesion in a number of species are in agreement that frogs adhere to substrates by the means of an area-dependent wet adhesion system (Emerson and Diehl 1980; Green 1981; Hanna and Barnes 1991; Barnes 1999).

This presents large species of tree frogs with a potential problem: Emerson (1978) comments on the suitability of frogs for allometric studies, quoting Inger (1967) on the uniformity of shape between species: 'a frog is a frog is a frog'. If hylids do have geometric similarity and scale following isometric predictions then, with increasing linear dimensions, mass will increase at a significantly greater rate than toe pad area. Specifically, area (and hence adhesive force) is expected to increase as the square of the linear dimension, whilst mass will increase as the cube. Large species of frog are therefore expected to either show reduced adhesive ability in comparison to small species, or to have proportionally greater area toe pads than small species. In two tree frog families investigated previously, the Microhylidae (Green and Simon 1986) and the Rhacophoridae (McAllister and Channing 1983), larger species were reported to have proportionally larger toe pads which may help them to maintain adhesive ability to around the same degree as smaller species. In contrast, there is no evidence that such a trend exists between adult frogs in Trinidadian hylid species (Barnes 1999; Smith 2003).

If the 'structural complexity' of the pad is correlated with increasing arboreality, it seems reasonable to assume that development of specialised cells is a response to increased pressures to counteract falling and thus confers increased adhesive ability. If this were the case it may be possible for large species to counter the detrimental effects of their lowered area-volume ratio through changes to pad morphology. This study aims to examine whether there is a demonstrable link between pad morphology and adhesive function, through a combination of adhesion measurements and scanning electron microscopy (SEM) studies in species from a single genus, representative of a range of sizes as adult frogs.

## Methods

### Study animals

Seven species of frog from the genus *Hyla* are found in Trinidad (Murphy 1997); *H. boans* (Linnaeus), *H. crepitans* Wied-Neuwied, *H. geographica* Spix, *H. microcephala* Fouquette, *H. minuscula* Rivero, *H. minuta* Peters, *H. punctata* (Schneider). For all species, adult frogs were collected from calling aggregations at breeding sites at various locations on the island. Frogs were taken back to laboratories at the University of the West Indies or to Simla Research Station and maintained at ambient temperature in vivaria with foliage gleaned from their collection sites, fed on crickets and insects daily and misted regularly. Snout–vent lengths (SVL) were measured to 0.1 mm using callipers. Immediately prior to experiments to determine adhesive ability, masses were measured using an electronic balance accurate to 0.01 g. In each species, the toe pads of front and back feet of at least one adult frog were photographed together with a 1-mm square grid using a Polaroid MicroSLR camera attached to a binocular microscope. Toe pad areas were then determined using a digitiser program (Cherry Digitiser).

### Measurement of sticking ability

The adhesive abilities of at least ten frogs from each species were determined following the protocol of Hanna and Barnes (1991) (modified from Emerson and Diehl 1980). Each frog is placed on a ‘rotation platform’ consisting of a Perspex sheet clipped to a wooden board attached to a kymograph spindle. The platform was smoothly rotated from 0° (horizontal) through 90° (vertical) to 180° (upside down) at an angular velocity of 3°/s. The angle ( $\alpha_{\text{detachment}}$ ) at which frogs fell from the platform was recorded. Frogs were caught as they fell and rotations repeated until ten detachments were recorded, and the average calculated. As the mass ( $m$ ) of the frogs was determined immediately prior to rotations,  $\alpha_{\text{detachment}}$  could, according to the simple trigonometry of the experimental set-up, then be used to calculate the maximum adhesive force ( $F$ ) (the force normal to the surface) according to the following equation (Barnes 1999):  $F = \cos(180 - \alpha_{\text{detachment}}) \times m \times g$  where  $g$  = the earth gravitational acceleration, 9.81 m/s<sup>2</sup>.

Frogs were placed on the rotation platform facing ‘head upwards’ as it turned and records were only taken if they maintained this orientation throughout. This precaution was taken because in the Cuban tree

frog, *Osteopilus septentrionalis*, individuals forced to maintain a downwards-facing orientation had impaired sticking ability (Hanna and Barnes 1991). Frogs were prevented from jumping by the light cupping of hands around the frog as the platform rotated. This stopped the frogs from seeing objects onto which they might jump and had a calming effect. Any instances where the frog jumped from the platform rather than fell were not recorded. Many frogs use areas of skin additional to toe pads, on the stomach and upper thighs, when adhering naturally. Frogs were allowed to adhere for an initial observation rotation to determine the natural adhesive behaviours of each species. For rotations used in the calculation of adhesive ability, frogs were prevented from using their stomach and thigh skin by gently prodding the hindquarters—an action which caused the frogs to walk with these accessory areas clear of the rotation platform. This means that all recorded adhesive forces were directly attributable to the toe pads only.

### Scanning electron microscopy

Feet were removed from one or two frogs per species, following adhesive ability determination; numbers killed were kept to a minimum because of concerns over world-wide decline in amphibian populations. Frogs were killed via a lethal dose of Benzocaine. Nine toes from each frog, four from the front and five from the back, were fixed in 2.5% glutaraldehyde buffered at pH 7.4 for 24 h. Specimens were then rinsed in 0.1 M phosphate-buffered sucrose, followed by immersion in buffered 1% osmium tetroxide for 1 h. After washing in distilled water, specimens were dehydrated in an alcohol series and critical point dried. Samples were mounted and gold-coated before viewing with a Philips SEM 500 scanning electron microscope.

### Image analysis

Toe pad cell size for each species was determined from images taken at 1,600× magnification, using Cherry Digitiser and the Scion Image Analysis Programs. Images were taken from a central portion of the pad where curvature of the surface was minimal, and selected to minimise effects of mucosal pore presence, as atypical cells often border these. Ten cells per pad were measured. Cell densities were calculated from counts within a fixed area of an image taken at 1,600× magnification. In the same area, the total length of intercellular channels was measured, and densities determined from this were expressed as perimeter

length per unit area. Mucosal pore size was similarly determined from a number of pores per pad. Pore structure and numbers of cells bordering the lumen were noted. Pore counts were made from whole pad images and translated to pore densities using the area measurements obtained from the living frog. Complexity scores (0–3) were recorded for several structural elements, in particular the degree of elevation and the definition of grooves on the circumferential margin, proximal margins and along the lateral edges of the digits (see Table 2) as follows:

Scores	Elevation	Groove
0	Completely flat (flush with ventral surface of digit)	Not present
1	Slightly raised (raised but not significantly curved)	Shallow
2	Rounded (raised and gently curved)	Defined
3	Globular (raised and semicircular in profile)	Prominent

#### Statistical analysis

All statistics were carried out using SPSS Version 10.05 for Windows (SPSS Inc.). Regressions used linear models unless otherwise stated.

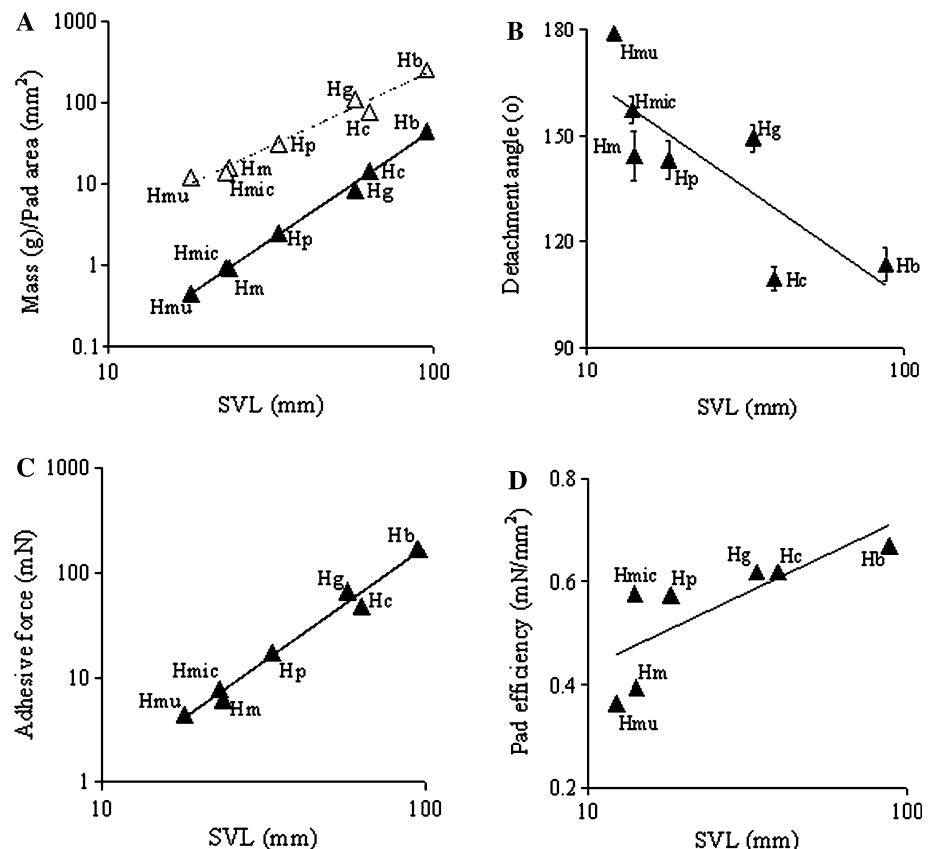
## Results

### Scaling and adhesion

Frogs ranged in size from 20 to 100 mm SVL (Table 2). Allometric relationships between SVL, mass and toe pad area (Fig. 1) showed that frogs had a similar geometry, though there was a tendency for larger species to be proportionally lighter. Thus, toe pad area scaled as  $SVL^{1.88}$  (Fig. 1a—logarithmic model;  $r = 0.99$ ,  $y = 1.88x - 1.36$ ,  $t = 13.42$ ,  $P < 0.01$ , 6 d.f.), at a rate no different to  $SVL^2$ , as would be predicted with isometry (difference from slope of  $2 - t = 0.86$ , N.S. 6 d.f.). Mass, however, increased as  $SVL^{2.71}$  (Fig. 1a—logarithmic model;  $r = 0.99$ ,  $y = 2.71x - 3.76$ ,  $t = 38.14$ ,  $P < 0.01$ , 6 d.f.), at a slightly lesser rate than the increase of  $SVL^3$  expected through isometry (difference from slope of  $3 - t = 4.14$ ,  $P < 0.01$ , 6 d.f.). However, even this lowered rate of mass increase was still significantly greater than the rate of increase in toe pad area across the same range of frog sizes (difference between area and mass— $t = 5.19$ ,  $P < 0.01$ , 12 d.f.).

There were lower mean angles of detachment in large species (Table 1), and a negative correlation between SVL and  $\alpha_{\text{detachment}}$  (Fig. 1b— $r = 0.80$ ,  $y = 173.04 -$

**Fig. 1** Relationships between mass, toe pad area, adhesive ability and body length in seven species of tree frog: **a** log–log plot of toe pad area (open triangles, broken line), mass (filled triangles, unbroken line) vs. snout–vent length (SVL); **b** detachment angles vs. SVL; **c** log–log plot of adhesive force vs. SVL; **d** force per unit area vs. SVL. Statistics in text. Data labels—Hmu *H. minuscula*, Hmic *H. microcephala*, Hm *H. minuta*, Hp *H. punctata*, Hg *H. geographica*, Hc *H. crepitans*, Hb *H. boans*



$0.68x$ ,  $t = 3.00$ ,  $P = 0.03$ , 6 d.f.). There was, therefore, a decrease in adhesive ability in larger species. Adhesive forces calculated from these angles (Table 1) scaled as  $SVL^{2.19}$  (Fig. 1c—logarithmic model;  $r = 0.99$ ,  $y = 2.19x - 2.12$ ,  $t = 15.99$ ,  $P < 0.001$ , 6 d.f.), no different to the increase in toe pad area (difference between slopes— $t = 1.55$ , N.S. 12 d.f.) and lower than the increase in mass (difference between slopes— $t = 3.33$ ,  $P < 0.01$ , 12 d.f.). However, if the effect of area was controlled by considering force per  $mm^2$ , there was a positive relationship that indicated that pads in larger species were more adhesively ‘efficient’ (Fig. 1d— $r = 0.78$ ,  $y = 0.003x + 0.40$ ,  $t = 2.77$ ,  $P = 0.04$ , 6 d.f.).

### Scaling and toe pad morphology

The surface of the toe pad in all seven species was defined by the characteristic presence of a pavement-like arrangement of specialised cells at the distal end of the digit. There was variation in the degree to which the area covered with specialised cells was ‘developed’,

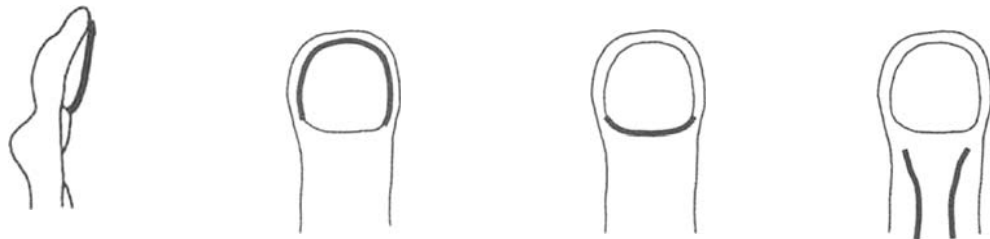
in terms of the grooves delineating its margins and in the elevation of the pad from the ventral surface (Table 2); but there were no correlations between SVL and circumferal grooves ( $r = 0.07$ ,  $t = 0.16$ , N.S. 6 d.f.), proximal margin grooves ( $r = 0.002$ ,  $t = 0.005$ , N.S. 6 d.f.) or lateral grooves ( $r = 0.08$ ,  $t = 0.18$ , N.S. 6 d.f.). There was a tendency towards a flattening of the pad in larger species but this was not statistically significant ( $r = 0.66$ ,  $t = 1.98$ ,  $P = 0.10$ , 6 d.f.).

Cell architecture was highly similar in all species; cells were columnar with hexagonal apices and striated sides. Superficially, cells appeared virtually indistinguishable between species (Fig. 2) but the measurements revealed significant differences in cell areas (Table 3). There was a strong positive correlation between SVL and cell area (Fig. 3a— $r = 0.86$ ,  $y = 86.55 + 0.40x$ ,  $t = 3.81$ ,  $P = 0.01$ , 6 d.f.) so that larger species had significantly larger specialised toe pad cells. Similarly, cell densities were lower in larger species (Fig. 3b— $r = 0.84$ ,  $y = 11,800 - 40x$ ,  $t = 3.51$ ,  $P = 0.02$ , 6 d.f.).

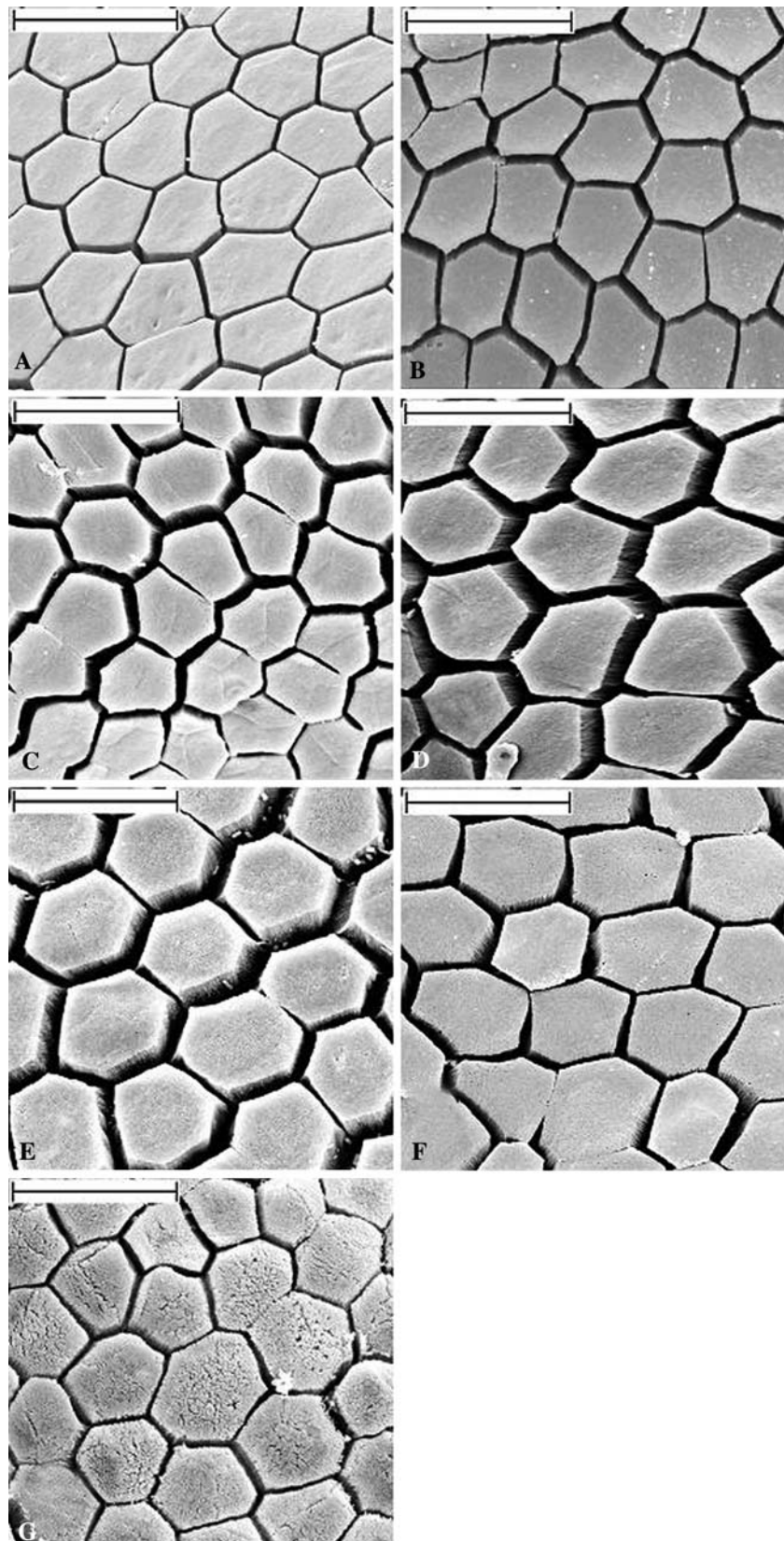
**Table 1** Species average values for morphological parameters [snout–vent length (SVL), mass and toe pad area], detachment angles and adhesive forces in seven species of tree frog

Species	SVL (mm) Mean (s.e.) n	Mass (g) Mean (s.e.) n	Toe pad area ( $mm^2$ ) Mean (s.e.) n	Detachment angle ( $^\circ$ ) Mean (s.e.) n	Adhesive force (mN) Mean (s.e.) n
<i>H. minuscula</i>	18.0 (0.7) 12	0.45 (0.0) 12	12.1 (2.3) 2	179 (0.3) 12	4.4 (0.3) 12
<i>H. microcephala</i>	23.6 (0.8) 12	0.91 (0.1) 12	15.6 (0.0) 1	144 (6.8) 12	6 (0.7) 12
<i>H. minuta</i>	23.1 (0.4) 22	0.93 (0.1) 22	13.5 (1.4) 2	157 (3.8) 22	8 (0.6) 22
<i>H. punctata</i>	33.4 (0.2) 12	2.43 (0.1) 12	30.4 (0.0) 1	143 (5.3) 12	18 (1.4) 12
<i>H. geographica</i>	57.4 (1.7) 14	8.43 (0.5) 14	107.4 (17) 2	150 (3.8) 14	66 (4.0) 14
<i>H. crepitans</i>	63.3 (1.0) 12	14.14 (0.7) 12	76.6 (0.0) 1	110 (3.5) 12	47 (7.6) 12
<i>H. boans</i>	94.9 (1.2) 10	44.72 (2.3) 10	254 (0.0) 1	114 (4.7) 10	170 (27) 10

**Table 2** Complexity scores for pad grooves and elevations in seven species of tree frog

Species				
	Pad elevation Mean (s.e.) n	Circumferal groove Mean (s.e.) n	Proximal groove Mean (s.e.) n	Lateral grooves Mean (s.e.) n
<i>H. minuscula</i>	1.33 (0.08) 18	1.64 (0.10) 18	1.03 (0.08) 18	1.69 (0.13) 18
<i>H. microcephala</i>	1.63 (0.10) 15	2.00 (0.18) 15	0.96 (0.11) 14	1.61 (0.16) 14
<i>H. minuta</i>	1.56 (0.10) 9	1.83 (0.12) 9	1.00 (0.12) 9	1.56 (0.29) 9
<i>H. punctata</i>	1.14 (0.14) 14	1.79 (0.25) 14	1.27 (0.18) 13	1.54 (0.27) 12
<i>H. geographica</i>	1.25 (0.09) 8	2.06 (0.11) 8	3.00 (0.00) 6	2.25 (0.16) 8
<i>H. crepitans</i>	1.50 (0.09) 8	2.06 (0.15) 8	1.69 (0.16) 8	1.67 (0.17) 3
<i>H. boans</i>	0.88 (0.13) 8	1.69 (0.16) 8	0.31 (0.09) 8	1.50 (0.50) 2

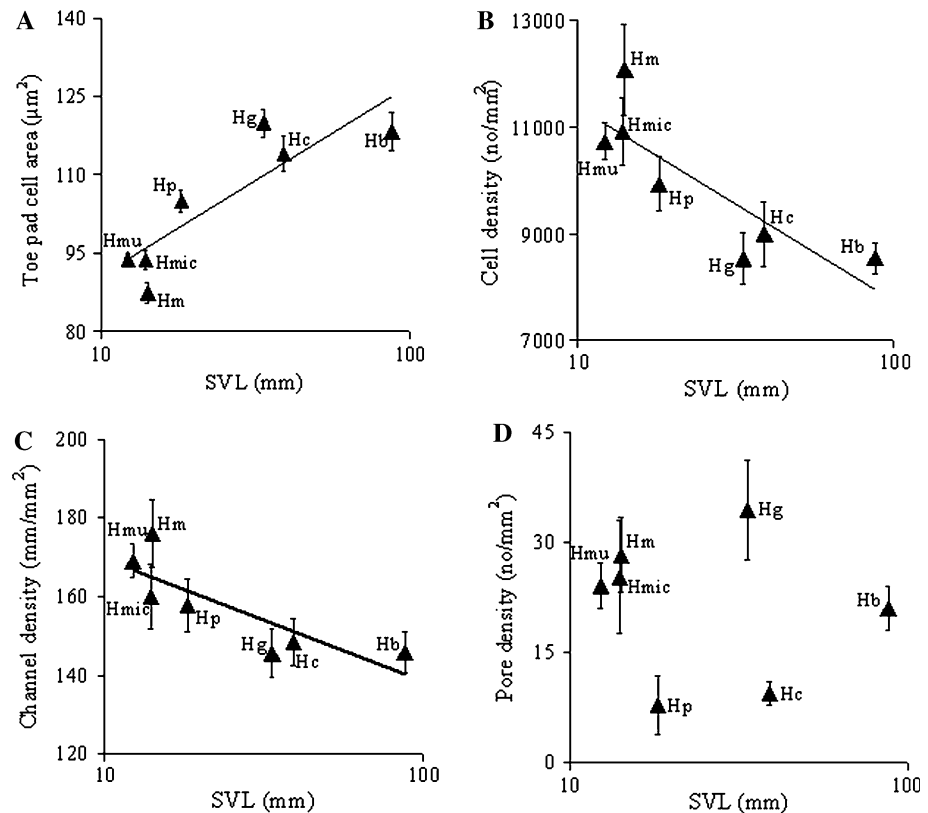
**Fig. 2** Toe pad cells in seven species of tree frog:  
**a** *H. minuscula*,  
**b** *H. microcephala*,  
**c** *H. minuta*, **d** *H. punctata*,  
**e** *H. geographica*,  
**f** *H. crepitans*,  
**g** *H. boans*. All scale bars  
 25  $\mu\text{m}$



**Table 3** Cell sizes, densities, intercellular channel lengths, average pore sizes and densities on toe pads in seven species of tree frog

Species	Cell area ( $\mu\text{m}^2$ ) Mean (s.e.) n	Cell density (no./mm <sup>2</sup> ) Mean (s.e.) n	Channel density (mm/mm <sup>2</sup> ) Mean (s.e.) n	Pore area ( $\mu\text{m}^2$ ) Mean (s.e.) n	Pore density (no./mm <sup>2</sup> ) Mean (s.e.) n
<i>H. minuscula</i>	94 (1) 174	10,733 (348) 18	169 (4) 18	38 (10) 8	24 (3) 13
<i>H. microcephala</i>	87 (2) 138	12,068 (854) 14	176 (9) 14	136 (20) 6	28 (5) 9
<i>H. minuta</i>	94 (2) 90	10,910 (623) 9	160 (8) 9	104 (23) 8	25 (8) 7
<i>H. punctata</i>	105 (2) 136	9,931 (511) 14	158 (7) 13	71 (8) 11	8 (4) 2
<i>H. geographica</i>	120 (3) 80	8,538 (477) 8	146 (6) 8	312 (22) 5	34 (7) 6
<i>H. crepitans</i>	114 (3) 78	8,992 (598) 8	148 (6) 8	168 (27) 6	9 (2) 6
<i>H. boans</i>	118 (4) 87	8,538 (297) 9	146 (5) 8	176 (36) 6	21 (3) 5

**Fig. 3** Relationships between measured toe pad morphology variables and body length in seven species of tree frog: **a** toe pad cell area vs. snout–vent lengths (SVL); **b** cell densities vs. SVL; **c** intercellular channel densities vs. SVL; **d** mucosal pore densities vs. SVL. Statistics in text. Data labels as in Fig. 1

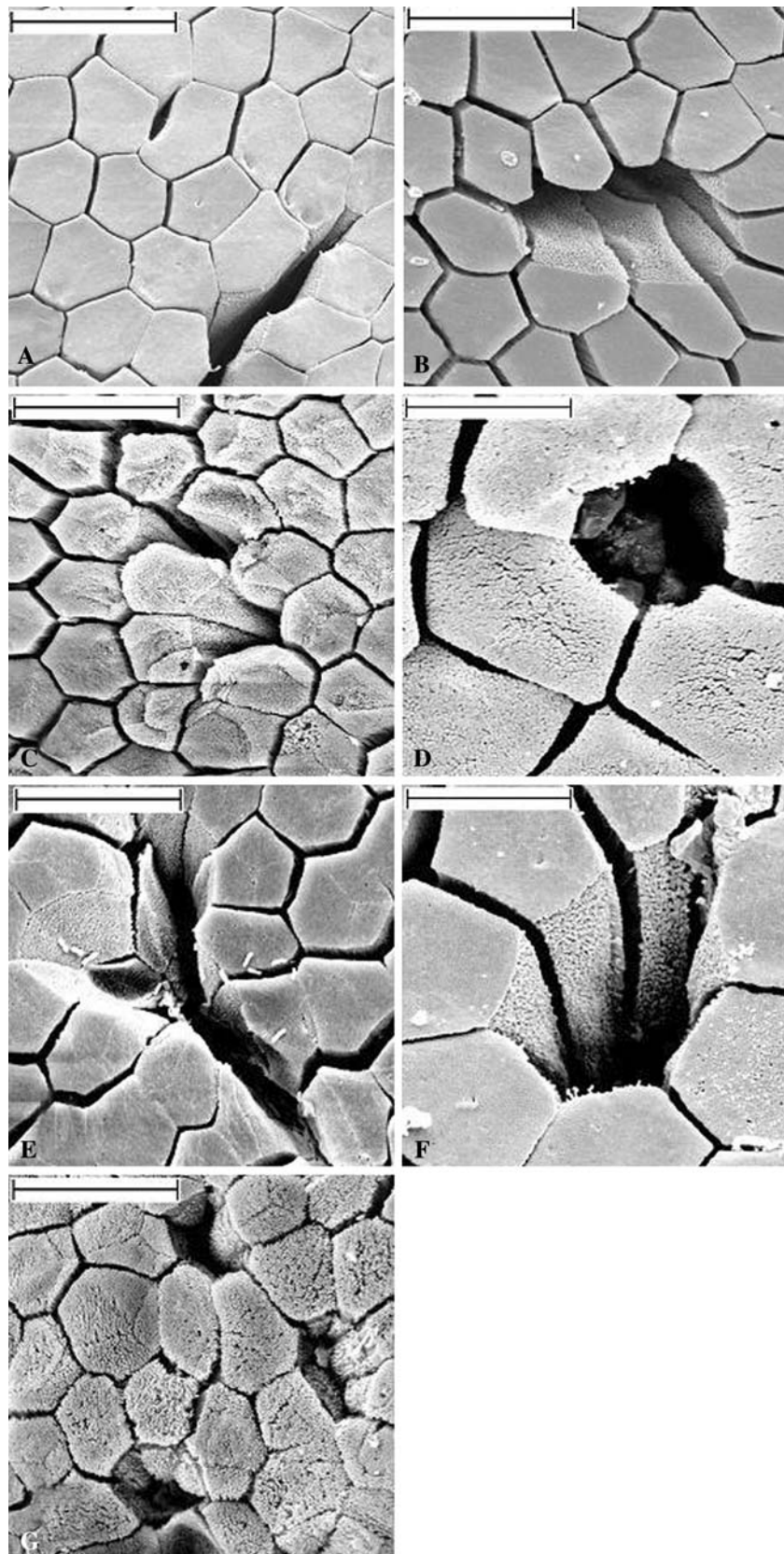


Channels between the columnar cells of the toe pad were highly variable in width (Fig. 2), with typical values ranging from 1 to 5  $\mu\text{m}$ . As channel length is dependent on the combined lengths of the cell perimeters it was expected that channel densities would increase with cell densities. Consequently, the relationships between channel densities and SVL were similar to those for cell densities; larger frogs showed a decrease in the length of channel per unit area (Fig. 3c— $r = 0.83$ ,  $y = 173.11 - 0.35x$ ,  $t = 3.32$ ,  $P = 0.02$ , 6 d.f.).

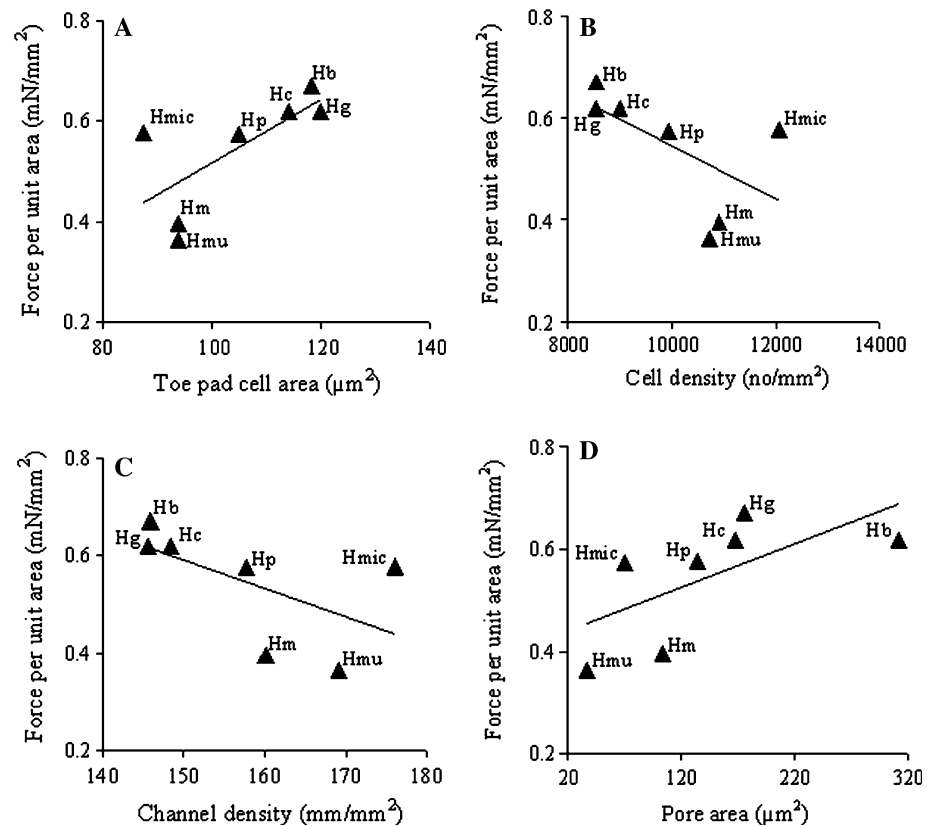
All mucosal pores were ‘Type II’ pores, *sensu* Green (1979); i.e. pores in which the sides of the cell facing the

lumen were modified in comparison to the normal cell striations. Both within and between species, pore size and shape was extremely variable (Fig. 4); but there was no correlation between pore area and SVL ( $r = 0.59$ ,  $y = 1.86x - 60.36$ ,  $t = 1.62$ ,  $P = 0.17$ , 6 d.f.). Mucus pore counts were higher in large species ( $r = 0.87$ ,  $y = 3.96x - 86.25$ ,  $t = 3.94$ ,  $P = 0.01$ , 6 d.f.) though as they also had larger toe pads this was expected. Controlling for size by dividing counts by toe pad area gave pore densities that differed from one another but were not correlated with SVL (Fig. 3d— $r = 0.14$ ,  $y = 23.40 - 0.05x$ ,  $t = 0.31$ ,  $P = 0.77$ , 6 d.f.).

**Fig. 4** SEM images of typical mucus pores in **a** *H. minuscula*\*, **b** *H. microcephala*\*, **c** *H. minuta*\*, **d** *H. punctata*\*\*, **e** *H. geographica*\*, **f** *H. crepitans*\*, **g** *H. boans*\*\*. \*Scale bars 25  $\mu\text{m}$ . \*\*Scale bar 12.5  $\mu\text{m}$



**Fig. 5** Relationships between pad efficiency and morphology in seven species of tree frog: **a** force per unit area vs. cell area; **b** force per unit area vs. cell density; **c** force per unit area vs. channel density; **d** force per unit area vs. mucosal pore size. Statistics in text. Data labels as in Fig. 1



### Toe pad morphology and adhesion

There were strong log–log correlations between adhesive force and cell size ( $r = 0.93$ ,  $y = 10.1x - 19.0$ ,  $t = 5.78$ ,  $P = 0.002$ , 6 d.f.), cell density ( $r = 0.92$ ,  $y = 39.1 - 9.5x$ ,  $t = 5.42$ ,  $P = 0.003$ , 6 d.f.) and channel density ( $r = 0.92$ ,  $y = 38.6 - 17.0x$ ,  $t = 5.07$ ,  $P = 0.004$ , 6 d.f.). There was also a trend towards an increase in adhesive force with mucosal pore area (logarithmic model— $r = 0.72$ ,  $y = 1.5x - 1.7$ ,  $t = 2.32$ ,  $P = 0.07$ , 6 d.f.). There was no demonstrable correlation between mucosal pore density and adhesive force (logarithmic model— $r = 0.15$ ,  $t = 0.32$ , N.S. 6 d.f.).

Force per unit area, as a measure of pad efficiency, and cell size were significantly positively correlated (Fig. 5a— $r = 0.82$ ,  $y = 0.007x - 0.23$ ,  $t = 3.24$ ,  $P = 0.02$ , 6 d.f.). Significant negative correlations were found between force per unit area and both cell density (Fig. 5b— $r = 0.81$ ,  $y = 1.25 - 0.001x$ ,  $t = 3.06$ ,  $P = 0.03$ , 6 d.f.) and channel density (Fig. 5c— $r = 0.92$ ,  $y = 2.00 - 0.01x$ ,  $t = 5.32$ ,  $P = 0.003$ , 6 d.f.). Thus adhesive force was positively correlated with cell area, and negatively correlated with both cell density and channel density. However, the relationship between mucosal pore size and adhesive efficiency was not significant statistically (Fig. 5d— $r = 0.63$ ,  $y = 0.001x + 0.42$ ,  $t = 1.95$ ,  $P = 0.11$ ,

6 d.f.), nor was there any effect of mucosal pore density on pad efficiency ( $r = 0.25$ ,  $t = 0.57$ , N.S. 6 d.f.).

Running a multiple regression analysis of channel density, cell size, cell density, mucosal pore size, mucosal pore density against adhesive efficiency confirmed that the most significant three variables were channel density ( $t = 38.42$ ,  $P = 0.02$ , 6 d.f.), cell density ( $t = 20.32$ ,  $P = 0.03$ , 6 d.f.) and cell area ( $t = 16.03$ ,  $P = 0.04$ , 6 d.f.). Mucosal pore size ( $t = 14.13$ ,  $P = 0.05$ , 6 d.f.) was less significant and there was no significant correlative effect of mucosal pore density ( $t = 10.65$ ,  $P = 0.06$ , 6 d.f.).

### Discussion

#### Scaling and adhesion

The seven species of Trinidadian hyliid had toe pads with very similar morphology. There were slight variations in the details of a number of pad features, such as extra-pad grooves and elevations, but these were not correlated to increasing size and perhaps reflect differences in ecology, i.e. the degree of arboreality that the frogs typically exhibit (Hertwig and Sinsch 1995). In general though, the structure was highly conserved.

As all the frogs are from a single genus in the family Hylidae, this might well be an expected finding; but pad morphology is also very similar to climbing frogs in the Hyperoliidae, Rhacophoridae and Microhylidae (McAllister and Channing 1983; Green and Simon 1986; Hertwig and Sinsch 1995; Mizuhira 2004), supporting the theory that the structure of the pad has become highly evolved for a specific adaptive function. Climbing frogs have long attracted scientific curiosity but, in spite of this, there been surprisingly few studies that consider pad morphology in relation to function in the wet adhesive mechanism that allows the frogs to scale smooth vertically inclined surfaces.

Wet adhesion is facilitated by two component forces; capillarity ( $F_c$ ) and Stefan adhesion ( $F_{sa}$ ). For two rigid flat plates the relative contributions of each type of wet adhesion to the overall force differs according to the following formula (adapted from Denny 1993):

$$F = F_c + F_{sa} \rightarrow \frac{2\pi r^2 \gamma}{h} + \frac{3\pi r^4 \eta v}{2h^3} \rightarrow \frac{2(\text{area})\gamma}{h} + \frac{(\text{area})^2 \eta v}{2h^3} \quad (1)$$

where  $r$  = pad radius,  $\eta$  = viscosity,  $\gamma$  = surface tension,  $h$  = meniscal height,  $v$  = speed of separation.

Although not the best model of adhesion for the tree frog system which is based on a flexible, domed and subdivided surface area, the formula introduces the basic principle that the components of wet adhesion are differentially affected by mucus properties; capillarity is dominant where a low viscosity, high surface tension fluid is the intervening liquid layer and Stefan adhesion where there are high viscosity, low surface tension fluids. There are also significant differences in the effects of decreasing the thickness of the fluid layer and increasing the contact area on the relative contributions of the two component forces, though both will increase. The fact that adhesive forces in tree frogs increase directly with toe pad area rather than with area squared has been interpreted as suggesting that capillarity is the dominant force involved (Emerson and Diehl 1980). However, some contribution of Stefan adhesion cannot be ruled out—even for a flat-plate model with water as the intervening fluid there is some influence of viscosity on the separation forces (Hanna and Barnes 1991).

Allometric relationships in Trinidadian tree frogs belonging to the genus *Hyla* were such that species are roughly geometrically similar. There was a tendency towards reduced mass in larger species, but not to a sufficient extent as to be matched by the change in toe pad area, which increased isometrically, as the square of the length. As adhesive forces were strongly corre-

lated to toe pad area, there was a quantitative decrease in the ability of larger species to maintain a hold upon a smooth rotation platform and the average detachment angle in the largest species was significantly lower than that in the smallest. However, there was an increase in the force per unit area that suggested large species had more efficient toe pads. It thus seemed sensible to consider the changes in toe pad structure and function with size that might influence wet adhesion. These fall into three categories: strategies to avoid peeling of the pads, differences in mucus properties and differences in pad morphology.

Since pads detach by peeling (Hanna and Barnes 1991), behavioural strategies must exist to prevent peeling occurring when the frog is adhering to a smooth surface. Although we have made no systematic attempt to study such behaviours as yet, many frogs do assume a sprawling posture when trying to maintain a hold on the platform. As well as adjusting the centre of mass to a position close to the surface, this may also be an attempt to keep the angle of pull on the pad as acute as possible, for peeling occurs spontaneously during locomotion when pads are pulled at angles exceeding 90° to the surface (Hanna and Barnes 1991). We cannot exclude that such behavioural strategies are better developed in larger hylids, but on the other hand there is no evidence in favour of such a hypothesis either.

Interspecific differences in terms of the properties of the mucus (e.g. in its viscosity or surface tension) are also entirely plausible. Many invertebrates can make rapid changes to the fluid secretions that effect adhesion by the addition of specific proteins (Smith et al. 1999; Smith and Morin 2002). Studies of mucosal secretions sampled from tree frogs suggest that protein content is highly variable (Kikuyama et al. 2002) and if the protein component of mucus could be altered quickly, large frogs might be able to compensate for pressures of increasing mass on the adhesive system. However, the effect on other locomotory modes, particularly jumping, also needs to be considered. Viscous mucus has been shown to have a detrimental effect on jump distance in the Cuban tree frog, *O. septentrionalis* (Barnes et al. 2002). It is thus no surprise that the fluid secreted by the toe pads in White's tree frog, *Litoria caerulea*, is a thin mucus, comparable in its viscosity to water (Federle et al. 2006). The properties of the mucus in the hylid species in this study are, as yet, unknown and while this remains a gap in our understanding it does seem likely that changes to mucosal properties, rather than being driven simply by the need for an efficient mechanism to withstand dislodgement, will be limited by other functional requirements.

## Toe pad morphology and adhesion

Finally, turning to toe pad morphology, this study has shown that unit force (toe pad efficiency) was positively correlated with toe pad cell size and mucus pore size, and negatively correlated with toe pad cell density and channel length. If the chief effect of increasing the network of intercellular channels were an increased drainage (Green 1979), then we would expect channel density to be positively correlated with adhesive efficiency rather than the other way around. However, an increase in mucus pore size could make sense since, under dry conditions, they could supply the increased amount of mucus needed to provide a continuous fluid layer under the larger pads of larger species, another requirement of good adhesion (Emerson and Diehl 1980; Öhler 1995; Barnes 1999). The positive correlation between unit force and cell size in this study is particularly interesting when compared to an earlier study by Green (1981) of the *Hyla versicolor* complex. He found a 5% increase in the ‘stickiness’ of the tetraploid *H. versicolor* in comparison to the diploid *Hyla chrysoscelis*. These species are almost identical in all respects other than cell size, which are larger in *H. versicolor* due to its greater degree of ploidy (Green 1980). Although Green (1981) dismisses the link between cell size and adhesion in his study as due to variability or experimental error, the positive correlation between these parameters in our study supports his observations and raises interesting questions about the function of polyploidy, seen in many amphibian genera.

How might increased cell size increase adhesive efficiency? The influence of the capillarity component is dependent on the presence of an air–water interface (Denny 1993) so whilst this may be important across the whole of the toe pad its contribution will not be significant under individual cells. The main effect is therefore likely to be due to the influences of Stefan adhesion. It is clear from Eq. 1 that changes in the thickness of the fluid layer significantly affect both components of wet adhesion, but will particularly increase the contribution of Stefan adhesion (as the denominator is  $2h^3$  as compared to  $h$ ). Whilst Stefan adhesion is likely to be relatively low in intercellular channels, the cell apices themselves are applied more closely and so the contribution of the viscosity-dependent component across the cell surface is likely to be significantly higher. The Stefan adhesion component ( $F_{sa}$ ) of a single toe pad is therefore likely to be proportional to  $n \times a^2$ , where  $n$  is the number of cells and  $a$  is cell area (see Eq. 1). From this it follows that, for a given toe pad area,  $F_{sa}$  will be positively correlated

with cell area. Thus our findings and those of Green (1981) may point to a contribution for Stefan adhesion, in spite of the low viscosity of the fluid layer effecting adhesion. Since cell size and channel density are inter-related, it is also clear that the advantages of increased cell size outweigh the advantages of increased channel length.

In a fixed area ‘tiled’ with a regular array of hexagonal cell apices, there is a second way in which increases in cell area might be facilitated—through a decrease in the width of the intercellular spaces. In SEM images of a fixed area from toe pads of all seven *Hylids*, channel width appears extremely variable both within and between species. Recent work using interference reflectance microscopy techniques to image the feet of live frogs suggests that it is dynamically variable even within individuals (Smith 2003; Federle et al. 2006).

Whilst the above arguments provide a good explanation for the advantages of increasing cell size to produce increased adhesion through viscosity effects, it is worth noting that the epithelial cells on the toe pads are smaller than those on much of the skin. Presumably there is a trade-off between adhesion and detachment in that further increases in cell size would reduce detachment rate and thus jumping ability.

This study has considered the specialised adhesive toe pads in several related species of tree frog to provide a link between morphology and function and to determine how frogs attempt to compensate for detrimental effects of increasing size on their adhesive mechanism. There are limitations to this study; it is not possible to determine properties of the mucus by considering morphology alone nor can one predict the volumes of mucus being released onto the pad to influence the meniscal profile. However, the study of relative morphology has detected a number of ways in which large frogs are able to increase the area available for close contact in order to increase the adhesive efficiency of the pad. Studies that consider the influence of scaling and morphology often focus on locomotion or feeding ability (Zani 2000) but the relationships between morphology and adhesion may be particularly illuminating in that the evolution of specialised adhesive structures facilitates niche expansion. In this case, adhesive ability has allowed frogs to exploit the abundant food sources available in arboreal habitats. Furthermore, the ability of tree frogs to adhere well in the presence of a fluid layer has already been considered with respect to the applications to wet weather tyre technology (Barnes et al. 2002), and there is vast potential for other biomimetic applications in situations that require an enhanced ability to adhere under wet

conditions (shoes for rock-climbing being only one of the most obvious). If this potential is to be realised then the relative importance of factors such as the shape and size of the pad cells, the mechanical properties of the pad and properties of the fluid layer, must be better understood and quantified. Differences in toe morphology between species may appear small but they have significant effects on the efficiency of the pad in terms of the adhesive force generated per unit area.

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