Convergent developmental patterns underlie the repeated evolution of adhesive toe pads among lizards

AARON H. GRIFFING[1,*,•], TONY GAMBLE[1,2,3,•], MARTIN J. COHN[4,•] and THOMAS J. SANGER[4,5,•]

[1]*Department of Biological Sciences, Marquette University, PO Box 1881, Milwaukee, WI 53201, USA*
[2]*Milwaukee Public Museum, 800 W. Wells St., Milwaukee, WI 53233, USA*
[3]*Bell Museum of Natural History, University of Minnesota, 2088 Larpenteur Ave. W., St. Paul, MN 55113, USA*
[4]*Department of Molecular Genetics and Microbiology, UF Genetics Institute, University of Florida, Gainesville, FL 32610, USA*
[5]*Department of Biology, Loyola University Chicago, 1032 W. Sheridan Rd, Chicago, IL 60660, USA*

How developmental modifications produce key innovations, which subsequently allow for rapid diversification of a clade into new adaptive zones, has received much attention. However, few studies have used a robust comparative framework to investigate the influence of evolutionary and developmental constraints on the origin of key innovations, such as the adhesive toe pad of lizards. Adhesive toe pads evolved independently at least 16 times in lizards, allowing us to examine whether the patterns observed are general evolutionary phenomena or unique, lineage-specific events. We performed a high-resolution comparison of plantar scale development in 14 lizard species in *Anolis* and geckos, encompassing five independent origins of toe pads (one in *Anolis*, four in geckos). Despite substantial evolutionary divergence between *Anolis* and geckos, we find that these clades have undergone similar developmental modifications to generate their adhesive toe pads. Relative to the ancestral plantar scale development, in which scale ridges form synchronously along the digit, both padded geckos and *Anolis* exhibit scansor formation in a distal-to-proximal direction. Both clades have undergone developmental repatterning and, following their origin, modifications in toe pad morphology occurred through relatively minor developmental modifications, suggesting that developmental constraints governed the diversification of the adhesive toe pad in lizards.

ADDITIONAL KEYWORDS: *Anolis*– convergent evolution – digital adhesion – embryo – evo-devo – gecko.

INTRODUCTION

Morphological evolution involves processes both intrinsic and extrinsic to the organism (Wake & Roth, 1989; Müller & Wagner, 1991; Wake, 1991; Wake & Larson, 2003). Processes external to an organism, such as selection, shape a species to its environments and drive adaptation. The intrinsic rules of development dictate the type, scale and frequency of variation produced by a species (Alberch, 1980, 1989; Oster & Alberch, 1982; Cheverud *et al.*, 1983; Maynard-Smith *et al.*, 1985; Brakefield, 2006; Olson, 2012, 2019). However, the variation that is produced by a given developmental system is not distributed equally in all directions – some variants are produced more often than others and some conceivable options are not observed at all. These developmental biases, or constraints, have the potential to affect the rate or direction of morphological evolution over both small and large timescales (Maynard-Smith *et al.*, 1985; Wake, 1991; Beldade & Brakefield, 2002; Brakefield, 2003; Arthur, 2004; Losos, 2011; Wake *et al.*, 2011). Understanding the ways that the variation-generating mechanisms of development change over the history of a diversifying lineage may shed light on the way integrated systems respond to selection and what combination of developmental processes the variation that selection acts upon arises from. Yet, relative to studies describing patterns of phenotypic diversity, comparatively little research has investigated how the

*Corresponding author. E-mail: aaron.griffing@marquette.edu

developmental mechanisms that produce selectable variation change during the origin and adaptive diversification of complex morphological traits.

Evolutionary innovations are of particular interest to those investigating morphological diversity. An innovation is defined as an evolutionarily novel structure that provides an organism access to new adaptive zones (see Müller & Newman, 2005 for a nuanced distinction between novelty and innovation). One such example of morphological innovation is the adhesive toe pads of lizards. Digital adhesion has independently evolved at least 16 times in three major squamate groups: multiple times within geckos, and once each in *Anolis* lizards and the *Prasinohaema+Lipinia* clade of skinks (Peterson, 1983; Irschick *et al.*, 1996; Skinner *et al.*, 2011; Gamble *et al.*, 2012; Higham *et al.*, 2017; Russell & Gamble, 2019). Digital adhesion is facilitated by expanded regions of

the plantar surface: toe pads. Externally, these pads consist of modified scales (lamellae and scansors) that bear setae (hair-like, hypertrophied projections of the epidermis) and foster adhesion via frictional interactions and van der Waals forces (Fig. 1; Ruibal & Ernst, 1965; Maderson, 1970; Williams & Peterson, 1982; Autumn *et al.*, 2002; Russell, 2002; Autumn, 2006). Although both lamellae and scansors are highly specialized and possess fields of setae, scansors differ from lamellae by exhibiting tendinous connections to the digits and in some cases vascular networks or adipose pads that facilitate control of the individual units (Russell, 1981, 1986; Bergmann & Russell, 2003; Russell *et al.*, 2019). Toe pads of *Anolis* and geckos are composed of both scansors and lamellae (Bergmann & Russell, 2003; Russell & Delaugerre, 2017; Russell & Gamble, 2019; Russell *et al.*, 2019; Russell & Garner, 2021).
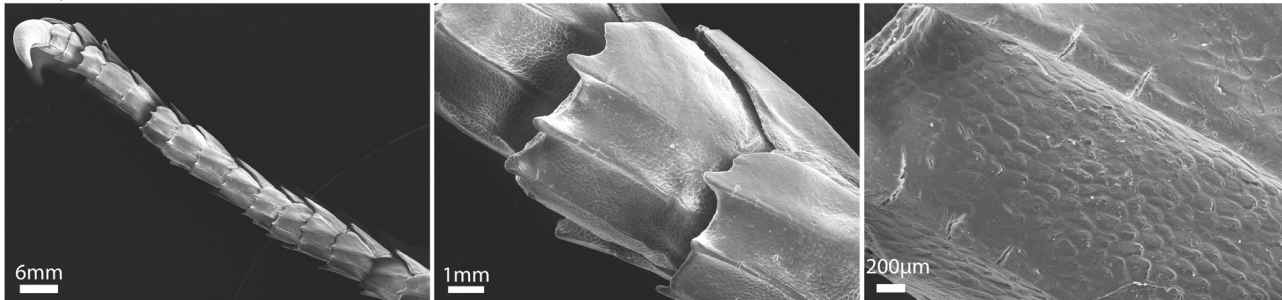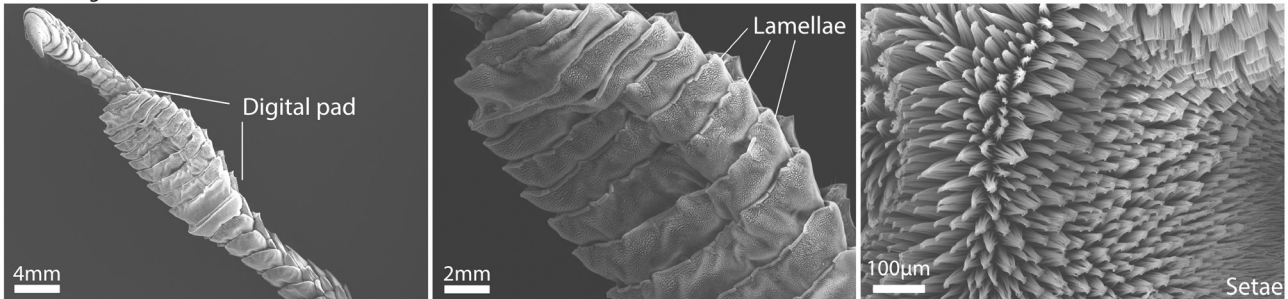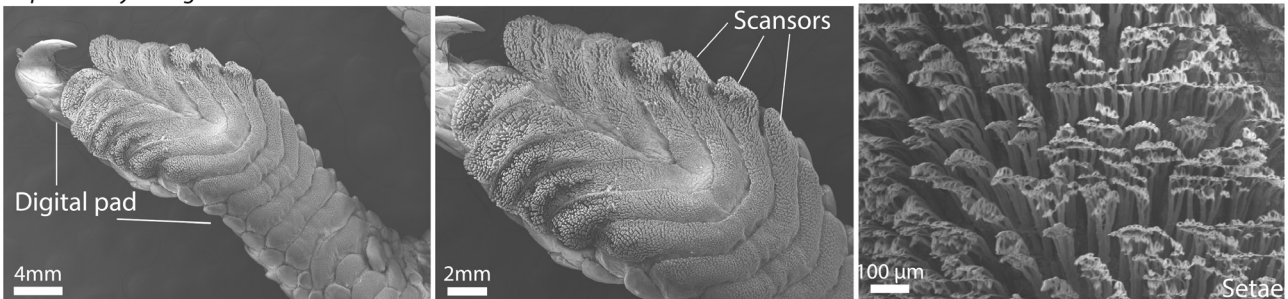

Figure 1. Morphology of adult lizard digits. Scanning electron micrographs depicting plantar views of adult *Sceloporus undulatus* (padless iguanian), *Anolis sagrei* (toe pad-bearing iguanian) and *Lepidodactylus lugubris* (toe pad-bearing gecko) pes, digit IV. *Sceloporus undulatus* exhibits imbricate scales without setae. *Anolis sagrei* and *L. lugubris* exhibit digital pads composed of seta-bearing scansors.

Toe pads increased the efficiency of locomotion in vertical habitats in the ancestral lineages of these now diverse clades (Russell, 1979; Bauer *et al.*, 2005; Losos, 2009; Gamble *et al.*, 2012; Higham *et al.*, 2017; Fontanarrosa *et al.*, 2018; Russell & Gamble, 2019; Miller & Stroud, 2021). Within toe pad-bearing groups, markedly divergent toe pad forms allow different species to further partition niche space by accessing different micro-habitats and/or substrates (Elstrott & Irschick, 2004; Russell & Delaugerre, 2017).

Geckos (infraorder Gekkota) comprise nearly 2100 described species (Uetz *et al.*, 2021), around 1100 of which exhibit adhesive toe pads (Gamble *et al.*, 2012; Russell & Gamble, 2019). Ancestrally, geckos were padless, yet adhesive pads independently arose approximately 14 times in this clade (Gamble *et al.*, 2012; Russell & Gamble, 2019). Perhaps just as striking as the repeated gains and losses of toepads is the diversity of overall toepad morphologies within and among the independent evolutionary origins. Gecko toepad morphologies are typically divided into three categories: *basal* pads where the scansors and lamellae are located ventral to the intermediate phalanges, *terminal fan* pads where the adhesive apparatus is at the tip of the digit in a broad, fan-like shape, and *terminal leaf* pads where flaring, paired adhesive apparatuses are located around the tip of the digit (Gamble *et al.*, 2012; Russell & Gamble, 2019). However, this is a gross oversimplification as species with basal pads can exhibit dramatically different numbers of scansors, phalangeal shapes and angle, presence of paraphalangeal elements, tendinous arrangement, and muscle arrangement (Russell, 1972, 1976, 1979). Differences in scansor numbers have been associated with differences in gecko toe pad area and, in turn, different maximum body sizes and exploitable habitats (e.g. Hecht, 1952; Johnson & Russell, 2009). However, the relationships between scansor numbers, toe pad areas and niche partitioning between congeners have not been studied to the degree in which they have been studied in *Anolis* lizards.

Adhesive toe pads evolved once within iguanian lizards, at least 70 Mya in the ancestor to extant *Anolis* lizards (Collette, 1961; Peterson, 1983; Losos, 2009; Gamble *et al.*, 2012; Román-Palacios *et al.*, 2018). This genus has over 400 recognized species, residing primarily within arboreal habitats of the Caribbean islands, and Central and South America (Losos, 2009). The toe pads of *Anolis* are less diverse in scansor width, orientation and elaboration than those of geckos; however, these toe pads diversified in pad size and in the number of scansors as species occupied different parts of the arboreal canopy. *Anolis* species that live near the top of the canopy tend to have larger toe pads with more scansors compared to

anoles that live closer to the ground and have smaller toe pads with fewer scansors (Fig. 2; Glossip & Losos, 1997; Beuttell & Losos, 1999; Macrini *et al.*, 2003). A species living close to the ground (e.g. trunk-ground anoles) may have as few as 15–20 scansors on pedal digit IV while a species living higher in the canopy may have as many as 50 scansors (e.g. crown giant anoles; Glossip & Losos, 1997).

Despite their diversity of form, unequivocal importance in locomotion and presence in hundreds of lizard species, only a handful of disparate studies have focused on the development of lizard toe pads (Rosenberg *et al.*, 1992; Khannoon, 2015; Khannoon *et al.*, 2015; Alturk & Khannoon, 2020; Griffing *et al.*, 2021). To date, no studies have addressed toe pad development in a comparative context, particularly comparing the independent origins of anole and gecko toe pads. A 'model clade' approach is required to determine the extent of similar and unique developmental modifications that occurred between anole and gecko toe pads as well as within the multiple origins of gecko toe pads (Sanger & Rajakumar, 2019). Herein we investigate how developmental processes have evolved during the repeated origin and diversification of adhesive toe pads in *Anolis* lizards and geckos. These clades are hypothesized to have diverged from one another ~200 Mya (Zheng & Wiens, 2016). The pattern of phenotypic convergence provides us with the opportunity to ask whether there are multiple ways to develop an adhesive pad. By including ancestrally padless outgroups in this analysis we also address whether there was a dramatic restructuring in development at the origin of these phenotypic innovations. Due to the diversity in mature toe pad morphologies (Fig. 2) and the phylogenetic distance between taxa with adhesive digits, we hypothesized that these taxa develop pads through distinct developmental processes.

MATERIALS AND METHODS

We collected embryos from 14 lizard species. These included four independent origins of adhesive toe pads in geckos, an ancestrally padless gecko (*Eublepharis macularius*), eight *Anolis* species representing the full diversity of toe pad proportions and a padless iguanian outgroup (*Sceloporus undulatus*). We dedicated most of our sampling efforts to seven focal species: *Anolis carolinensis*, *A. sagrei*, *S. undulatus*, *Correlophus ciliatus*, *E. macularius*, *Lepidodactylus lugubris* and *Hemidactylus turcicus*. These species readily produced eggs in captivity and could provide complete developmental series. We opportunistically sampled embryos from the seven additional species that produced fewer eggs or whose developmental staging
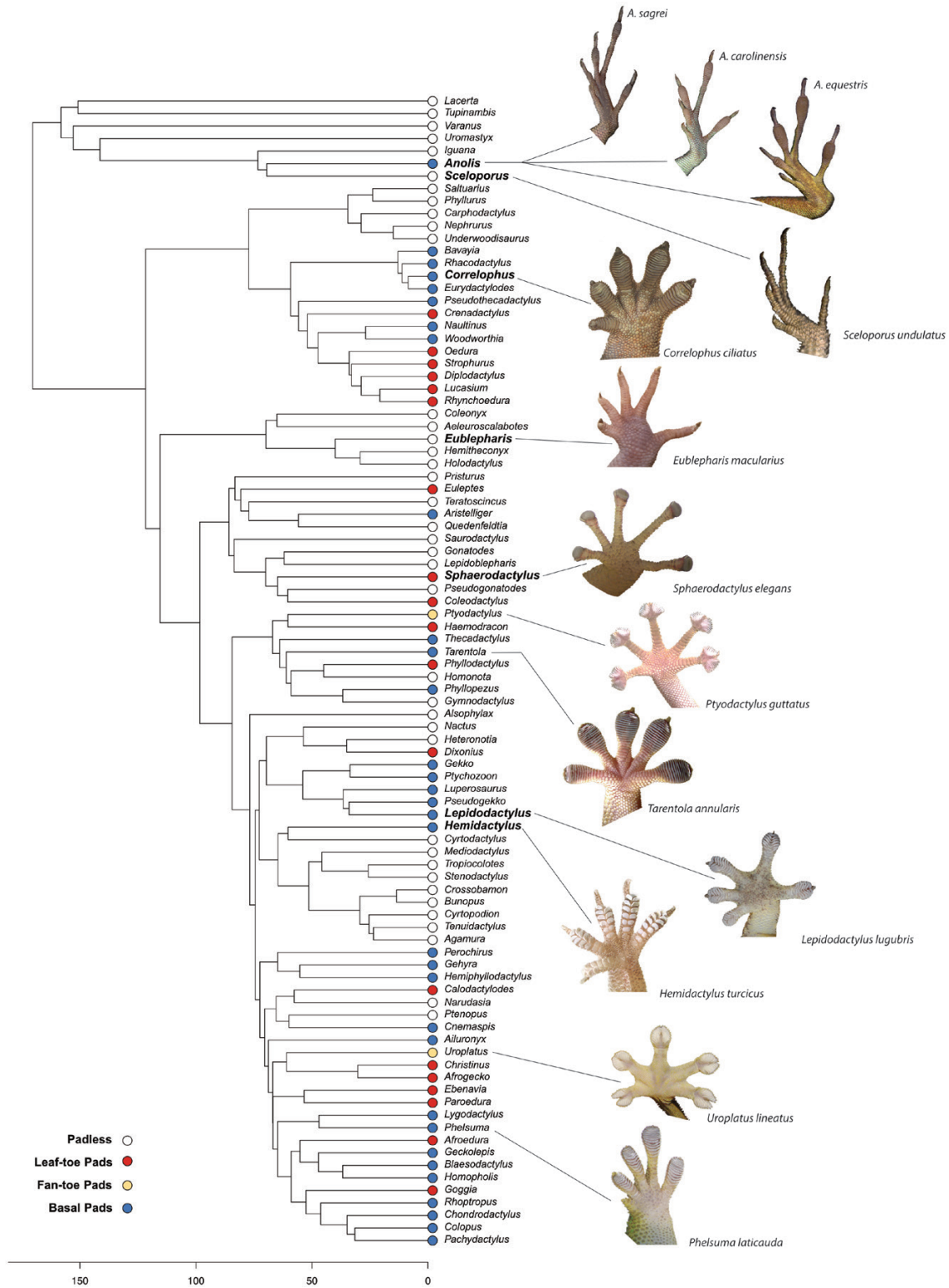
Figure 2. Convergence and divergence in adhesive toe pad morphology. Time-calibrated (millions of years) phylogeny illustrating generic relationships of geckos with squamate outgroups. Tree topology and tip colours correspond to toe pad character states defined by Russell & Gamble (2019). Bold type indicates genera investigated in this study.

criteria were not well established. This allowed us to test whether our evolutionary hypotheses are broadly generalizable or limited in scope. Among anoles, the toe pads of the longest digit (pes, digit IV) has been studied extensively in comparative and functional studies (e.g. Losos & de Queiroz, 1997; Knox *et al.*, 2001; Pinto *et al.*, 2008). Herein, we follow this tradition but also acknowledge that not every toe has the same number of lamellae/scansors and some digits may lack them altogether.

Embryonic staging series differ in how developmental stages are discretized and named for different species. For example, 19 stages (referred to as St 1–19) were previously discretized for *A. sagrei* (Sanger *et al.*, 2008b), while most gecko staging tables follow the numbering of Dufaure & Hubert (1961) and are discretized into 14–16 stages (Wise *et al.*, 2009; Griffing *et al.*, 2019, 2021). Furthermore, anoles exhibit a markedly shorter post-ovipostional time *in ovo* than geckos (e.g. 28 vs. 66 days post-ovipostion; Sanger *et al.*, 2008b; Griffing *et al.*, 2019). For ease of comparison, we allocate our sampled embryos into larger categories, using the approximate numbering scheme of Griffing *et al.* (2019, 2021): late stage 35 – early stage 36, late stage 36 – early stage 38, late stage 37 – late stage 38, early stage 39 – late stage 40, and stage 41 (Fig. 3). Following Griffing *et al.* (2019), stage 35 is partially characterized by a reduction in the latter half of digital webbing. Webbing is completely recessed by stage 36. Between stages 36 and 37, the first signs of plantar scales and scansors appear. Plantar digital development progresses and is superficially complete by stage 41. Specific days post-oviposition for different stages are included in the Supporting Information (Tables S1 and S2).

ANOLIS AND *SCELOPORUS* HUSBANDRY AND ADULT MORPHOLOGY

We describe toe pad morphogenesis for two anole species with variations in the general anole toe pad morphology, *A. sagrei* and *A. carolinensis* (Fig. 2). *Anolis sagrei* tends to live close to the ground and has relatively narrow toe pads with few scansors (15–20; Schoener, 1975; Glossip & Losos, 1997). Scansor counts begin at the distalmost extremity pad and continue to the joint between the third and fourth phalanx from the base of the longest toe (pes, digit IV). In contrast, *A. carolinensis* lives relatively high in the canopy and has wider toe pads with many scansors (24–27; Schoener, 1975; Glossip & Losos, 1997). *Sceloporus undulatus* is a padless, semi-arboreal iguanian lizard from the eastern USA. Females of *A. sagrei* and *Sceloporus undulatus* were wild caught from Gainesville, Florida, USA. We purchased gravid female *A. carolinenesis* from a commercial supplier (Candy's Quality Reptiles,

Reserve, LA, USA). We maintained gravid females of these species in captivity as previously described (Sanger *et al.*, 2008a). Briefly, females were housed in cages of four to six individuals with perches and an artificial potted plant for the lizards to lay eggs in. We checked the pots for eggs every morning, after which time we incubated eggs in moist vermiculite at 27 °C until the time of dissection (12–20 days). We removed the embryos from eggs following protocols detailed in Sanger *et al.* (2008a) and the developmental stage of each embryo was identified by reference to previous characterization of *Anolis* embryonic development (Sanger *et al.*, 2008b).

GECKO HUSBANDRY AND ADULT MORPHOLOGY

We describe toe pad morphogenesis for three geckos, the arboreal *C. ciliatus* (Diplodactylidae), the arboreal *L. lugubris* (Gekkonidae) and the semi-arboreal *H. turcicus* (Gekkonidae). These species have independently evolved the basal toe pad morphology (Gamble *et al.*, 2012; Russell & Gamble, 2019). The scansors of *C. ciliatus* are undivided, with 16–21 mediolaterally broad and distoproximally short scansors (Bauer & Sadlier, 2000). The toepads of *L. lugubris* possess 10–12 scansors, of which the two distalmost are medially divided, forming half-scansors (Russell, 1972). The toe pads of *H. turcicus* possess 8–11 scansors and are considered 'complex' with regard to their scansoral morphology (Russell, 1976; Leviton *et al.*, 1992). With the exception of the most distal and the most proximal scansors, the toe pads of *H. turcicus* are medially divided, forming two separate columns of half-scansors (Russell, 1972, 1976). As with anoles, we focus our descriptions on the longest toe (pes, digit IV). Furthermore, we describe plantar scale morphogenesis of the ancestrally padless and terrestrial gecko, *E. macularius* (Eublepharidae; Russell & Gamble, 2019).

The details of gecko husbandry are described elsewhere (*C. ciliatus*, Seipp & Henkel, 2000; De Vosjoli *et al.*, 2003; *E. macularius*, Thorogood & Whimster, 1979; Vickaryous & Gilbert, 2019; *H. turcicus*, Konečný, 2002; *L. lugubris*, Griffing *et al.*, 2018). We collected embryos of hard-shelled gecko species (*H. turcicus*, *L. lugubris*) following Griffing *et al.* (2018) and soft-shelled species (*C. ciliatus*, *E. macularius*) following Sanger *et al.* (2008a) and Vickaryous & Gilbert (2019). Briefly, at the time of embryo removal, hard-shelled eggs are removed from the cage using a sharp scalpel when glued to the cage (*L. lugubris*) or gently by hand (all other species) to avoid damaging the embryo. The developmental stage of each embryo was identified by reference to previous characterizations of gecko embryonic development (Wise *et al.*, 2009; Griffing *et al.*, 2019, 2021).
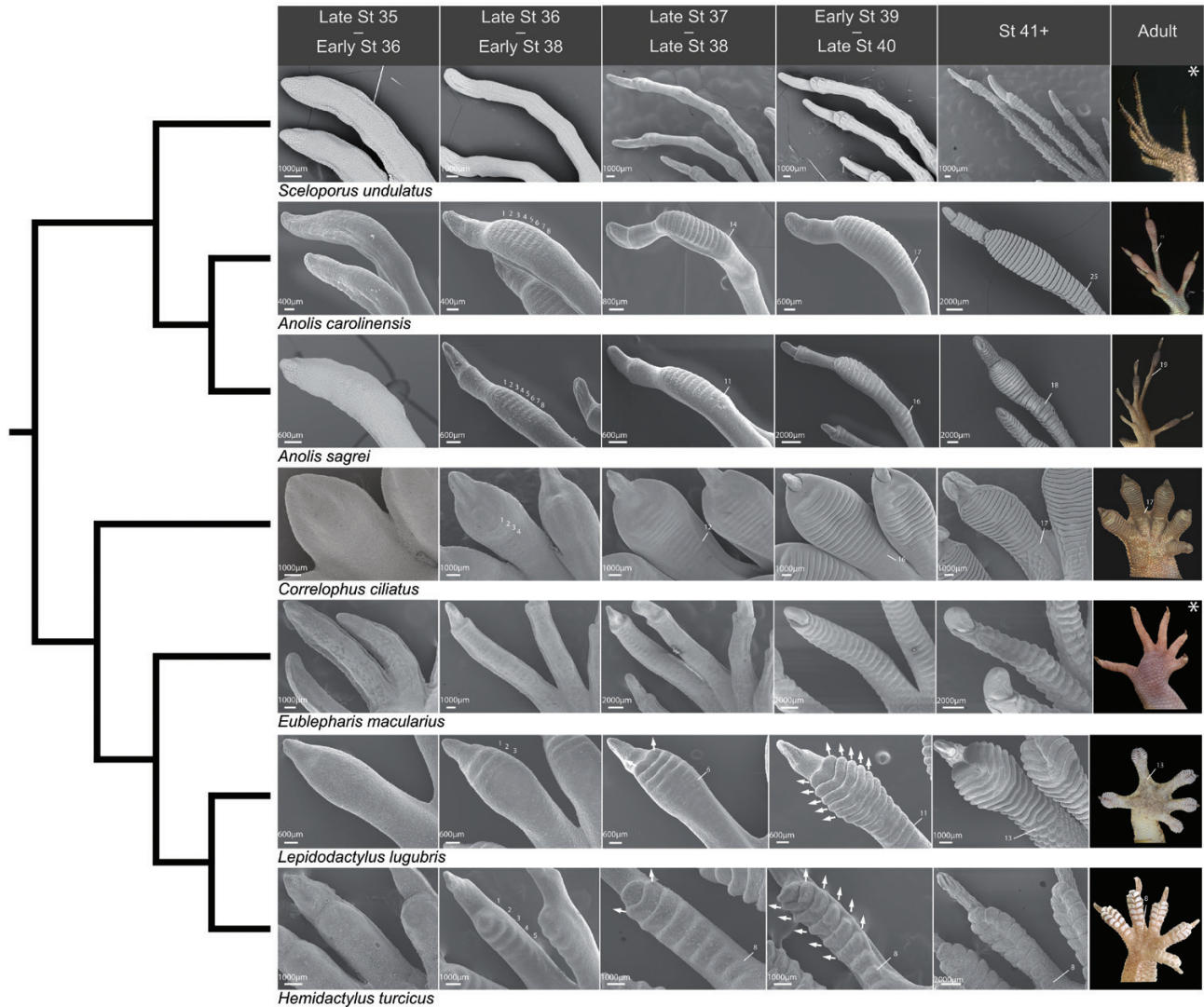
Figure 3. Embryology of lizard digits. Scanning electron micrographs depicting plantar views of *Sceloporus undulatus*, *Anolis carolinensis*, *Anolis sagrei*, *Correlophus ciliatus*, *Eublepharis macularius*, *Lepidodactylus lugubris* and *Hemidactylus turcicus* embryonic pes, digit IV. Development proceeds from left (early digit morphogenesis) to right (digital development complete) with the right-most photograph illustrating adult digital morphology. Phylogenetic relationships depicted to the left of scanning electron micrographs are based on the topology of Zheng & Wiens (2016). Numbers correspond to the developing ridges, scansors or lamellae. Asterisks correspond to padless taxa. Arrows illustrate the direction of inferred digital ridge expansion.

OPPORTUNISTIC SAMPLING OF ADDITIONAL LIZARD SPECIES

We opportunistically collected embryos of additional anole and gecko species to test (1) whether species with distinct toe pad morphologies pass through similar stages as our focal species and (2) whether species-specific morphologies (e.g. scansor number) are established during toe pad morphogenesis. We collected informative embryos from the leaf-litter specialist gecko, *Sphaerodactylus macrolepis* (Sphaerodactylidae), which represents an additional

independent origin of the gecko adhesion system (Fig. 2). *Sphaerodactylus macrolepis* has an asymmetric, distal toe pad that is composed of a single scansor. Setae form directly on an enlarged pad lateral to the claw. Aspects of *Sphaerodactylus* husbandry are detailed by Bruse *et al.* (2005). We also integrate toe pad development data from previously published reports of two gecko species for which we were unable to acquire new embryos, *Ptyodactylus guttatus* (Rosenberg *et al.*, 1992) and *Tarentola annularis* (Khannoon, 2015; Khannoon *et al.*, 2015).

Ptyodactylus guttatus exhibits a terminal fan toe pad with 10 or 11 scansors on each side of the fan (Rosenberg *et al.*, 1992) while *T. annularis* exhibits a basal pad with 17–19 undivided scansors. We opportunistically sampled six additional anole species across the range of toe pad morphologies ranging from pads with relatively few scansors to those with many: *Anolis angusticeps* (14 scansors), *A. brevirostris* (19 scansors), *A. cybotes* (12 scansors), *A. lineatopus* (13 scansors), *A. grahami* (27 scansors) and *A. equestris* (50 scansors). With the exception of embryos of *A. angusticeps* and *A. equestris*, these embryos were collected as part of a previous study (Sanger *et al.*, 2012).

SPECIMEN PREPARATION AND SCANNING ELECTRON MICROSCOPY

To visualize the early development of the adhesive apparatus we used scanning electron microscopy. After the embryos were separated from their shell and yolk, we severed the lower leg between the knee and ankle, which we preserved and stored in 1% glutaraldehyde at 4 °C. We subsequently treated the embryonic tissue in 1% osmium tetroxide for 1–2 h (depending on size) at room temperature followed by critical point drying. We sputter coated all specimens with a 0.1-nm-thick coating of gold/palladium or palladium. We then imaged the specimens on a Hitachi SU500 field emission electron microscope (University of Florida) or an SU3500 scanning electron microscope (Loyola University Chicago). In all cases, sample sizes only reflect embryos that were informative to our study of toe pad development, not the total number of embryos collected (i.e. those significantly earlier than the beginning of toe pad morphogenesis or close to hatching). In the context of the embryo, this stage of development indicates the time that digital webbing regresses to the time the mature toe pads, fully covered by lamellae/scansors, are observed.

ETHICS

All research was performed in compliance with the US National Research Council's Guide for the Care and Use of Laboratory Animals and the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals and under the supervision of the Institutional Animal Care and Use Committees (IACUC) of Marquette University, Loyola University Chicago and University of Florida. All specimens were collected with approval of the appropriate Institutional Animal Care and Use Committee [University of Florida (T.J.S. and M.J.C.), Loyola University Chicago (T.J.S.) and Marquette University (A.H.G. and T.G.)].

RESULTS

SCELOPORUS PLANTAR SCALE MORPHOGENESIS

We examined plantar scale development in 12 *Sceloporus undulatus* embryos. Digital webbing is fully reduced by embryonic day 24. The digital scales of *Sceloporus undulatus* form between embryonic days 27 and 35. Digital scale development begins several days after the interdigital webbing is fully reduced at approximately the same time claw formation begins. The ridges of the first scales form near the base of the digit (Fig. 3; Supporting Information, Fig. S1). Scales then proceed to develop synchronously along the length of the digit, becoming increasingly distoproximally asymmetrical throughout development. Plantar scale development is complete by stage 41.

ANOLIS TOE PAD MORPHOGENESIS

We examined toe pad morphogenesis in 19 embryos of *A. sagrei* spanning 12–18 embryonic days and 27 embryos of *A. carolinensis* spanning 14–20 embryonic days. In spite of the differences in adult morphology, early toe pad morphogenesis follows the same stereotypical pattern (Fig. 3; Supporting Information, Figs S2, S3). Immediately following regression of digital webbing, an enlarged pad forms ventral to the third phalanx that is lateromedially expanded. This pad is then subdivided by the emergence of seven to eight horizontal ridges across the width of the pad. These ridges preceded any signs of plantar scale development. No individuals were observed with fewer than seven ridges. New ridges form in the distoproximal direction as the pad expands towards the fourth phalanx. As new ridges form proximally, distal ridges begin to take on the exaggerated distoproximal asymmetry of mature scansors. The number of scansors observed in adults of these species is established by the end of toe pad morphogenesis.

EUBLEPHARIS PLANTAR SCALE MORPHOGENESIS

We examined plantar scale development in nine *E. macularius* embryos. Digital webbing is fully reduced by embryonic day 26. The digital scales of *E. macularius* form from embryonic days 27–41. The ridges of all plantar scales form synchronously along the length of the digit, becoming increasingly distoproximally asymmetrical throughout development (Fig. 3; Supporting Information, Fig. S4). We did not observe scales forming at the base of the digit preceding the synchronous development along the digit as observed in *Sceloporus undulatus*.

GECKO TOE PAD MORPHOGENESIS

We examined toe pad morphogenesis in 36 embryos of *C. ciliatus* spanning 28–49 embryonic days, 34 embryos

of *L. lugubris* spanning 28–48 embryonic days and 12 embryos of *H. turcicus* spanning 20–38 embryonic days. Like *Anolis* spp., species-specific morphologies of *C. ciliatus*, *L. lugubris* and *H. turcicus* are established during toe pad morphogenesis (Fig. 3).

In all species of toe pad-bearing gecko we examined, ridges indicating scansor development preceded any signs of plantar scale development. In *C. ciliatus*, regression of digital webbing leaves a thin margin of tissue along the edge of each digit, giving them a wide, flat appearance. Four horizontal ridges then form along the widest portion of the digit, excluding the thin margin of tissue adjacent to the digital condensation (Fig. 3; Supporting Information, Fig. S5). We did not observe *C. ciliatus* with fewer than four ridges. After the initial ridges form, additional ridges form both proximally and distally until the entire plantar surface of the digit is covered by immature scansors and lamellae. During this ridge extension period, pre-existing ridges expand in a mediolateral direction, taking up the full width of the digit. As this expansion unfolds, ridges begin to take on their distoproximally asymmetrical appearance.

In *L. lugubris*, an enlarged pad can be readily observed on the ventral portion of the digit shortly after digital web reduction (Fig. 3; Supporting Information, Fig. S6). The most distal portion of the pad is then subdivided by three to four small, horizontal ridges. These ridges extend across the entire width of the pad. We did not observe *L. lugubris* embryos with fewer than three ridges. A small furrow then splits these ridges medially. Additional ridges form proximally starting from this initial set of ridges. As these new ridges form, the most distal ridges acquire their characteristic distoproximal asymmetry, appearing to grow in a distolateral/distomedial direction and becoming angled towards the midline furrow.

Following digital web reduction, an enlarged pad forms on the ventral portion of the digit of *H. turcicus* (Fig. 3; Supporting Information, Fig. S7). The pad is then subdivided by four to five horizontal ridges that extend across the entire width of the pad. Additional ridges form proximally starting from this initial set of ridges while expanding in size in both distoproximal and mediolateral directions. The ridges become 'V'-shaped as a furrow appears down the midline of the digit and the scansorial ridges appear to grow in a distolateral/distomedial direction. The process subdivides five of the eight total scansor rows – each left and right component of the scansor rows takes on a rounded shape.

OPPORTUNISTIC SAMPLING

We collected informative embryos for six additional *Anolis* species representing the spectrum of toe pad dimensions and scansor/lamellar number (Fig. 4; Supporting Information, Fig. S8). The morphologies we observed were consistent with the detailed descriptions of *A. sagrei* and *A. carolinensis* (Fig. 3). We observed a digital pad without ridges in one of these species, *A. equestris*, a species with relatively large pads and as many as 50 scansors. Individuals of four of the six species we sampled were found with seven to eight horizontal ridges subdividing an enlarged pad. No individuals with scansor rows present were observed with fewer than seven ridges in any species. Individuals of all six of the *Anolis* species we opportunistically sampled were observed with an intermediate number of ridges compared to what is observed in adults of these species. In all cases, the adult number of scansors is established by the end of embryonic development in all species.

We also sampled an additional gecko species, *Sphaerodactylus macrolepis* (eight informative embryos), which has mediolaterally asymmetric distal 'leaf-toe' pads. As above, the species-specific morphologies of this species are established during toe pad morphogenesis. Toe pad development in *Sphaerodactylus macrolepis* begins with the formation of an enlarged pad on one side of the toe (Fig. 4; Supporting Information, Fig. S9). At this time the claw retains its mid-sagittal location. Digital scale development proceeds from proximal to distal. The mature pad, which is not subdivided by multiple scansors, continues to expand until it displaces the claw to the side of its original mid-sagittal location.

DISCUSSION

Despite diverging ~200 Mya (Zheng & Wiens, 2016) and dramatic differences in adult morphology (Fig. 2), the early development of anole and gecko toe pads is remarkably similar. The early development of scales and their evolutionary derivatives, such as feathers and hair, are broadly conserved among vertebrates (Headon & Overbeek, 1999; Chuong *et al.*, 2000; Harris *et al.*, 2002, 2008; Widelitz, 2008; Di-Poï & Milinkovitch, 2016; Wu *et al.*, 2017; Cooper *et al.*, 2019). Adhesive lamellae and scansors have long been considered modified plantar scales (Collette, 1961; Hiller, 1968; Russell, 1975); however, the process by which toe pad scansors/lamellae form is distinct from the presumably ancestral pattern of non-adhesive plantar scale development observed in *Sceloporus undulatus*, *E. macularis* (Figs 3, 5A) and *Pogona vitticeps* (Cooper *et al.*, 2019). In each of these species, representing three families of lizards, scales form nearly synchronously across the length of the digit. In contrast, the toe pads of all *Anolis* and gecko species we investigated pass through four similar stages
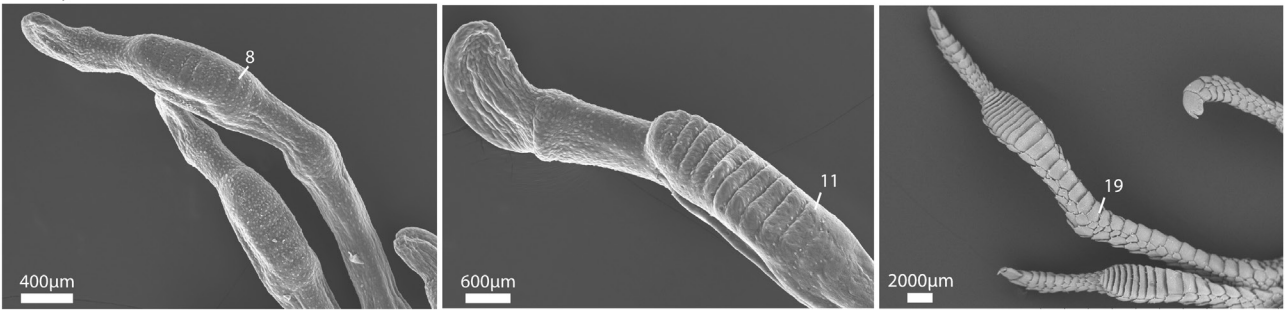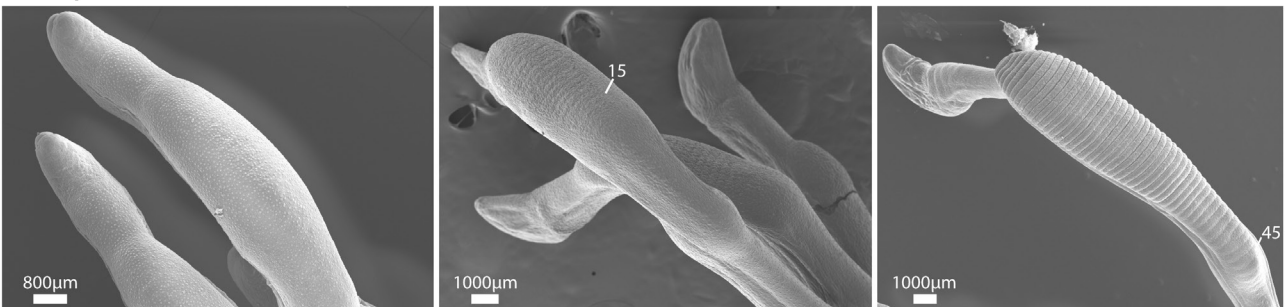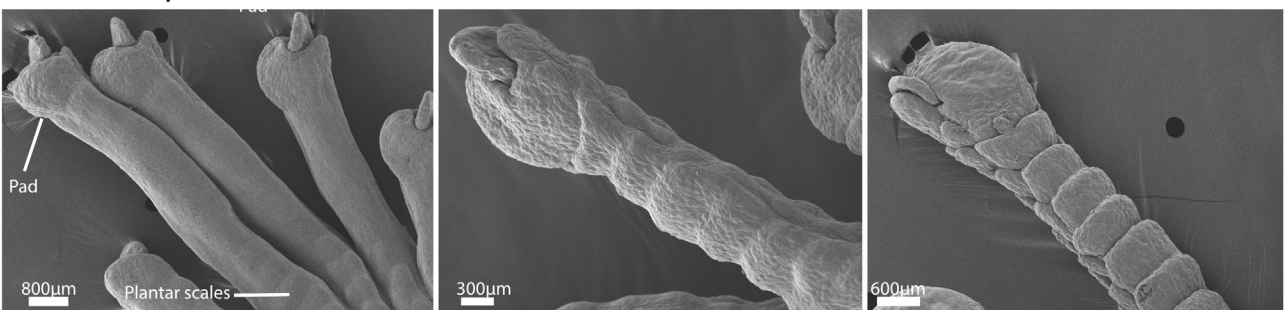
A. cybotes

A. equestris

S. macrolepis

Figure 4. Opportunistic sampling of *Anolis* and gecko embryos. Scanning electron micrographs depicting plantar views of *Anolis cybotes*, *Anolis equestris* and *Sphaerodactylus macrolepis* embryonic pes, digit IV. Numbers correspond to the developing ridges or lamellae.

following digital webbing reduction (Fig. 5B): (1) pad formation through hypertrophy of digital tissues, (2) pad subdivision in the distal portion of the digit, (3) pad extension in a distal-to-proximal direction and (4) elaboration. The results suggest that *Anolis* and geckos have independently converged on a similar developmental pattern to generate their adhesive toe pads. The distal-to-proximal extension of the scale ridges deviates from the majority of vertebrate limb development patterns, which often develop from proximal to distal. For example, within the hand and foot, digits develop in a proximal-to-distal sequence (Saunders, 1948; Summerbell, 1974). After the origin of the toe pad, diversity in toe pad morphology is generated by 'tinkering' with this newly established developmental shift. More specifically, the diversity

observed in adult toe pad morphology is determined by modifying the position of the toe pad or adjusting developmental timing, whereby some species progress to later stages of the developmental sequence than others (Fig. 5B). Both geckos and anoles underwent a dramatic reorganization of their plantar scale developmental programmes at the origin of toe pads. Based on this observation, we hypothesize that the ancestral scale development mechanism is not capable of generating variation along the axes necessary to form an adhesive pad.

Our results show that a pad is formed before lamellae or scansors arise. This pattern is present in anoles, geckos with terminal pads, and geckos with basal pads, all of which arose independently (Rosenberg *et al.*, 1992; Khannoon, 2015; Khannoon *et al.*, 2015;
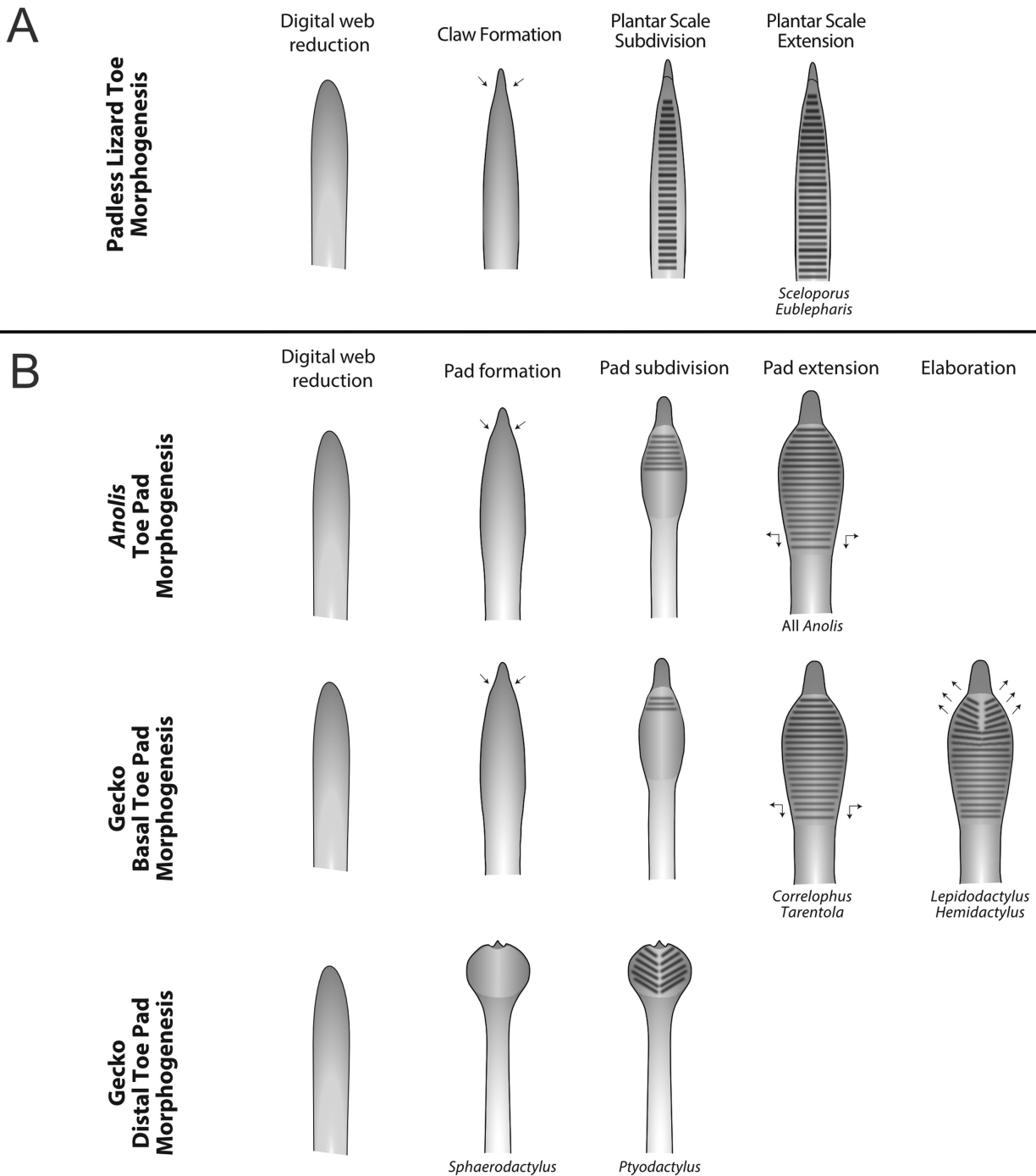
Figure 5. Schematics illustrating the current understanding of plantar scale morphogenesis of lizards. A, morphogenesis of padless lizard toes. B, morphogenesis of pad-bearing lizard toes. Arrows illustrate regions and direction of growth.

Alturk & Khannoon, 2020; Griffing *et al.*, 2021). We speculate that enlarged lamellae/scansors cannot be supported by a relatively narrow toe. The supportive structure of the enlarged pad is a necessary prerequisite for the development and function of lamellae and scansors. Future manipulative experiments that attempt to dissociate pad development from lamellae/scansor development and/or attempt to grow an adhesive pad on a non-padded toe will be needed to fully test this hypothesis.

Following the origin of adhesive toe pads, adaptive changes in toe pad morphology are consistently

generated through similar developmental modifications. This agrees with the predictions of previous authors (e.g. Jacob, 1977; Duboule & Wilkins, 1998; Müller & Newman, 2005) that adaptive variation would arise from relatively small modifications to developmental programmes, not large-scale developmental repatterning. This pattern is most prominent in our data on *Anolis* toe pad development. Within *Anolis*, the toe pad evolved only once, ~70 Mya (Losos, 2009; Román-Palacios *et al.*, 2018). Our study examined development of species with ~15–50 scansors. Despite this range, variation in scansor number is consistently the result of modifications to the stages of pad expansion. Species with relatively few scansors, such as *A. sagrei*, stop producing scansors at an earlier stage than species with more scansors, such as *A. carolinensis* or *A. equestris*. Furthermore, regardless of adult morphology, all *Anolis* species begin with the same base-state of seven to eight ridges (Figs 3, 4). Although our intra-clade sampling of geckos is not as dense as it is in anoles, we predict that the same pattern will be found in other species from within each independent origin of toe pads.

Compared to anoles, geckos exhibit a striking diversity of adult pad morphologies, varying in both their position and their complexity (Fig. 2; Russell, 1972; Russell & Gamble, 2019). The spinulate Oberhäutchen layer of the epidermis is ancestral to geckos, including padless taxa, which has facilitated repeated elaborations into adhesive setae (Maderson, 1970; Stewart & Daniel, 1972; Russell, 1976, 1979; Peattie, 2008). The full spectrum of gecko toe pad diversity is reflected in the characteristic developmental sequence of toe pads (Fig. 5B); species with relatively simple scansors stop at earlier developmental stages than those with more complex toe pad elaborations. For example, following reduction of the interdigital webbing, *Sphaerodactylus* stops relatively early in the developmental sequence, creating a bulbous, distal toe pad covered in setae. Its remaining digital scales develop in the typical ancestral pattern (Figs 4, 5B; Supporting Information, Fig. S9). Additional toe pad complexity is added in *Correlophus* (Griffing *et al.*, 2021), *Tarentola* (Khannoon, 2015) and *Ptyodactylus* (Rosenburg *et al.*, 1992) as horizontal rows of scansors are laid down from distal to proximal. Several key stages are missing from the *Ptyodactylus* embryological series to know whether the scansors form perpendicular, or at an angle, to the long axis of the toe. Finally, following pad extension, *H. turcicus* and *L. lugubris* develop toe pad elaborations, leading to the varying degrees of toe pad scansor bifurcation (Figs 3, 5B). These elaborations appear to be achieved through distolateral growth of individual scansorial ridges which create the appearance of scansor bifurcation. Through these simple modifications, geckos have repeatedly 'escaped' the ancestral constraint of epidermal appendage development (Fig. 5).

The newfound evolutionary and developmental lability, relative to non-padded lizards, is evident in the remarkable diversity in toe pad morphology not observed in other clades (Fig. 2; Russell & Gamble, 2019). In spite of other developmental possibilities and the dramatic divergence away from the ancestral pattern of toe pad development, this pattern suggests that inherent developmental constraints limit the ways in which adhesive toe pads and scales can form. Further research into the histological patterns and molecular regulation of toe pad morphogenesis will help us to understand the contingencies of pad and scale development and will be critical to further unravelling the complexities of adhesive toe pad evolution.

Innovations, such as the adhesive toe pad, have played a central role in studies examining the dynamic process of phenotypic evolution (Müller, 1990; Müller & Wagner, 1991; Heard & Hauser, 1995; Müller & Newman, 2005; Rabosky, 2017). Previous authors have predicted that key innovations arise following a significant repatterning of development while adaptive changes in morphology occur through relatively minor tinkering of established developmental processes (Jacob, 1977; Wake & Roth, 1989; Müller, 1990; Müller & Wagner, 1991; Wagner & Müller, 2002; West-Eberhard, 2003). Our results on the origin and diversification of the adhesive toe pad provide robust evidence in support of these predictions – toe pads are initially formed through a repatterning of digital development and subsequently diversify in shape and size through slight modifications. Future studies should carefully consider the roles that evolutionary history and constraint play in shaping the developmental bases of adaptive variation. To decipher the relative roles that evolutionary history and constraint play in the diversification of morphology, it is critical to integrate robust phylogenetic sampling of developmental processes across micro-, meso- and macroevolutionary timescales (Abouheif, 2008; Wake *et al.*, 2011; Sanger & Rajakumar, 2019). We do not yet know whether this pattern of conserved variation is limited to rapidly diversifying adaptive radiations, such as *Anolis* (Sanger *et al.*, 2012, 2013) and Darwin's finches (Abzhanov *et al.*, 2004, 2006; Mallarino *et al.*, 2012), or whether it is a general phenomenon of morphological evolution. Testing this hypothesis will require the developmental bases of adaptive variation to be studied in additional groups that have diversified over different timescales. Once vetted, the hypothesis of conserved variation may provide a general rule regarding the processes that govern the production of selectable phenotypic variation.

DATA AVAILABILITY

The data underlying this work are available in the article and in its online Supporting Information.

REFERENCES

Abouheif E. 2008. Parallelism as the pattern and process of mesoevolution. *Evolution & Development* **5:** 3–5.

Abzhanov A, **Kuo WP**, **Hartmann C**, **Grant BR**, **Grant PR**, **Tabin CJ. 2006.** The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. *Nature* **442:** 563–567.

Abzhanov A, **Protas M**, **Grant BR**, **Grant PR**, **Tabin CJ. 2004.** Bmp4 and morphological variation of beaks in Darwin's Finches. *Science* **305:** 1462–1465.

Alberch P. 1980. Ontogenesis and morphological diversification. *American Zoologist* **20:** 653–667.

Alberch P. 1989. The logic of monsters: Evidence for internal constraint in development and evolution. *Geobios* **22:** 21–57.

Arthur W. 2004. The effect of development on the direction of evolution: Toward a twenty-first century consensus. *Evolution & Development* **6:** 282–288.

Alturk WIM, **Khannoon ER. 2020.** Ontogeny of the Moorish gecko *Tarentola mauritanica* with emphasis on morphogenesis of the skin and its derivatives. *Journal of Experimental Zoology, Part B: Molecular and Developmental Evolution* **334:** 294–310.

Autumn K. 2006. Frictional adhesion: a new angle on gecko attachment. *Journal of Experimental Biology* **209:** 3569–3579.

Autumn K, **Sitti M**, **Liang YA**, **Peattie AM**, **Hansen WR**, **Sponberg S**, **Kenny TW**, **Fearing R**, **Israelachvili JN**, **Full RJ. 2002.** Evidence for van der Waals adhesion in gecko setae. *Proceedings of the National Academy of Sciences* **99:** 12252–12256.

Bauer AM, **Böhme W**, **Weitschat W. 2005.** An Early Eocene gecko from Baltic amber and its implications for the evolution of gecko adhesion. *Journal of Zoology, London* **265:** 327–332.

Bauer AM, **Sadlier RA. 2000.** *The herpetofauna of New Caledonia*. Ithaca: Society for the Study of Amphibians and Reptiles.

Beldade P, **Brakefield PM. 2002.** The genetics and evo-devo of butterfly wing patterns. *Nature Reviews Genetics* **3:** 442–452.

Bergmann PJ, **Russell AP. 2003.** Lamella and scansor numbers in *Thecadactylus rapicauda* (Gekkonidae): Patterns revealed through correlational analysis and implications for systematic and functional studies. *Amphibia-Reptilia* **24:** 379–385.

Beuttell K, **Losos JB. 1999.** Ecological morphology of Caribbean anoles. *Herpetological Monographs* **13:** 1–28.

Brakefield PM. 2003. The power of evo-devo to explore evolutionary constraints: Experiments with bufferfly eyespots. *Zoology* **106:** 283–290.

Brakefield PM. 2006. Evo-devo and constraints on selection. *Trends in Ecology and Evolution* **21:** 362–368.

Bruse F, **Meyer M**, **Schmidt W. 2005.** *Day geckos*. Frankfurt: Edition Chimaira.

Cheverud JM, **Rutledge JJ**, **Atchley WR. 1983.** Quantitative genetics of development: Genetic correlations among age-specific trait values and the evolution of ontogeny. *Evolution* **37:** 895–905.

Chuong C-M, **Chodanker R**, **Widelitz RB**, **Jiang T-X. 2000.** Evo-devo of feathers and scales: Building complex epithelial appendages. *Current Opinion in Genetics & Development* **10:** 449–456.

Collette BB. 1961. Correlations between ecology and morphology in anoline lizards from Havana, Cuba, and southern Florida. *Bulletin of the Museum of Comparative Zoology* **125:** 135–162.

Cooper RL, **Lloyd VJ**, **Di-Poï N**, **Fletcher AG**, **Barrett PM**, **Fraser GJ. 2019.** Conserved gene signaling and a derived patterning mechanism underlie the development of avian footpad scales. *EvoDevo* **10:** 19.

De Vosjoli P, **Fast F**, **Repashy A. 2003.** *Rhacodactylus: the complete guide to their selection and care*. Vista: Advanced Visions.

Di-Poï N, **Milinkovitch MC. 2016.** The anatomical placode in reptile scale morphogenesis indicates shared ancestry among skin appendages in amniotes. *Science Advances* **2:** e1600708.

Duboule D, **Wilkins AS. 1998.** The evolution of 'bricolage'. *Trends in Genetics* **14:** 54–59.

Dufaure J, **Hubert J. 1961**. Table de development du lézard vivipare: *Lacerta* (*Zootoca*) *vivipara* Jacquin. *Archives d'Anatomie Microscopique et de Morphologie Expérimentale.* **50:** 309–327.

Elstrott J, **Irschick DJ. 2004.** Evolutionary correlations among morphology, habitat use and clinging performance in Caribbean *Anolis* lizards. *Biological Journal of the Linnean Society* **83:** 389–398.

Fontanarrosa G, **Daza JD**, **Abdala V. 2018.** Cretaceous fossil gecko hand reveals a strikingly modern scansorial morphology: qualitative and biometric analysis of an amber-preserved lizard hand. *Cretaceous Research* **84:** 120–133.

Gamble T, **Greenbaum E**, **Jackman TR**, **Russell AP**, **Bauer AM. 2012.** Repeated origin and loss of adhesive toepads in geckos. *PLoS One* **7:** e39429.

Glossip D, **Losos JB. 1997.** Ecological correlates of number of subdigital lamellae in anoles. *Herpetologica* **53:** 192–199.

Griffing AH, **Sanger TJ**, **Daza JD**, **Nielsen SV**, **Pinto BJ**, **Stanley EL**, **Gamble T. 2019.** Embryonic development of a parthenogenetic vertebrate, the mourning gecko (*Lepidodactylus lugubris*). *Developmental Dynamics* **248:** 1070–1090.

Griffing AH, **Sanger TJ**, **Epperlein L**, **Bauer AM**, **Cobos A**, **Higham TE**, **Naylor E**, **Gamble T. 2021.** And thereby hangs a tail: Morphology, developmental patterns, and biomechanics of the adhesive tails of crested geckos (*Correlophus ciliatus*). *Proceedings of the Royal Society B: Biological Sciences* **288:** 20210650.

Griffing AH, **Sanger TJ**, **Matamoros IC**, **Nielsen SV**, **Gamble T. 2018.** Protocols for husbandry and embryo collection of a parthenogenetic gecko, *Lepidodactylus lugubris* (Squamata: Gekkonidae). *Herpetological Review* **49:** 230–235.

Harris MP, **Fallon JF**, **Prum RO. 2002.** *Shh-Bmp*2 signaling module and the evolutionary origin and diversification of feathers. *Journal of Experimental Zoology* **294:** 160–176.

Harris MP, **Rohner N**, **Schwartz H**, **Perathoner S**, **Konstantinidis P**, **Nüsslein-Volhard C. 2008.** Zebrafish eda and edar mutants reveal conserved and ancestral roles of ectodysplasin signaling in vertebrates. *PLoS Genetics* **4:** e1000206.

Headon DJ, **Overbeek PA. 1999.** Involvement of a novel Tnf receptor homologue in hair follicle induction. *Nature Genetics* **22:** 370–374.

Heard SB, **Hauser DL. 1995.** Key evolutionary innovations and their ecological mechanisms. *Historical Biology* **10:** 151–173.

Hecht MK. 1952. Natural selection in the lizard genus *Aristelliger*. *Evolution* **6:** 112–124.

Higham TE, **Gamble T**, **Russell AP. 2017.** On the origin of frictional adhesion in geckos: Small morphological changes lead to a major biomechanical transition in the genus *Gonatodes*. *Biological Journal of the Linnean Society* **120:** 503–517.

Hiller U. 1968. Untersuchungen zum Feinbau und zur Funktion der Haftborsten von Reptilien. *Zeitschrift für Morphologie der Tiere* **62:** 307–362.

Irschick DJ, **Austin CC**, **Petren K**, **Fisher RN**, **Losos JB**, **Ellers O. 1996.** A comparative analysis of clinging ability among pad-bearing lizards. *Biological Journal of the Linnean Society* **59:** 21–35.

Jacob F. 1977. Evolution and tinkering. *Science* **196:** 1161–1166.

Johnsn MK, **Russell AP. 2009.** Configuration of the setal fields of *Rhoptropus* (Gekkota: Gekkonidae): Functional, evolutionary, ecological and phylogenetic implications of observed pattern. *Journal of Anatomy* **214:** 937–955.

Khannoon ER. 2015. Developmental stages of the climbing gecko *Tarentola annularis* with special reference to the claws, pad lamellae, and subdigital setae. *Journal of Experimental Zoology, Part B: Molecular and Developmental Evolution* **324:** 450–464.

Khannoon ER, **Russell AP**, **Tucker AS. 2015.** Developmental mechanisms underlying differential claw expression in the autopodia of geckos. *EvoDevo* **6:** 8.

Knox AK, **Losos JB**, **Schneider CJ. 2001.** Adaptive radiation versus intraspecific differentiation: Morphological variation in Caribbean *Anolis* lizards. *Journal of Evolutionary Biology* **14:** 904–909.

Konečný P. 2002. Natural history and captive husbandry of the Turkish gecko *Hemidactylus turcicus*. *Gekko* **2:** 19–20.

Leviton AE, **Anderson SC**, **Adler K**, **Minton SA. 1992.** *Handbook to Middle East amphibians and reptiles*. Ithaca: Society for the Study of Amphibians and Reptiles.

Losos JB. 2009. *Lizards in an evolutionary tree: ecology and adaptive radiation of anoles*. Berkeley: University of California Press.

Losos JB. 2011. Convergence, adaptation, and constraint. *Evolution* **65:** 1827–1840.

Losos JB, **de Queiroz K. 1997.** Evolutionary consequences of ecological release in Caribbean *Anolis* lizards. *Biological Journal of the Linnean Society* **61:** 459–483.

Macrini TE, **Irschick DJ**, **Losos JB. 2003.** Ecomorphological differences in toepad characteristics between mainlined and island anoles. *Journal of Herpetology* **37:** 52–58.

Maderson PFA. 1970. Lizard hands and lizard glands: models for evolutionary study. *Forma et Functio* **3:** 179–204.

Mallarino R, **Campàs O**, **Fritz JA**, **Burns KJ**, **Weeks OG**, **Brenner MP**, **Abzhanov A. 2012.** Closely related bird species demonstrate flexibility between beak morphology and underlying developmental programs. *Proceedings of the National Academy of Sciences* **109:** 16222–16227.

Maynard-Smith J, **Burian R**, **Kauffman S**, **Alberch P**, **Campbell J**, **Goodwin B**, **Lande R**, **Raup D**, **Wolper L. 1985.** Developmental constraints and evolution: a perspective from the Mountain Lake conference on development and evolution. *The Quarterly Review of Biology* **60:** 265–287.

Miller AH, **Stroud JT. 2021.** Novel tests of the key innovation hypothesis: Adhesive toepads in arboreal lizards. *Systematic Biology*. doi:10.1093/sysbio/syab041.

Müller GB. 1990. Developmental mechanisms at the origin of morphological novelty: A side-effect hypothesis. In: Müller GB, Nitecki MH, eds. *Evolutionary innovations*. Chicago: University of Chicago, 99–130.

Müller GB, **Newman SA. 2005.** The innovation triad: An evo devo agenda. *Journal of Experimental Zoology, Part B: Molecular and Developmental Evolution* **304B:** 487–503.

Müller GB, **Wagner GP. 1991.** Novelty in evolution: restructuring the concept. *Annual Review of Ecology and Systematics* **22:** 229–256.

Olson ME. 2012. The developmental renaissance in adaptationism. *Trends in Ecology and Evolution* **27:** 278–287.

Olson ME. 2019. Overcoming the constraint–adaptation dichotomy: long live the adaptation–constraint dichotomy. In: Fusco G, ed. *Perspectives on evolutionary and developmental biology.* Padua: University of Padova Press, 123–139.

Oster G, **Alberch P. 1982.** Evolution and bifurcation of developmental programs. *Evolution* **36:** 444–459.

Peattie AM. 2008. Subdigital setae of narrow-toed geckoes, including a eublepharid (*Aeluroscalabotes felinus*). *The Anatomical Record* **291:** 869–875.

Peterson JA. 1983. The evolution of the subdigital pad of *Anolis* I. Comparisons among the anoline genera. In: Rhodin AGJ, Miyata K, eds. *Advances in herpetology and evolutionary biology: essays in honor of Ernest E. Williams.* Cambridge: Museum of Comparative Zoology, 245–283.

Pinto G, **Mahler DL**, **Harmon LJ**, **Losos JB. 2008.** Testing the island effect in adaptive radiation: Rates and patterns of morphological diversification in Caribbean and mainland *Anolis* lizards. *Proceedings of the Royal Society, B: Biological Sciences* **275:** 2749–2757.

Rabosky DL. 2017. Phylogenetic tests for evolutionary innovation: The problematic link between key innovations and exceptional diversification. *Philosophical Transactions of the Royal Society B* **372:** 20160417.

Román-Palacios C, **Tavera J**, **Castañeda MdR. 2018.** When did anoles diverge? An analysis of multiple dating strategies. *Molecular Phylogenetics and Evolution* **127:** 655–668.

Rosenberg HI, **Russell AP**, **Cavey MJ. 1992.** Development of the subdigital adhesive pads of *Ptyodactylus guttatus* (Reptilia: Gekkonidae). *Journal of Morphology* **211:** 243–258.

Ruibal R, **Ernst V. 1965.** The structure of the digital setae of lizards. *Journal of Morphology* **117:** 271–294.

Russell AP. 1972. *The foot of gekkonid lizards: a study in comparative and functional anatomy.* Unpublished PhD thesis, University of London.

Russell AP. 1975. A contribution to the functional analysis of the foot of the Tokay, *Gekko gecko* (Reptilia: Gekkonidae). *Journal of Zoology, London* **176:** 437–476.

Russell AP. 1976. Some comments concerning interrelationships amongst gekkonine geckos. In: Bellairs Ad'A, Cox CB, eds. *Morphology and biology of reptiles.* London: Academic Press, 217–244.

Russell AP. 1979. Parallelism and integrated design in the foot structure of gekkonine and diplodactyline geckos. *Copeia* **1979:** 1–21.

Russell AP. 1981. Descriptive and functional anatomy of the digital vascular system of the Tokay, *Gekko gecko*. *Journal of Morphology* **169:** 293–323.

Russell AP. 1986. The morphological basis of weight-bearing in the scansors of the tokay gecko (Reptilia: Sauria). *Canadian Journal of Zoology* **64:** 948–955.

Russell AP. 2002. Integrative functional morphology of the gekkotan adhesive system (Reptilia: Gekkota). *Integrative & Comparative Biology* **42:** 1154–1163.

Russell AP, **Delaugerre M-J. 2017.** Left in the dust: differential effectiveness of the two alternative adhesive pad configurations in geckos (Reptilia: Gekkota). *Journal of Zoology* **301:** 61–68.

Russell AP, **Gamble T. 2019.** Evolution of the gekkotan adhesive system: does digit anatomy point to one or more origins?. *Integrative & Comparative Biology* **59:** 131–147.

Russell AP, **Garner AM. 2021.** Setal field transects, evolutionary transitions and gecko–anole convergence provide insights into the fundamentals of form and function of the digital adhesive system in lizards. *Frontiers in Mechanical Engineering* **6:** 111.

Russell AP, **Stark AY**, **Higham TE. 2019.** The integrative biology of gecko adhesion: Historical review, current understanding, and grand challenged. *Integrative & Comparative Biology* **59:** 101–116.

Sanger TJ, **Hime PM**, **Johnson MA**, **Diani J**, **Losos JB. 2008a.** Laboratory protocols for husbandry and embryo collections of *Anolis* lizards. *Herpetological Review* **39:** 58–63.

Sanger TJ, **Losos JB**, **Gibson-Brown JJ. 2008b.** A developmental staging series for the lizard genus *Anolis*: a new system for the integration of evolution, development, and ecology. *Journal of Morphology* **269:** 129–137.

Sanger TJ, **Rajakumar R. 2019.** How a growing organismal perspective is adding new depth to integrative studies of morphological evolution. *Biological Reviews* **94:** 184–198.

Sanger TJ, **Revell LJ**, **Gibson-Brown JJ**, **Losos JB. 2012.** Repeated modification of early limb morphogenesis programmes underlies the convergence of relative limb length in *Anolis* lizards. *Proceedings of the Royal Society B* **279:** 739–748.

Sanger TJ, **Sherratt E**, **McGlothlin JW**, **Brodie ED**, **Losos JB**, **Abzhanov A. 2013.** Convergent evolution of sexual dimorphism in skull shape using distinct developmental strategies. *Evolution* **67:** 2180–2193.

Saunders JW. 1948. The proximo-distal sequence of origin of the parts of the chick wing and the role of the ectoderm. *Journal of Experimental Zoology* **108:** 363–403.

Schoener TW. 1975. Presence and absence of habitat shift in some widespread lizard species. *Ecological Monographs* **45:** 233–258.

Seipp R, **Henkel FW. 2000.** *Rhacodactylus: Biology, natural history, & husbandry*. Frankfurt: Edition Chimaira.

Skinner A, **Hugall AF**, **Hutchinson MN. 2011.** Lygosomine phylogeny and the origins of Australian scincid lizards. *Journal of Biogeography* **38:** 1044–1058.

Stewart GR, **Daniel RS. 1972.** Scales of the lizard *Gekko gecko*: surface structure examined with scanning electron microscope. *Copeia* **1972:** 252–257.

Summerbell D. 1974. Interaction between the proximo-distal and antero-posterior co-ordinates of positional value during the specification of positional information in the early development of the chick limb bud. *Development* **32:** 227–237.

Thorogood J, **Whimster IW. 1979.** The maintenance and breeding of the leopard gecko, *Eublepharis macularius*, as a laboratory animal. *International Zoo Yearbook* **19:** 74–78.

Uetz P, **Freed P**, **Hošek J. 2021.** *The reptile database.* Available at www.reptile-database.org.

Vickaryous, MK, **Gilbert EAB. 2019.** Reptile embryology and regeneration. In: Pelegri F, ed. *Vertebrate embryogenesis.* New York: Humana Press, 219–246.

Wagner GP, **Müller GB. 2002.** Evolutionary innovations overcome ancestral constraints: a re-examination of character evolution in male sepsid flies (Diptera: Sepsidae). *Evolution & Development* **4:** 1–6.

Wake DB. 1991. Homoplasy: the result of natural selection, or evidence of design limitations? *The American Naturalist* **138:** 543–567.

Wake DB, **Larson A. 2003.** Multidimensional analysis of an evolving lineage. *Science* **238:** 42–48.

Wake DB, **Roth G. 1989.** The linkage between ontogeny and phylogeny in the evolution of complex systems. In: Wake DB, Roth G, eds. *Complex organismal functions: integration and evolution in vertebrates.* New York: Wiley, 361–377.

Wake DB, **Wake MH**, **Specht CD. 2011.** Homoplasy: from detecting pattern to determining process and mechanism of evolution. *Science* **331:** 1032–1035.

West-Eberhard MJ. 2003. *Developmental plasticity and evolution.* Oxford: Oxford University Press.

Widelitz RB. 2008. Wnt signaling in skin organogenesis. *Organogensis* **4:** 123–133.

Williams EE, **Peterson JA. 1982.** Convergent and alternative designs in the digital adhesive pads of scincid lizards. *Science* **215:** 1509–1511.

Wise PAD, **Vickaryous MK**, **Russell AP. 2009.** An embryonic staging table for *in ovo* development of *Eublepharis macularius*, the leopard gecko. *The Anatomical Record* **292:** 1198–1212.

Wu P, **Yan J**, **Lai YC**, **Ng CS**, **Li A**, **Jiang X**, **Elsey RM**, **Wideltiz R**, **Bajpai R**, **Li WH**, **Chuong CM. 2017.** Multiple regulatory modules are required for scale-to-feather conversion. *Molecular Biology and Evolution* **35:** 417–430.

Zheng Y, **Wiens JJ. 2016.** Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4162 species. *Molecular Phylogenetics and Evolution* **94:** 537–547.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Scanning electron micrographs depicting plantar views of the developing padless *Sceloporus undulatus* pes, digit IV. Light microscope image of adult pes.

Figure S2. Scanning electron micrographs depicting plantar views of the developing basal padded *Anolis sagrei* pes, digit IV. Numbers correspond to the developing ridges, scansors or lamellae. Light microscope image of adult pes.

Figure S3. Scanning electron micrographs depicting plantar views of the developing basal padded *Anolis carolinensis* pes, digit IV. Numbers correspond to the developing ridges, scansors or lamellae. Light microscope image of adult pes.

Figure S4. Scanning electron micrographs depicting plantar views of the developing padless *Eublepharis macularius* pes, digit IV. Light microscope image of adult pes.

Figure S5. Scanning electron micrographs depicting plantar views of the developing basal padded *Correlophus ciliatus* pes, digit IV. Numbers correspond to the developing ridges, scansors or lamellae. Light microscope image of adult pes.

Figure S6. Scanning electron micrographs depicting plantar views of the developing basal padded *Lepidodactylus lugubris* pes, digit IV. Numbers correspond to the developing ridges, scansors or lamellae. Arrows illustrate the direction of inferred digital ridge expansion. Light microscope image of adult pes.

Figure S7. Scanning electron micrographs depicting plantar views of the developing basal padded *Hemidactylus turcicus* pes, digit IV. Numbers correspond to the developing ridges, scansors or lamellae. Arrows illustrate the direction of inferred digital ridge expansion. Light microscope image of adult pes.

Figure S8. Opportunistic sampling of *Anolis* embryos. Scanning electron micrographs depicting plantar views of *Anolis angusticeps*, *Anolis grahami*, *Anolis cybotes*, *Anolis lineatopus*, *Anolis equestris* and *Anolis brevirostris* embryonic pes, digit IV. Numbers correspond to the developing ridges or lamellae.

Figure S9. Opportunistic sampling of gecko embryos. Scanning electron micrographs depicting plantar views of distal-padded *Sphaerodactylus macrolepis* embryonic pes, digit IV.

Table S1. Comparative digital development time table of non-padded species investigated in this study in days post-oviposition (DPO).

Table S2. Comparative digital development time table of padded species investigated in this study. Both days post-oviposition (DPO) and stages (St) are included when appropriate. Stages are further divided and labelled as either early (e) or late (l). A dash indicates data are not available for opportunistically sampled species(*). DPO data were unavailable for *Sphaerodactylus macrolepis*. DPO should be used with caution as relatively few individuals of each stage were observed for some species, making an evaluation of variation difficult or impossible.